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Evaluation of Dentists' Anxiety Levels During the Coronavirus Pandemic

Koronavirüs Salgını Esnasında Diş Hekimlerinin Anksiyete Düzeylerinin Değerlendirilmesi

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Abstract

Objective: Coronavirus disease-2019 (COVID-19) is a highly contagious infection whose possible transmission routes are airborne droplets, close contact with an infected person or contaminated surface, blood, or saliva. These possible routes of transmission are closely related to the work conditions of dentists. The present study assessed the anxiety levels of dentists and related factors during the COVID-19 pandemic using the Beck Anxiety Inventory (BAI).

Materials and Methods: A questionnaire consisting of 33 questions on the anxiety levels of dentists related to infection COVID-19 was used. The questionnaires were sent to dentists via online platforms. Mann-Whitney U and Kruskal-Wallis tests were used to compare quantitative variables.

Results: In total, 260 dentists were included in the study. The median (minimum-maximum) BAI score of participants was 28 (19-75), which displayed moderate anxiety. There was a statistically significant difference between the genders in terms of BAI scores ($p<0.001$). No statistically significant difference was found between ages ($p=0.79$). Of the participants, 64.6% (n=168) stated that the level of income decreased, and 69.2% stated that safe working conditions decreased.

Conclusions: During the COVID-19 pandemic, dentists were one of the most affected groups by the pandemic due to dental settings. Gender and the presence of chronic disease were the main factors that negatively affected the anxiety level of dentists. As the developments regarding the pandemic are updated daily, dentists should follow them and update their information through various platforms.

Öz

Amaç: Koronavirüs hastalığı-2019 (COVID-19), olası bulaşma yolları havadaki damlacıklar, enfekte bir kişiyle ya da kontamine bir yüzeyle yakın temas, kan veya tükürük olan oldukça bulaşıcı bir enfeksiyondur. Bu olası bulaşma yolları diş hekimlerinin çalışma şartları ile yakından ilişkilidir. Bu çalışmanın amacı, diş hekimlerinin COVID-19 salgını esnasındaki anksiyete düzeylerini ve ilişkili faktörleri Beck Anksiyete Envanteri (BAE) ile değerlendirmektir.

Gereç ve Yöntemler: Çalışmada, diş hekimlerinin COVID-19 enfeksiyonuna ilişkin anksiyete düzeyleri ile ilgili 33 sorudan oluşan anket formu kullanıldı. Anketler online platformlar aracılığıyla diş hekimlerine gönderildi. Kantitatif değişkenleri karşılaştırmak için Mann-Whitney U ve Kruskal-Wallis testleri kullanıldı.

Bulgular: Toplamda 260 diş hekimi çalışmaya dahil edildi. Katılımcıların ortanca (minimum-maksimum) BAE skoru 28 (19-75) olarak bulundu ve bu skor orta

derecede anksiyeteyi ifade etmektedir. BAE skorlarına göre cinsiyetler arasında istatistiksel olarak anlamlı fark vardı (p<0,001). Ancak yaşlara göre istatistiksel olarak anlamlı bir fark bulunmadı (p=0,79). Katılımcıların %64,6'sı (n=168) gelir düzeylerinin düştüğünü, %69,2'si ise güvenli çalışma koşullarının azaldığını belirttiler.

Sonuç: COVID-19 salgını esnasında, çalışma ortamlarının doğası gereği salgından en çok etkilenen gruplardan biri diş hekimleridir. Çalışmamızda, cinsiyet ve kronik hastalık varlığı diş hekimlerinin anksiyete düzeyini olumsuz etkileyen temel faktörler olarak belirlendi. Salgın ile ilgili gelişmeler günden güne değişiklik gösterebildiği için, diş hekimlerinin gelişmeleri yakından takip etmeleri ve çeşitli platformlar üzerinden bilgilerini güncellemeleri gerekmektedir.

Introduction

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) or better known as the 2019 coronavirus disease (COVID-19) is a viral infection that first started in December 2019 in Wuhan, China (1). This newly discovered highly contagious infection spread very rapidly to other countries of the world and became a worldwide pandemic in a few weeks (2,3). The first positive case in our country was detected on March 11, 2020, the same day the World Health Organization officially declared the COVID-19 outbreak (4).

The common clinical symptoms of COVID-19 are fever, dry cough, dyspnea, myalgia, fatigue, headache, diarrhea after 3-5 days incubation period. Anosmia and ageusia were also reported in most patients for early diagnosis of COVID-19 infection. Radiological findings of the disease are ground-glass opacities in lower lung lobes (5-7). Since the symptoms of most patients (about 80%) are not very obvious, the number of undiagnosed patients increases. These patients can easily transmit the infection to other people (8,9).

Airborne droplets, close contact to an infected person or a contaminated surface, blood or saliva are reported routes of possible transmission. These transmission routes pose a great risk in dental practice, which has a similar transmission route (10). Dental procedures can cause bleeding, aerosol and droplet formation, causing the infection to be transmitted from the patient to the dentist and other people in the same area (10,11). On the other hand, the dental office also potentially may expose patients to cross-infection. Due to the risk of cross-infection, emergency dental care was recommended in dentistry (12). Therefore, the risk of contamination and reduced working hours due to infection may lead to anxiety in dentists.

The aim of this study was to evaluate the anxiety levels of dentists and related factors during COVID-19 infection using the Turkish version of Beck Anxiety Inventory (BAI) (13).

Materials and Methods

Ethical approval of the study was obtained from, Ethics Committee of the Faculty of Medicine of Aydın Adnan Menderes University (protocol number: 2020/88, date: 07.05.2020). In addition, Republic of Turkey, Ministry of Health, Scientific Research Platform also approved the study. In this study, a questionnaire consisting of 33 questions on the anxiety levels of dentists related to infection of COVID-19 was composed and converted into an on-line format by using the Google forms (Google Inc., Mountain View, CA, USA) and sent through WhatsApp to the dentists. The dentists who participated in the study were accessed from institution database. This questionnaire was conducted between August to September 2020. A statement about the details of the study was added to the beginning of the questionnaire and it was stated that participation was voluntary. The first 12 questions addressed personal and professional information, including their age, gender, marital status, number of children, institution, presence of expertise, smoking status, chronic disease, income level, work position, safe working conditions, and the remaining 21 questions were the items of the BAI (14).

Beck Anxiety Inventory

The BAI evaluates the extent of anxiety containing 21 questions related to common symptoms of anxiety. Each question is scored between 0 and 3 points, so the total score ranges from 0 to 63. A total score of 0-21 indicates very low anxiety, 22-35 represents moderate anxiety, and 36 and higher means potentially concerning levels of anxiety. The reliability and validity of the scale for the Turkish population was tested by Ulusoy et al. (13).

Statistical Analysis

The statistical package of SPSS version 23.0 for Windows (SPSS, Inc, Chicago, IL, USA) was used for analysis of the data. Mann-Whitney U test was used to compare for two independent groups and Kruskal-Wallis test was used to compare for more than two independent groups for quantitative variables. Pairwise comparisons were performed using Dunn's test. A p-value of less than 0.05 was considered to indicate a statistically significant difference.

Results

A total of 260 responses were received with a response rate of 80%. The median [minimum (min)-maximum (max)] BAI score of participants was 28 (19-75) which displays moderate anxiety. The distribution of anxiety levels of the participants according to BAI scores was represented in Table 1. Of the participants, 56.5% were female (n=147), and 43.5% (n=113) were male. There was statistically significant difference between the genders in terms of BAI scores (p<0.001). The median (min-max) BAI score of female dentists was 32 (19-75), while that of male dentists was 25 (21-52). The age range of the participants was 25-60. No statistically significant difference was found between ages (p=0.79). A statistical analysis revealed that there was significant difference between the participants' BAI scores in terms of chronic diseases (p=0.01). The median (min-max) BAI score of participants with chronic disease was 33 (22-75), and those without chronic disease was 28 (19-59). In addition, there was a statistically significant difference in terms of working conditions (work positions, work security and safe working conditions) of the participants. Of the participants, 64.6% (n=168) stated that the level of income decreased, 33.8% (n=88) did not change, and 1.5% (n=4) increased. However, no statistical difference was found related to income levels (p=0.79). The demographic and professional features of the participants are provided in Table 2.

Table 1. The distribution of anxiety levels of the participants

Anxiety levels	BAI score	n	%
Very low anxiety	0-21	25	9.6
Moderate anxiety	22-35	170	65.4
Concerning levels of anxiety	≥36	65	25.0
	Total	260	100.0

BAI: Beck Anxiety Inventory

Discussion

Among healthcare professionals, dentists are more vulnerable to infectious diseases, as they work in routine clinical procedures close to the patients' oral and nasal cavities that contain body fluids such as saliva, mucus and blood (15). Dentists treating COVID-19 positive patients who are asymptomatic or in the incubation period are at serious risk of exposure to the virus and cross-infection (16,17). This possibility may cause fear and anxiety in physicians. Additionally, economic concerns may trigger anxiety in this group due to recommended emergency dental care procedures or postponed practices until an uncertain period (18,19). In this study, approximately two-thirds of the participants stated that their income level (64.6%) and safe working conditions (69.2%) decreased. Depending on the risk of virus contamination and economic concerns, participants presented moderate anxiety using the BAI. This scale is a standard protocol which was used in the present study to measure anxiety levels of various dental practitioners. In their study using the BAI, Zhao et al. (19) stated that frontline dental staff is more likely to suffer from anxiety disorders than the general public. According to the study findings of Suryakumari et al. (18), the mean anxiety scores of the dental practitioners were high, but a high percentage of the population presented with a low level of anxiety. In a different study applied to dentists through a questionnaire in various countries, Ahmed et al. (20) suggested that dentists have high anxiety levels. Similarly, Mahdee et al. (21) also reported a high level of anxiety in Iraqi dentists. In a recently published study, Oliveri et al. (22) reported high level of general anxiety during the first weeks of pandemic in endodontists and dental staff. In a different study (23), the authors declared that the participants have low level of comfort related to the COVID-19 pandemic similar to the study of Yilmaz and Ozbilen (24) which was performed to orthodontists. According to the study of Tysiąc-Miśta and Dziedzic (25) dentists who suspended their clinical work expressed high anxiety in comparison to dentists who continued their practice.

Table 2. The demographic and professional features of the participants

Sociodemographic characteristics	n (%)	median (min-max)	p-value
Gender			
Female	147 (56.5)	32 (19-75)	p<0.001
Male	113 (43.5)	25 (21-52)	
Age			
25-35	112 (43.1)	28 (21-75)	
36-45	99 (38.1)	28 (21-59)	0.79
46-60	49 (18.8)	31 (19-53)	
Institution			
State agency	170 (65.4)	29 (21-59)	0.21
Private practice	90 (34.6)	28 (19-75)	
Presence of expertise			
Yes	128 (49.6)	28 (21-75)	0.93
No	130 (50.4)	28 (19-59)	
Marital status			
Married	180 (69.2)	28.5 (19-75)	0.15
Not married	80 (30.8)	27 (21-52)	
Chronic disease			
Yes	38 (14.6)	33 (22-75)	0.01
No	222 (85.4)	28 (19-59)	
Number of children			
0	114 (43.8)	28 (21-75)	
1	79 (30.4)	31 (21-53)	0.07
2	57 (21.9)	27 (19-52)	
>2	10 (3.8)	30.5 (24-59)	
Smoking status			
Before pandemic, No; Now, No	177 (68.3)	28 (19-75)	
Before pandemic, No; Now, Yes	7 (2.7)	29 (24-36)	0.94
Before pandemic, Yes; Now, Yes	62 (23.9)	27.5 (21-59)	
Before pandemic, Yes; Now, No	13 (5.0)	30 (22-41)	
Income level			
Decreased	168 (64.6)	28 (19-75)	
Not changed	88 (33.8)	28 (21-57)	0.79
Increased	4 (1.5)	36 (23-49)	
Work security			
Can lose	30 (11.6)	34 (23-53)	0.007
Not changed	228 (88)	28 (19-59)	
Lost*	1 (0.4)		
Work position			
Cannot work on my position[a]	66 (25.4)	28 (21-75)	
Working on my position[a]	170 (65.4)	28 (19-59)	0.008
Working out of my position[b]	24 (9.2)	34.5 (21-57)	
Safe working conditions			
Decreased[a]	180 (69.2)	30 (19-75)	
Not changed[b]	35 (13.5)	25 (21-41)	0.001
Increased[b]	45 (17.3)	27 (21-45)	

[a,b]Significant differences are represented by different superscript letters, *not analyzed, min-max: Minimum-maximum

Various findings were reported as potential factors associated with the anxiety levels of dentists. In this study, it was determined that age, specialty, income level, and smoking status did not affect anxiety level, but gender, work position, work security, safe working conditions and presence of chronic disease negatively affected. In contrast, Zhao et al. (19) stated that while age and protective measures were inversely related to anxiety level, workload, potential infectious substance exposure and aerosol formation were not associated

with anxiety. Age, gender, qualification, type of practice, and years in practice were not effective on the high levels of fear and anxiety according to findings of Suryakumari et al. (18). No significant relationship was reported by Ahmed et al. (20) between the anxiety levels of dentists and their gender and education levels. Age and gender affected the anxiety levels of dentists according to study of Mahdee et al. (21). As mentioned, various anxiety levels and related factors were reported for dentists in the literature. These differences may arise from study population, sample size, country conditions, economical status, and the questionnaires (self-structured or validated) used in these studies.

If the necessary protective measurements are not undertaken, dental staff can be exposed to infection because of the nature of dental settings (15). In fact, dental staff is very familiar with personal protection and infection control measures and risk assessment. However, further protective measures should be required to protect against this pandemic. N95 or FFP2/FFP3 masks were uncommon for infection control in dentistry until COVID-19 pandemic (23). Medical uniform, disposable protective clothing and cap, N95 or FFP2/FFP3 masks, protective goggles and face shield, and gloves are the most commonly used personal protective equipment to protect against COVID-19 infection (3,11,12,22). Surface disinfection, ventilation of the operating room, and washing hands frequently are also recommended precautions. Before dental procedures, mouth washing with chlorhexidine or hydrogen peroxide and rubber dam usingare necessary for aerosol-producing procedures (8,10,15). Despite taking personal protective measures, dentists may be concerned about the risk of infection. Taking necessary preventive measures and having sufficient knowledge on this subject will reduce the anxiety level of dentists. By controlling infection, dentists can play an important role in preventing the transmission of COVID-19. Additionally, thanks to the application of COVID-19 vaccine to healthcare professionals, the anxiety levels of physicians may also decrease (3,5). There are some limitations of the study. The effect of the vaccine on the anxiety level of dentists was not evaluated. There was no study in the literature that measured the anxiety levels of dentists after vaccination against COVID-19 infection. In addition, anxiety levels of dentists were not measured before the pandemic.

Conclusion

In the present study, gender and the presence of chronic disease adversely affected the anxiety level of dentists. The changes in work positions and the decrease in safe working conditions of dentists during the pandemic have also negatively affected the level of anxiety of this group. The experiences obtained during the unprepared COVID-19 pandemic clearly revealed the necessity of preparing appropriate protocols for each healthcare institution to set an example for similar global pandemics that may occur in the future. Being prepared for such extraordinary conditions will help reduce the anxiety levels of dentists.

Ethics

Ethics Committee Approval: Ethical approval of the study was obtained from, Ethics Committee of the Faculty of Medicine of Aydın Adnan Menderes University (protocol number: 2020/88, date: 07.05.2020).

Informed Consent: Informed consent was not applicable for this type of study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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Original Article / Özgün Araştırma

Retrospective Evaluation of the Effect of Tenoxicam and Paracetamol Applied as Intraoperative Analgesics During Orthognathic Surgery on Postoperative Pain

Ortognatik Cerrahi Sırasında İntraoperatif Analjezik Olarak Uygulanan Tenoksikam ve Parasetamolün Postoperatif Ağrı Üzerine Etkisinin Retrospektif Değerlendirilmesi

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Abstract

Objective: Postoperative pain caused by orthognathic surgery significantly affects patient comfort. Non-steroidal anti-inflammatory drugs, corticosteroids and opioid analgesics can be used to relieve postoperative pain. The aim of this study was to retrospectively evaluate the effect of tenoxicam and paracetamol administered intraoperatively during orthognathic surgery on postoperative pain.

Materials and Methods: The study was conducted with 63 files in accordance with the determined criteria. For postoperative pain control, patients were divided into two groups according to the types of analgesics administered. Patients who were administered tenoxicam 20 mg (35 patients) or paracetamol 10 mg/kg (28 patients) IV without extubating were included in the study. In the follow-ups in the postoperative period, the values at 0, 1, 3, 6, 12, 24, 36, and 48 h from the visual analogue scale (VAS) data, the presence of nausea and vomiting and the need for opioid analgesics were evaluated in the files recorded.

Results: There was no statistically significant difference in VAS values between the tenoxicam group and the paracetamol group in the postoperative follow-up. Nausea and vomiting were higher in the tenoxicam group than in the paracetamol group, but no statistically significant difference was observed. Also found no significant difference in rescue opioid requirements.

Conclusion: When intraoperative tenoxicam administration and paracetamol administration were compared in patients undergoing orthognathic surgery, lower VAS scores were observed and analgesic efficacy was found to be sufficient in patients in the tenoxicam group, but no statistically significant difference was found. Similarly, statistically close results were found between the presence of nausea and vomiting and the need for rescue opioids.

Keywords
Pain, orthognathic surgery, analgesia

Anahtar Kelimeler
Ağrı, ortognatik cerrahi, analjezi

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Öz

Amaç: Ortognatik cerrahi ile oluşan postoperatif ağrı hasta konforunu önemli ölçüde etkilemektedir. Postoperatif ağrının giderilmesi amacıyla non-steroid antienflamatuvar ilaçlar, kortikosteroidler ve opioid analjezikler kullanılabilmektedir. Bu çalışmanın amacı, ortognatik cerrahi sırasında intraoperatif olarak uygulanan tenoksikam ve parasetamolün ağrı üzerine etkisini retrospektif olarak değerlendirmektir.

Gereç ve Yöntemler: Belirlenen kriterlere uygun 63 dosya verisi ile çalışma gerçekleştirildi. Postoperatif ağrı kontrolü için, uygulanan analjezik türlerine göre hastalar iki gruba ayrıldı. Ekstübe edilmeden IV olarak tenoksikam 20 mg (35 hasta) veya parasetamol 10 mg/kg (28 hasta) uygulanan hastalar çalışmaya dahil edildi. Postoperatif dönemdeki saatlik takiplerde vizüel analog skalası (VAS) verilerinden 0, 1, 3, 6, 12, 24, 36 ve 48. saatteki değerler ile bulantı kusma varlığı ve opioid analjezik ihtiyacı kaydedilen dosyalardaki veriler değerlendirildi.

Bulgular: Tenoksikam grubu ve parasetamol grubu arasında postoperatif saatlik takiplerde VAS değerleri arasında istatistiksel olarak anlamlı fark bulunmadı. Tenoksikam grubunda bulantı ve kusma parasetamol grubundan daha yüksek görülürken istatistiksel olarak anlamlı bir fark gözlenmedi. Ek opioid gereksinimlerinde de tenoksikam grubu ile parasetamol grubu arasında anlamlı bir fark bulunamadı.

Sonuç: Ortognatik cerrahi geçiren hastalarda intraoperatif tenoksikam uygulaması ile parasetamol uygulaması karşılaştırıldığında, tenoksikam grubu hastalarında daha düşük VAS skorlarının gözlendiği ve analjezik etkinliğin yeterli olduğu tespit edilmiş, ancak istatiksel olarak anlamlı bir fark bulunamamıştır. Benzer şekilde bulantı kusma varlığı ile ek opioid gereksinimi arasında da istatiksel olarak yakın sonuçlar edildi.

Introduction

Orthognathic surgery is a treatment method that includes invasive, major operations that are frequently applied to repair maxillofacial deformities. Pain, edema, and loss of function, which are symptoms of widespread inflammation, may occur in the postoperative period as a result of tissue damage in orthognathic surgery (1). Postoperative pain after orthognathic surgery significantly affects patient comfort. Therefore, good management of the inflammatory process is very important.

Intraoperative analgesia, one of the preventive analgesia methods, is a comprehensive analgesia method aiming to control the development of central sensitization in the postoperative period. It is a practice that adopts the conventional perioperative strategy for anticipated physiological pain.

Tenoxicam, an oxicam derivative among non-steroidal anti-inflammatory drugs (NSAIDs), can be used once a day with its 100% bioavailability, approximately 99% blood protein binding, adequate penetration into synovial fluid, low systemic clearance, and long elimination half-life. Therefore, it can be used as an intraoperative analgesic and as the preferred analgesic in the treatment of postoperative pain (2).

Paracetamol (acetaminophen) is a para-aminophenol derivative drug with antipyretic analgesic properties. Unlike tenoxicam, it has no antithrombotic activity and its anti-inflammatory activity is negligible. The therapeutic effect of acetaminophen begins quickly, is short-lived, and does not prolong bleeding time, as it does not affect platelet function. It has no potential toxic effects on the cardiovascular, respiratory, and gastrointestinal systems (3).

The main purpose of this study is to evaluate the efficacy of tenoxicam and paracetamol intraoperatively in orthognathic surgery for postoperative pain control. Also, secondarily, it is aimed to evaluate the effect of these drugs on nausea and vomiting, as well as the need for additional analgesia during the postoperative period.

Materials and Methods

In this study; The aim was to retrospectively evaluate the patients who underwent orthognathic surgery. Our study was analysed by the Aydın Adnan Menderes University Faculty of Dentistry Clinical Research Ethics Committee with protocol number 2020/11 (date: 13.01.2021).

Inclusion criteria for the study:

- Le Fort 1, patients undergoing bilateral sagittal split osteotomy (BSSO) and bimaxillary surgery,
- Patients undergoing routine anesthesia protocol,
- Patients with The American Society of Anesthesiology (ASA) I-II group were included in the study.

The exclusion criteria for the study were as follows:

- Patients with ASA III and above, hepatic or renal dysfunction
- Patients who use long-term NSAIDs or opioid-derived drugs and have a history of allergic reactions to drugs,
- Patients with symptoms of pain, swelling, inflammation in the head and neck region before the operation,
- Pregnant and lactating patients,
- Patients with missing file data were not included in the study.

Data Recording

Demographic Data and Surgical Characteristics

Age, gender, weight, and ASA data of the patients were noted. Surgery time, type of surgery (single or double chin surgery), and demographic data were recorded as surgical characteristics.

Type of Analgesic Used

Patients using tenoxicam in the intraoperative period were enrolled in the tenoxicam group, and patients using paracetamol were enrolled in the paracetamol group.

Evaluation of Opioid Requirement and Presence of Nausea and Vomiting

The presence or absence of need for opioid analgesia in the postoperative period and the presence or absence of nausea and vomiting in addition to the amount of opioids used were recorded.

Measuring the Level of Pain

Visual analogue scale (VAS), a visual technique, was used for pain level measurement. The VAS score was accepted as 0 in the preoperative period. All individuals whose post-surgical pain levels were included in the study were told that on the pain scale arranged in the form of a 10 cm horizontal marker on the prepared forms, they stated that there was no pain at the "zero" level and the most severe pain at the "10" level. The values recorded at the 0^{th}, 1^{st}, 3^{rd}, 6^{th}, 12^{th}, 24^{th}, 36^{th}, and 48^{th} hours of the postoperative routine measurements for each individual were used in our study.

Anesthesia Protocol and Surgical Method

Our routine general anesthesia protocol was applied to all included patients. After the patients were taken to the operating room, an vascular access was opened and anesthesia induction was performed with 1 µg/kg fentanyl, 2 mg/kg propofol, and 0.8 mg/kg-1 rocuronium. During the maintenance of anesthesia, all patients were administered sevoflurane in a volume of 1-2% in 50% O_2 and 50% N_2O. Electrocardiography, non-invasive blood pressure, oxygen saturation, and end-tidal carbon dioxide monitoring were performed for each patient. Nasal intubation was performed with an endotracheal tube in all patients. The patients were taken to the recovery room after extubation following the end of the surgery. All hemodynamic parameters, vital signs, and early postoperative complications of each patient were recorded.

Bilateral buccal, inferior alveolar, and lingual nerve block anesthesia in the mandible, bilateral buccal local infiltrative and posterior superior alveolar block anesthesia in the maxilla were performed in all patients with 2% articaine 80 mg+1/200,000 epinephrine.

Le fort 1 osteotomy was performed with the Bell (4) method. BSSO was performed according to the Hunsuck (5) modification. For postoperative pain control, patients were administered intraoperative tenoxicam and paracetamol.

In the hourly follow-ups in the postoperative period, the values at 0^{th}, 1^{st}, 3^{rd}, 6^{th}, 12^{th}, 24^{th}, 36^{th}, and 48^{th} hours from the VAS data in the patient file, the presence of nausea and vomiting and the need for opioid analgesics were recorded.

Statistical Analysis

Data analysis was performed using SPSS (version 18.0, SPSS Inc., Chicago, Illinois, USA). The assumption of normal distribution for quantitative variables was tested with the Kolmogorov-Smirnov test. Correlations between categorical variables were analysed by the chi-square test. Comparisons between groups were analysed by independent sample t-test or Mann-Whitney U test. Descriptive statistics were presented as mean with standard deviation, number and percentage, quantitative and categorical variables, respectively. A p-value below 0.05 was considered significant for all comparisons.

Results

A total of 63 patients, 36 female, and 27 male, were included in the study. The patients were divided into two main groups according to the analgesia used in the file data. The group in which tenoxicam was used as intraoperative analgesia in the orthognathic surgery was called the "tenoxicam group" and the group in which paracetamol was used was called the "paracetamol group".

In Table 1, the demographic data and the mean and p-values of the surgical characteristics in our study are summarized. There was no statistically significant difference between the tenoxicam group and the paracetamol group in terms of age, gender, body weight, and ASA classification ($p>0.05$).

The VAS values of the tenoxicam and paracetamol groups in the hourly follow-up postoperatively are shown in Figure 1. Very close values were recorded in

Table 1. Demographic data and clinical characteristics of the tenoxicam group and paracetamol group

	Tenoxicam group n=35	Paracetamol group n=28	p-value
Age (years)	23.60±9.72	21.53±2.88	0.552
Body weight (kg)	68.97±17.93	64.67±11.15	0.663
Gender Female Male	 16 (45.7%) 19 (54.3%)	 20 (71.4%) 8 (28.6%)	0.073
Duration of anesthesia (min.)	316.42±96.01	325.35±82.61	0.819
Type of surgery			0.112
Single chin	11 (85.7%)	4 (14.29%)	
Double chin	24 (14.3%)	24 (85.71%)	
ASA			0.819
I	30	24	
II	5	4	
Total	35	28	

P<0.05 value was considered statistically significant. ASA: The American Society of Anesthesiology, Min: Minute

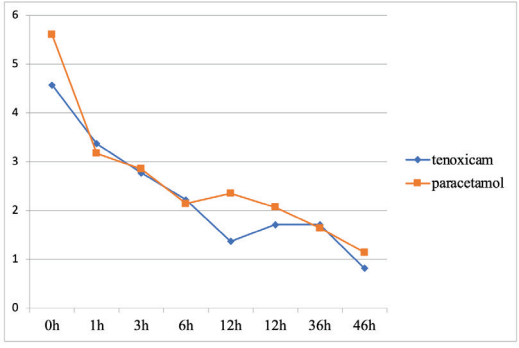

Figure 1. Mean VAS values of the groups in the postoperative period
VAS: Visual analog scale

the tenoxicam group and paracetamol group on average at the 6th hour postoperatively. However, the VAS value at the 12th hour followed in the paracetamol group was higher than in the tenoxicam group. The lower VAS score in the tenoxicam group at the postoperative 12th hour made us think that tenoxicam may be a longer-acting analgesic than paracetamol.

The variation of VAS values according to gender is summarized in Table 2. Except for the 3rd hour, mean VAS values were higher in females at every hour. In the 48 hours follow-up, the female gender gave statistically significant higher scores than the male gender (p<0.05).

According to the intraoperative analgesia preference, the VAS pain values recorded at the 0th hour did not show a statistically significant difference between the tenoxicam and paracetamol groups (p>0.05) (Table 3). However, statistical findings suggest that the results may be more significant in favor of tenoxicam when the sample size is larger. There was no statistically significant difference in postoperative VAS values between the tenoxicam group and the paracetamol group in the other hours followed.

The statistical data of nausea and vomiting and additional opioid requirements of the tenoxicam group and paracetamol group are shown in Table 4. Nausea and vomiting were more common in the

Table 2. Postoperative VAS values by gender

	Female	Male	p-value
VAS0	5.16±2.24	4.85±2.19	0.774
VAS1	3.38±1.94	3.14±1.61	0.927
VAS3	2.74±1.97	2.88±1.73	0.649
VAS6	2.27±1.70	2.07±1.49	0.690
VAS12	2.20±2.24	1.29±1.85	0.082
VAS24	2.23±1.81	1.40±1.75	0.093
VAS36	2.00±1.96	1.46±1.72	0.281
VAS48	1.31±1.22	0.53±0.94	0.013

P<0.05 value was considered statistically significant. VAS: Visual analogue scale

Table 3. Comparison of VAS means over time and between groups

	Tenoxicam group	Paracetamol group	p-value
VAS0	4.57±2.40	5.60±1.83	0.069
VAS1	3.37±1.97	3.17±1.58	0.955
VAS3	2.77±1.92	2.85±1.77	0.800
VAS6	2.22±1.62	2.14±1.60	0.843
VAS12	1.37±1.62	2.35±2.49	0.119
VAS24	1.71±1.74	2.07±1.86	0.422
VAS36	1.71±1.84	1.64±1.74	0.891
VAS48	0.82±0.98	1.14±1.26	0.388
P	0.023	0.001	

P-value <0.05 was considered statistically significant. VAS: Visual analog scale

Table 4. Nausea, vomiting, and need for additional opioids in the tenoxicam group and paracetamol group

	Tenoxicam group	Paracetamol group	p-value
PONV (n, %) Existent Non-existent	 23 (65.7%) 12 (34.3)	 14 (50%) 14(50%)	0.303
Additional opioid needs (n, %) Existent Non-existent	30 (85.7%) 5 (14.3%)	19 (64.3%) 9 (35.7%)	0.129
PONV: Postoperative nausea and vomiting			

tenoxicam group than in the paracetamol group, but no statistically significant difference was observed. No statistically significant difference was found in additional opioid requirements (p>0.05).

Discussion

Bimaxillary surgery is a well-executed orthognathic surgical procedure that includes BSSO and Le Fort I osteotomy. It has been reported in the literature that young adult women undergoing double chin surgery have higher pain scores following surgery. Mobini et al. (6) reported in their study conducted in 2018 that women felt more pain after bimaxillary orthognathic surgery compared to men. In our study, similar to these literatures, regardless of the groups, it was found that women felt more pain than men.

Although various accepted or approved analgesia protocols are used to keep pain under control after orthognathic surgery; Researchers could not reach a consensus regarding the ideal method of administering analgesia. Many drugs and methods such as steroids, patient-controlled analgesia method, opioids, NSAIDs, combination of NSAIDs and local anesthetics, antiemetics, antiepileptics, nerve blocks are used in order to provide postoperative analgesia in double maxillofacial surgery (7-10).

Tenoxicam is an effective agent that has an intravenous (IV) form of NSAIDs and has a longer duration of action than lornoxicam, and is frequently used in mild to moderate pain. The recommended daily dosing regimen for tenoxicam is 20-40 mg. Akca et al. (11) reported in their study that administration of IV 20 mg tenoxicam in 80 patients undergoing inguinal hernia repair and laparoscopic cholecystectomy is a simple, safe, and highly effective method for postoperative pain control. Vandermeulen et al., (12) in a multicenter, placebo-controlled study on 258 patients undergoing abdominal or orthopedic surgery, showed that IV administration of 40 mg tenoxicam was effective in postoperative pain control and reduced the use of postoperative morphine. In the light of similar studies, IV 20 mg tenoxicam was administered as a single dose in our study, and this application was found to be sufficient to provide effective analgesia in the early postoperative period.

Acetaminophen (para-aminophenol derivatives) is evaluated outside of NSAIDs because it does not have anti-inflammatory and antiplatelet activities. Although other central mechanisms of action have a role, it shows analgesic and antipyretic activity by inhibiting prostaglandin synthesis in the central nervous system. However, in peripheral tissues, acetaminophen is a weak inhibitor of cyclooxygenase (10 times less potency than acetylsalicylic acid) and therefore does not significantly affect prostaglandin synthesis, which plays a role in the development of inflammation. The weaker anti-inflammatory activity on peripheral inflammation compared to NSAIDs can be explained in this way (13). In a study by Wininger et al. (14) on 224 patients undergoing abdominal laparoscopic surgery, repeated administration of 2 separate dose regimens of IV paracetamol (1000 mg and 650 mg) was associated with statistically significant analgesic efficacy compared to placebo and after abdominal laparoscopic surgery, it was concluded that this drug is also effective in adults. Tuzuner Oncul et al. (15) in a study on buried third molars; showed that paracetamol provides postoperative analgesic efficacy similar to NSAIDs and is an appropriate analgesic for patient satisfaction. In our study, 10 mg/mL paracetamol was used intraoperatively; It was found to be effective as an analgesic in hourly VAS measurements and additional opioid requirement, consistent with other studies.

No study was found comparing tenoxicam and paracetamol for the control of postoperative pain in orthognathic surgery cases in the database searches. However, there are studies comparing the efficacy of tenoxicam and paracetamol in different applications. In a study conducted by Gunusen et al. (16) in patients with postoperative pain after elective abdominal hysterectomy, 120 patients who received 1 g paracetamol, 20 mg tenoxicam or placebo at the

end of the operation were randomly divided into three groups and all patients were administered postoperative principal component analysis with morphine. As a result of the study, it was found that a single dose of 20 mg tenoxicam after abdominal hysterectomy provided more effective analgesia and reduced total morphine consumption compared to paracetamol and placebo. In addition; side effects except nausea were found to be similar (16). In the study of Cheung and Rodrigo (17) in which they compared the efficacy of tenoxicam for pain relief with paracetamol after third molar surgery, it was observed that both drugs were effective as analgesics after third molar surgery and tenoxicam had comparable efficacy to paracetamol. In our study, the VAS data of the patients at 0^{th}, 1^{st}, 3^{rd}, 6^{th}, 12^{th}, 24^{th}, 36^{th}, and 48^{th} hours postoperatively were compared. It was determined that the VAS measurements of the tenoxicam group at the postoperative 0^{th} hour were less than the VAS measurements of the paracetamol group in the same periods. However, due to the small sample size, no statistically significant difference was found. In other time periods, VAS values were found to be similar in the two groups.

The most important factors affecting patient satisfaction in the postoperative period are nausea, vomiting, and inappropriate postoperative pain management. One of the common causes of high rates of nausea and vomiting is the use of opioid derivatives and opioid-like drugs such as tramadol (18,19). Huang et al. (20) compared intraoperative tenoxicam with placebo for the relief of postoperative pain after cesarean section. In this study, nausea and vomiting of patients were also evaluated compared to the placebo group, and they found that there was no difference. In our study, there was no statistically significant difference between the paracetamol group and the tenoxicam group in terms of postoperative nausea and vomiting, which was consistent with these studies.

One of the most important factors in avoiding the use of NSAIDs for postoperative analgesia is the concern that it will cause bleeding. During our study, we did not detect any side and toxic effects, hypotension, arrhythmia, cyanosis due to the drugs used in the intraoperative and postoperative period.

Conclusion

As a result, in patients undergoing orthognathic surgery; when intraoperative tenoxicam administration and paracetamol administration were compared, lower VAS scores were observed in tenoxicam group patients and analgesic efficacy was found to be sufficient. A single dose of intraoperative tenoxicam and paracetamol administration is an effective and safe method in the treatment of postoperative pain in patients undergoing orthognathic surgery. Various methods have been tried to reduce postoperative pain in these patients, and there is still no agreed-upon analgesia protocol. Future controlled prospective studies are needed to better evaluate the types, doses, and complications of analgesic agents, and to provide a better understanding of the results.

Ethics

Ethics Committee Approval: Our study was analysed by the Aydın Adnan Menderes University Faculty of Dentistry Clinical Research Ethics Committee with protocol number 2020/11 (date: 13.01.2021).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Z.B.D., B.G., Ö.K., Consept: B.G., Design: B.G., Data Collection or Processing: Z.B.D., Analysis or Interpretation: Ö.K., Literature Search: Z.B.D., B.G., Ö.K., Writing: Z.B.D., B.G.

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

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Original Article / Özgün Araştırma

Evaluation of Oral Administration of Pirfenidone in Preventing Epidural Fibrosis Formation in Rats

Sıçanlarda Epidural Fibroz Oluşumunu Önlemede Oral Pirfenidon Uygulamasının Değerlendirilmesi

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Keywords
Laminectomy, fibroblast growth factor, transforming growth factor, pirfenidone, epidural fibrosis

Anahtar Kelimeler
Laminektomi, fibroblast büyüme faktörü, dönüştürücü büyüme faktörü, pirfenidone, epidural fibrozis

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Abstract

Objective: To investigate the oral administration of pirfenidone on eperidural fibrosis formation (EF).

Materials and Methods: Twenty four rats were divided into three equal groups. Group 1 (control), group 2 (25 mg/kg pirfenidone), and group 3 (50 mg/kg). Thoracic laminectomies were performed in all rats. Pirfenidone was given orally once a day for 30 days. Vertebral column samples were removed surgically at the end of the procedure. EF grading, number of fibroblasts and arachnoidal involvoment were evaluated. TGF-β1 and basic fibroblast growth factor (bFGF) were analyzed using quantitative real-time polymerase chain reaction, and synthesized proteins were examined.

Results: In group 3, EF formation was less than the other groups. The factors in the emergence of antifibrotic activity at 50 mg/kg doses were investigated, and it was revealed that pirfenidone reduced fibroblast proliferation, and showed this effect by reducing TGF-β1 and bFGF expression in fibrotic tissue.

Conclusions: Pirfenidone can be a potential agent for EF treatment.

Öz

Amaç: Bu çalışmanın amacı pirfenidonun oral yoldan uygulanmasının eperidural fibroz (EF) oluşumu üzerindeki etkisini araştırmaktır.

Gereç ve Yöntemler: Yirmi dört sıçan üç eşit gruba ayrıldı: grup 1 (kontrol), grup 2 (25 mg/kg oral pirfenidon) ve grup 3 (50 mg/kg oral pirfenidon). Tüm sıçanlara torakal laminektomi yapıldı. Pirfenidon 30 gün boyunca günde bir kez ağızdan verildi. İşlemin sonunda vertebral kolon örnekleri cerrahi olarak alındı. EF derecesi, fibroblast sayısı ve araknoidal tutulum histopatolojik olarak değerlendirildi. Transforming büyüme faktörü-β1 (TGF-β1) ve bazik fibroblast büyüme faktörünün (bFGF) gen ekspresyonu, kontitatif gerçek zamanlı polimeraz zincir reaksiyonu kullanılarak analiz edildi ve sentezlenen proteinler incelendi.

Bulgular: Grup 3'te EF oluşumu diğer gruplardan azdı. Elli mg/kg dozlarında antifibrotik aktivitenin ortaya çıkmasında rol oynayan faktörler araştırıldığında pirfenidonun fibroblast proliferasyonunu azalttığı ve bu etkiyi TGF-β1 ve bFGF ekspresyonunu azaltarak gösterdiği ortaya konmuştur.

Sonuç: Pirfenidon, EF tedavisinde potansiyel bir ajan olabilir.

Introduction

Laminectomy is commonly performed to treat the spine pathologies (1). Depending on the dimensions of a laminectomy, removal of the soft tissues from the epidural space can cause many complications. Epidural fibrosis (EF) is one such common complication (2). This EF tissue can also be described as a healing tissue; however, this healing tissue increases in the epidural space after laminectomy, and may cause adhesion between tissues, and compression around neural tissue (3,4). These excessive healing tissue can also lead to the reappearance of the patient's existing symptoms before surgery (5,6). Therefore, reducing adhesion via EF is critical for reducing the success rate of surgery.

In the literature, many agents have been used (1-8). However, no effective treatment has been reported (2). Additional surgical interventions for the treatment of EF may also lead to many complications (5,7-9). Additionally, many surgical interventions after the first surgery can also cause more fibrotic tissue than was formed in the first operation (8). Therefore, the preventing the formation of EF is the best approach (9).

EF formation is occurs as a result of an inflammatory process (10). This complex inflammation process ends with a process in which cellularity decreases but the extracellular matrix component accumulates excessively (7,10). Basic fibroblast growth factor (bFGF) and transforming growth factor beta (TGF-β) have been showed to have a critical role in the formation of fibrosis (7,10,11). Therefore, researchers have focused on altering fibroblast proliferation and trans differentiation.

Pirfenidone, which is known as 5-methyl-N-phenyl-2-(1H)-pyridone, is an antifibrotic medicine (11-13). It has been approved for the treatment of idiopathic pulmonary fibrosis (11-14). According to previous studies, pirfenidone reduced the deposition of collagen and inflammatory cells, and inhibited the proliferation and differentiation fibroblasts by TGF-β1 (14). Therefore, pirfenidone, which has antifibrotic properties, can also be a potential agent for reducing EF formation.

Here we performed an experimental laminectomy in rats to show the effect of the pirfenidone on EF formation.

Materials and Methods

Animals

The experimental procedure was reviewed and approved by the animal Ethics Committee of Aydın Adnan Menderes University Animal Experiments Local Ethics Committee (approval no: 64583101/2019/017, date: 26.03.2019).). Twenty-four female Wistar albino rats, the average weight of which was 350±10 mg, were used in this study. Environmental conditions of the experimental study were 20-24 °C, 50±10% humidity and a 12-hour light/dark cycle. The subjects were ensured easy access to food and water.

Experimental groups

Group 1: Laminectomy; only T11 and T12 laminectomies were performed (n=8).

Group 2: Twenty-five mg/kg pirfenidone; T11 and T12 laminectomies and after hemostasis, surgical layers were closed in accordance with the normal anatomic layer. Then 25 mg/kg pirfenidone was given orally once a day for 30 days (n=8).

Group 3: Fifty mg/kg pirfenidone; the same procedure as group 2 was performed, and then 50 mg/kg pirfenidone was given orally once a day for 30 days (n=8).

Anesthesia and Surgical Procedure

A mixture of ketamine (50 mg/kg, Ketasol, Richter Pharma, Austria) and xylazine (10 mg/kg, Bioveta Plc, Czech Republic) was given intraperitoneally to the subjects in the groups for anesthesia. They were set up in the prone position. The laminectomy areas of the rat were wetted for shaving and then povidone-iodine was applied. After a longitudinal midline incision, the T11-T12 laminae were exposed and laminectomies were performed at the same levels. After hemostasis, the surgical wound was closed in accordance with anatomic layers. Cervical dislocation was performed under deep anesthesia for sacrifice at the end of the postoperative 30th day. Then, the vertebral column was removed en bloc.

Histopathologic Evaluation

After appropriate samples were taken from the materials, they were placed in cassettes, and routine tissue follow-up was performed in an automatic tissue tracking device within 14-16 hours. Serial sections, 3-4 µm in size were taken from the samples embedded in paraffin blocks. The slides were stained with Masson's

Trichrome Hematoxylin-Eosin (H&E), and then they were analyzed with a microscope.

The extent of fibrous tissue and its relationship with dura mater were scored according to He schame (15). The arachnoid involvement was also noted.

The cell density of scar tissue observed in the EF area was evaluated according to the Hinton method (16). The density of Vimentin were evaluated during the immunohistochemical examination.

Immunohistochemistry of Vimentin

Immunohistochemical staining was performed with a DAKO brand automatic staining device using an Avidin-Biotin complex system. Evaluations for vimentin immunohistochemical staining were performed under the light microscopy at x40, x100, x200, and x400 magnifications. If the staining rate was 10% or more, the staining was accepted as positive. The degree of staining was evaluated according to the staining rates that can be observed at different magnifications. The staining visible at x200 magnification was considered light, the stainingvisible at x100 magnification was considered medium, and the staining visible at x40 magnification was considered strong staining.

Western Blot Analysis for Proteins

Protein analysis was performed using the western blot analysis method previously defined by Arabacı Tamer et al. (17). The blotted membrane was blocked with 3% Bovine serum albumin for two hours at room temperature. The membrane was incubated with primary antibody (1:500 dilution monoclonal antibody anti-TGF-β, anti-FGF, anti-Bactin, Santa Cruz Biotechnology, Heidelberg, Germany) for 24 hours at a temparture of 4 °C temperature. Afterwards, the membrane was washed twice in TBST (Tris-buffered saline containing 0.1% Tween-20) and membrane was incubated with secondary antibody (1:2000 anti-mouse IgG1-HRP and anti-rabbit IgG-HRP Santa Cruz Biotechnology) for two hours at room temperature. Band analysis was performed using NIH-ImageJ software. For normalization, β-actin was used with respect to each band.

Quantitative Real-time Polymerase Chain Reaction (qRT-PCR) Analysis

The quantitative real-time PCR (qPCR) analysis method was used for gene analysis (18). The primers used were as follows: GAPDH Forward 5'-ATGACTCTACCCACGGCAAG-3', GAPDH Reverse 5'-CTGGAAGATGGTGATGGGTT-3': TGF-β Forward 5'-ACCTGCAAGACCATCGACATG-3', TGF-β-Reverse 5'-CGAGCCTTAGTTTGGACAGGAT-3', FGF Forward 5'-AAGCCCGTCGGTGTCCATGG-3', FGF Reverse 5'-GATGGCACAGTGGATGGGAC-3'. It was made using Applied Biosystem Software for the analysis. Relative quantitative data were calculated by the $2^{-\Delta\Delta}$Ct method. The calculation of gene expression ratios was expressed with GAPDH as a reference gene.

Statistical Analysis

Whether the quantitative variables were normally distributed among the groups was checked using the Kolmogorov-Smirnov test. In group comparisons, one-way analysis of variance (ANOVA) and Kruskal-Wallis H test was used. Pairwise comparisons of groups were made using the Tukey HSD and Bonferroni corrected multiple comparison test for ANOVA and Kruskal-Wallis H test, respectively. Descriptive statistics were expressed as mean ± standard deviation for variables with normal distribution and median (25^{th}-75^{th} percentile) for variables without normal distribution. $P<0.05$ value was evaluated as statistically significant.

Results

Effect of Pirfenidone on Epidural Fibrosis in Histopathological Analysis

When the groups were evaluated in according to EF, the grade 3 (Figure 1A and 1B) EF were dominant in group 1, grade 2 (Figure 1C and 1D) and grade 3 EF were predominant in group 2, and grade 1 (Figure 1E and 1F) and grade 2 EF were dominant in group 3. According to these results, group 3 showed a statistically significant difference between other groups ($p<0.001$) (Table 1). In addition to this, arachnoid involvement was detected in three rats in group 1 and two rats in group 2, but in group 3, no arachnoid involvement was detected (Table 2).

Effect of Pirfenidone on Average Fibroblast Numbers in Histopathological Analysis

When groups were compared in terms of fibroblast count, the highest number of fibroblasts was in group 1, while the least number of fibroblasts was in group 3. The number of fibrolasts in group 2 was found to be between group 1 and group 3 (Table 1). According to these results, a 50 mg/kg dose of pirfenidone provided a statistically significant in decreasing the fibroblasts number compared to other groups ($p<0.001$).

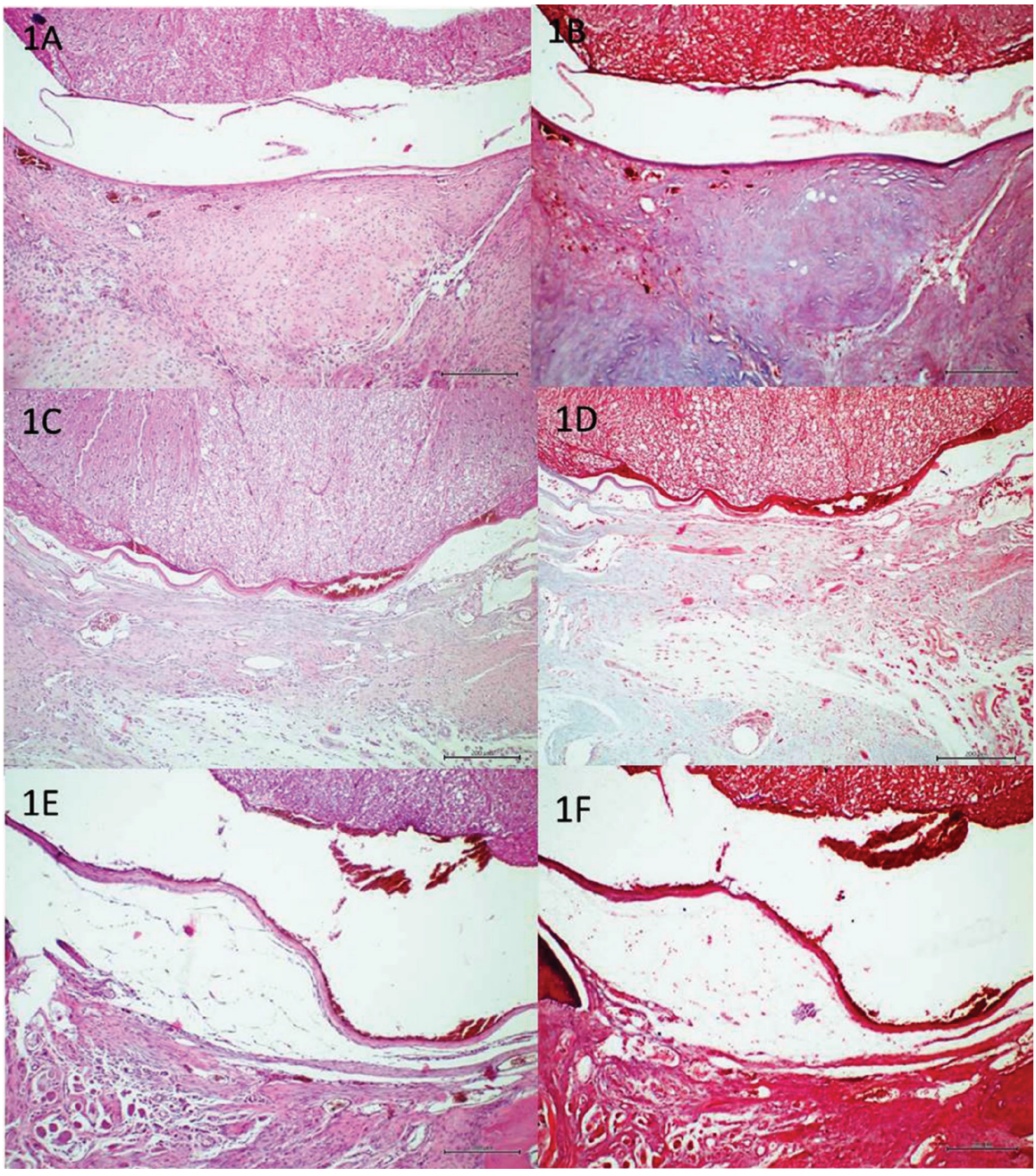


Figure 1. Dense scar tissue adherent to the dura mater was showed in the group 1 [H&E (1A) and Masson's trichrome (1B) staining, scale bar: 200 µm, x100 magnification]. Moderate scar tissue was observed in the group 2 [H&E (1C) and Masson's trichrome (1D) staining, scale bar: 200 µm, x100 magnification]. Loose scar tissue without adherence to the dura mater was found in the group 3 [H&E (1E) and Masson's trichrome (1F) staining, scale bar: 200 µm, x100 magnification]

Effect of Pirfenidone on Immunostaining in Histopathological Analysis

To compare the results of immunostaining, a comparison was made by scoring 3 for severe staining (Figure 2A), 2 for moderate staining (Figure 2B), and 1 for low staining (Figure 2C). When groups were compared in terms of vimentin staining, it was found that severe staining was dominant in group 1, and severe and moderate staining was dominant in group 2, whereas in group 3. According to these

results, there was no statistically significant difference between group 1 and group 2, but there was also a statistically significant difference between group 3 and other groups (p<0.001) (Table 1).

Western Blot Analysis Results

TGF-β1 protein expression levels were decreased with pirfenidone treatment in group 2 and group 3 (p<0.001) compared with group 1. Moreover, bFGF protein expression levels were suppressed in group 2 and group 3 (p<0.001). Both protein expression results showed that the treatment of a 50 mg/kg dose of pirfenidone in laminectomy suppressed bFGF and TGF-β1 protein expression levels more (Figure 2D) (Table 3).

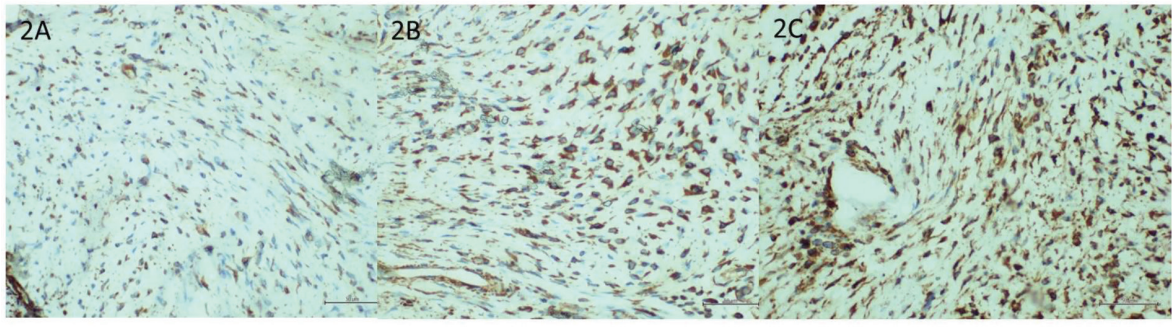
qRT-PCR analysis Results

qRT-PCR results revealed that *TGF-β1* gene expression significantly decreased in the pirfenidone treated groups when compared with group 1

Table 1. Comparison of the groups according to the epidural fibrosis grading, fibroblast counting, vimentin immunostaining, and qRT-PCR analysis results. Similar letters in the same line indicate similarity between groups; different letters represent difference between groups

	Group 1 (n=8)	Group 2 (n=8)	Group 3 (n=8)	p-value
Epidural fibrosis	3.0 (3.0-3.0)[a]	2.0 (2.0-3.0)[a]	1.5 (1.0-2.0)[b]	<0.001
Average fibroblast number	183.5±11.4[a]	153.5±28.9[b]	113.3±18.8[c]	<0.001
Vimentin immunostaining	3.0 (3.0-3.0)[a]	2.5 (2.0-3.0)[a]	1.5 (1.0-2.0)[b]	<0.001
bFGF/GAPDH	1.279±0.419[a]	0.618±0.174[b]	0.071±0.049[c]	<0.001
TGF-β1/GAPDH	1.454±0.402[a]	0.617±0.156[b]	0.111±0.060[c]	<0.001

Similar letters in the same line indicate similarity between groups, different letters represent difference between groups. qRT-PCR: Quantitative real-time polymerase chain reaction, TGF-β: Transforming growth factor beta, bFGF: Basic fibroblast growth factor



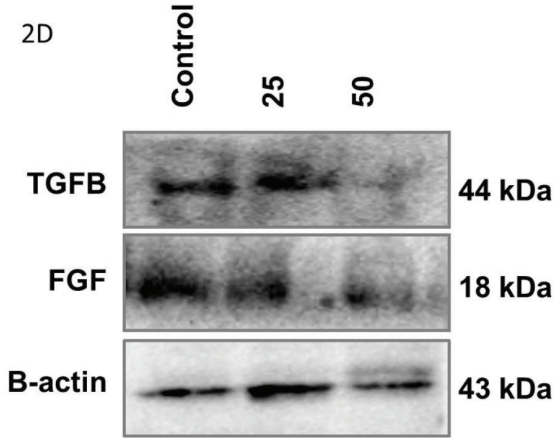


Figure 2. The density of vimentin cells was lowest in the group 3 (2A) and lower in the group 2 (2B) compared with the group 1 (2C). (Scale bar: 50 µm, x400 magnification). Comparison of TGF-β1 and FGF expression of the groups according to the Western blot analysis results (2D)
FGF: Fibroblast growth factor, TGF-β: Transforming growth factor beta

(p<0.001). *bFGF* gene expression levels were also significantly decreased in the pirfenidone-treated groups when compared with the group 1 (p<0.001). *TGF-β1* and *bFGF* gene expression results showed that administration of a 50 mg/kg dose of pirfenidone suppressed increasing gene levels in EF, thus showing a better effect (Table 1).

Discussion

Our study is the first to use pirfenidone at 25 mg/kg and 50 mg/kg doses in a laminectomy model and to examine its effect on the EF formation. According to the results of our study, it was revealed that, when pirfenidone was administered orally at 50 mg/kg doses, it statistically significantly decreased EF formation compared with controls and 25 mg/kg doses of pirfenidone. In addition, this study revealed that pirfenidone reduced fibroblast proliferation and showed this effect by reducing TGF-β1 and FGF expression in fibrotic tissue.

In addition to decreasing TGF-β expression, many factors may be effective in the emergence of this antifibrotic effect of pirfenidone. In previous studies related to EF, it was revealed that reducing the effect of factors such as interleukin (IL)-1, IL-6, tumor necrosis factor (TNF)-α, platelet-derived growth factor, FGF and vascular endothelial growth factor (VEGF) reduced EF formation (8,19-21). When the studies related with pirfenidone were examined, a study conducted by Oku et al. (11) revealed that pirfenidone decreased lung fibrosis by reducing the interferon (IF)-γ, bFGF and TGF-β expression. In another study conducted by Guo et al. (14) in a silica-induced lung fibrosis model, the authors reported that the use of pirfenidone reduced the expression of IL-1β, TNF-α and IL-6 in lung tissue. In addition to these, in one *in vitro* study conducted by Liu et al. (22), the authors reported that pirfenidone showed an antifibrotic effect by reducing angiogenesis by decreasing the expression of VEGF/VEGFR2. This reveals that pirfenidone has an active role in reducing EF formation in the proliferation phase, and may also have an effect as an anti-inflammatory agent.

Another possible factor related with the emergence of antifibrotic activity may be that pirfenidone uses different pathways in different tissues. Analyzing pirfenidone from this point of view, Sun et al. (13) reported that pirfenidone showed antifibrotic properties by using the TGF-β1/Smad/CTGF pathway. Liu et al. (23) suggested that pirfenidone could have an antifibrotic effect by antagonizing the MAPK signaling pathway. In another study, Sun et al. (24) reported that pirfenidone showed antifibrotic activity by antagonizing the Smad and PI3K/AKT pathway. In addition to these studies, in a study conducted by Guo et al. (14), the authors reported that pirfenidone showed antifibrotic activity by antagonizing the TGF-β/smad2/3 pathways. The fact that pirfenidone has an antifibrotic activity over different pathways reveals that pirfenidone has a pleiotropic feature. Therefore, pirfenidone may also be used in different diseases beyond lung fibrosis, such as EF.

Another factor in the emergence of its anti-inflammatory and antifibrotic activity may be due to its dose-related effect. Many studies have revealed the dose-dependent effect of pirfenidone. One of these experimental studies was by Sun et al. (13), who reported that pirfenidone decreased collagen accumulation and fibroblast proliferation both *in vivo* and *in vitro* depending on the dose. In another study was by Liu et al. (23), pirfenidone was shown to reduce TGF-β, type-3 collagen, α-SMA and fibronectin in a dose-dependent manner. In addition, Shi et al.

Table 2. Comparison of the groups according to the arachnoid involvement

		Group 1	Group 2	Group 3
Arachnoid involvement	-	5	6	8
	+	3	2	0

Table 3. Comparison of protein expression of the groups according to the Western blot analysis results. Similar letters in the same line indicate similarity between groups; different letters represent difference between groups

	Group 1	Group 2	Group 3	p-value
bFGF/B-actin	1.112±0.081[a]	0.770±0.076[b]	0.374±0.029[c]	<0.001
TGF-β1/B-actin	1.100±0.063[a]	0.824±0.054[b]	0.220±0.062[c]	<0.001

Similar letters in the same line indicate similarity between groups, different letters represent difference between groups, TGF-β: Transforming growth factor beta, bFGF: Basic fibroblast growth factor

(25) revealed that pirfenidone reduced fibroblast proliferation and collagen accumulation in a dose-dependent manner. In our study, pirfenidone reduced the EF grade, fibroblast proliferation and TGF-β1 and bFGF expression in a dose-dependent manner. The results of our experiment are supported by the literature.

In this study, although we show for the first time that the use of oral pirfenidone has a positive effect on reducing the formation of EF, the study has some limitations. First, only two different doses of pirfenidone were administered. Second, in this initial study, molecular mechanisms were not investigated to reveal the mechanism of action of pirfenidone. Finally, a small sample size was used.

Conclusion

According to the results of our study, it was revealed that the use of oral pirfenidone decreased EF formation. In the emergence of this effect of pirfenidone, it has been demonstrated that at least two growth factors, namely TGF-β1 and bFGF, reduce the level of both gene expression and protein production depending on the dose. Therefore, pirfenidone can be a potential agent for EF.

Ethics

Ethics Committee Approval: The experimental procedure was reviewed and approved by the animal Ethics Committee of Aydın Adnan Menderes University Animal Experiments Local Ethics Committee (approval no: 64583101/2019/017, date: 26.03.2019).

Informed Consent: Informed consent is not required.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Z.K., N.K.Ç., S.A., Concept: Z.K., S.A., Design: Z.K., Data Collection or Processing: Z.K., Ö.Ç., Analysis or Interpretation: N.K.Ç., Ö.Ç., H.Ö., Literature Search: Z.K., N.K.Ç., Ö.Ç., Writing: Z.K.

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

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Original Article / Özgün Araştırma

Assessment of Endodontic Emergency Care in a COVID-19 Pandemic

COVID-19 Pandemisinde Endodontik Acil Bakımın Değerlendirilmesi

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Abstract

Objective: This study compared the aerosol-generating and non-aerosol-generating endodontic emergency procedures' success and assessed the outcome of endodontic treatments initiated before a pandemic but could not be completed in the targeted time.

Materials and Methods: Emergency treatments were performed according to symptoms of teeth. Treatment success or failure was determined according to patients whether not re-referral with untimely pain. Short-term outcome and complications arising from teeth, which endodontic treatments were prolonged were also recorded. A chi-square test was used in the statistical analysis, and $p<0.05$ was considered significant.

Results: The aerosol-generating procedure group's success rate was 86.2%, while it was 70.0% in the non-aerosol generating procedure group ($p=0.050$). The short-term survival rate of teeth was 83.7% in patients whose endodontic treatment had been prolonged.

Conclusion: Considering the pros and cons, each emergency patient should be evaluated case-by-case.

Öz

Amaç: Bu çalışmada, aerosol oluşturan ve aerosol oluşturmayan endodontik acil prosedürlerin başarısı karşılaştırıldı ve pandemi öncesi başlatılan ancak hedeflenen sürede tamamlanamayan endodontik tedavilerin sonuçları değerlendirildi.

Gereç ve Yöntemler: Dişlerin semptomlarına göre acil tedavileri uygulandı. Tedavi başarısı veya başarısızlığı hastaların ağrı ile tekrar başvurup başvurmamasına göre belirlendi. Endodontik tedavileri uzamış dişlerde kısa dönem sonuçları ve komplikasyonlar da kaydedildi. İstatistiksel analizde ki-kare testi kullanıldı ve $p<0,05$ anlamlı kabul edildi.

Bulgular: Aerosol oluşturan prosedürler grubunun başarı oranı %86,2 iken, aerosol oluşturmayan prosedürler grubunda bu oran %70,0 idi ($p=0,050$). Endodontik tedavisinin tamamlanma süreci uzamış hastalarda kısa süreli diş sağkalım oranı %83,7 idi.

Sonuç: Artıları ve eksileri göz önünde bulundurularak her acil hasta, olgu bazında değerlendirilmelidir.

Keywords
Aerosols, SARS-CoV-2, emergencies, endodontics, pulpitis

Anahtar Kelimeler
Aerosoller, SARS-CoV-2, aciller, endodonti, pulpit

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Introduction

The coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has become a worldwide health problem in a short time and restricted

social life (1,2). SARS-CoV-2 enters the body through the respiratory system, and its interpersonal transmission occurs mainly through droplets generated when talking, coughing or sneezing (3,4). Other transmission routes have also been reported, such as aerosol, fecal-oral, and indirect transmission. Aerosols are in a diameter of less than 5 mm, while respiratory droplets are >5-10 mm in diameter (5). "The virus can stay in aerosols for hours and on multiple surfaces for days under laboratory conditions" (6). Most dental procedures pose a high contagion risk for dentists, patients, and auxiliary staff due to aerosol/droplet production and instrument contamination (7). Considering the risk of exposure for different work categories, dentists are at the greatest risk of coronavirus (8). While the first COVID-19 case in Turkey was seen on March 10, 2020, the World Health Organization declared a pandemic outbreak on March 11, 2020 (9). The Turkish Ministry of Health recommended solely performing emergency treatments and postponing non-urgent dental procedures on March 17, 2020 (10). The Turkish Dental Association (TDA) also recommended avoiding and minimizing aerosol-generating dental procedures (11).

On June 1, 2020, the Turkish Ministry of Health published "Guide on Working in Healthcare Institutions During the Normalization Period in COVID-19 Pandemic", and our dental polyclinic has adapted to the normalization process until June 15, 2020 (12).

Between these dates, considering the emergency care definition and recommendations of TDA, only emergency dental care was provided to the patients. Some patients who applied with the need for endodontic treatment received palliative treatment (prescription of drugs or temporary restoration) to avoid the aerosol generation as recommended (11). However, in some cases, it was almost impossible to avoid aerosols in the emergency intervention of severe toothache caused by pulp inflammation or acute apical abscess, which we frequently encounter in the endodontics clinic. We aimed to compare the success of aerosolized and non-aerosolized endodontic emergency procedures and to evaluate the short-term results of delayed endodontic treatments.

Materials and Methods

This study was approved by Medical Ethics Committee of Başkent University (project no: D-KA20/42, date: 22.12.2020) and the Turkish Ministry of Health's approval were taken following the Declaration of Helsinki. All clinical data of the patients who called or visited the dental polyclinic of Başkent University Adana Dr. Turgut Noyan Healthcare Center between March 17 and June 15, 2020, were collected. The patients referred to the endodontics clinic were selected and categorized as follows:

1) Treatment postponement was recommended in cases with at least one asymptomatic tooth needing endodontic treatment (n=149).

2) In cases with at least one tooth with mild symptoms that need endodontic treatment, non-aerosol-generating procedures (n=65) were performed. These procedures involved restoring a cavitated tooth with a sedative temporary filling (zinc oxide-eugenol cement; Cavex Holland BV, Haarlem, Netherlands) and prescribing analgesics and/or antibiotics in appropriate cases (Figure 1). The position statements of the international endodontic societies have been followed in the prescription for specific cases (13-15).

3) In cases with severe and spontaneous pain either with vital pulp, non-vital pulp or previous root canal filling, aerosol-generating procedures (n=65) were performed. In these cases, calcium hydroxide medication and temporary tooth restoration were applied after chemomechanical cleaning of the root canal system under local anaesthesia.

Only patients who had no symptoms related to COVID-19 and revealed no history of international travel or suspicious contact were referred. Before treatment, all patients read and signed the informed consent forms.

Patients' fever was measured prior to treatment, and they rinsed with 0.2% povidone-iodine. Six dentists and their assistants in the endodontics clinic

	Pain Management by Prescription
Vital Cases	Ibuprofen 600mg + Acetaminophen 325- 500mg
Non-vital Cases	• Amoxicillin with clavulanic acid b.i.d. 500-875 mg x 3-7 days/ Clindamycin 300mg q.i.d. x 3-7 days • Ibuprofen 600mg + Acetaminophen 325-500mg

Figure 1. Prescription procedure of analgesics, and/or antibiotics

used personal protection equipment (FPP3 mask, face shield, bonnet, gloves, protective clothing and rubber-dam isolation in aerosol-generating procedures) as recommended. During this period, there was no COVID-19 transmission to dental staff in our clinic, and antibody tests performed in our hospital during July were negative.

"Success" in both emergency procedures was defined as relief of pain and no need for additional intervention until the organized appointment during the normalization process. "Failure" in non-aerosol-generating procedures was defined as the need for an additional aerosol-generating procedure or the tooth having to be extracted due to the treatment process's prolongation.

"Failure" in aerosol-generating procedures was defined as the patient referring again with symptoms such as pain, swelling, or the tooth's extraction due to the prolongation of the treatment process.

"Follow-up" for both emergency procedures was defined as coming to our clinic to complete the relevant tooth's treatment during the normalization period.

The outcome of teeth which endodontic treatment was initiated in our clinic before March 17, 2020; however, it could not be completed due to the restriction of dental procedures only to emergency care was included in the present study. Complications arising from prolonging these patients' treatment process who came to their appointments to complete their treatment during the normalization period were evaluated. These complications were classified as loss of temporary filling, tooth fracture, flare-up and need for emergency treatment and tooth extraction. The success of endodontic treatment was defined as the accomplishment of root canal filling and coronal restoration.

Statistical Analysis

IBM SPSS Statistics Version 20.0 software was used in the statistical analyses. Categorical variables were described as numbers and percentages, whereas continuous variables were expressed as mean, standard deviation, median and minimum-maximum where appropriate. A chi-square test was used in the comparison of categorical variables. The statistical level of significance for all tests was considered to be 0.05.

Results

Demographics

The demographics of 2,574 patient attendance according to the departments were presented in Figure 2. The patients who called our clinic by phone were advised to come only for emergencies. Three on-duty dentists who changed every day examined the patients who visited the clinic. Patients were directed to the relevant departments according to their primary dental complaints or needs. A total of 279 patients were referred to the endodontics clinic. While 149 patients were recommended to postpone their treatment, the remaining 130 patients received endodontic emergency treatment with or without aerosol by six dentists. There was no application to the endodontics clinic with a history of trauma.

Attendance to Endodontics Clinics

Sixty seven teeth of 65 patients (28 male, 37 female patients; mean age 35±12.2 years, age range: 11-68 years) were treated with non-aerosol generating procedures. However, 27 patients did not apply to our clinic again after the procedure. They were excluded

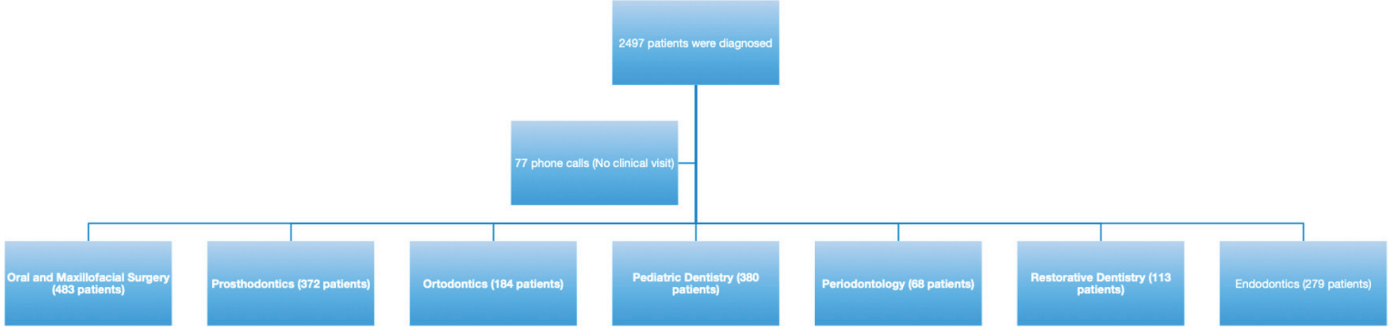

Figure 2. Demographics of the patient attendance according to the departments

from the evaluation in terms of success criteria because of the lack of follow-up data. Table 1 shows the detailed data of patients undergoing non-aerosol generating procedures.

Treatment of 58 in 65 patients (29 male, 36 female patients; mean age: 35.7±13 years, age range: 11-66 years) with aerosol-generating procedures was continued in our clinic and included in the success evaluation because the follow-up information was complete. Data of aerosolized procedures are summarized in Table 2. The aerosol-generating procedures group's success rate is 86.2%, while it is 70.0% in the non-aerosol-generating procedures group. Also, the rate of those followed up after the aerosol-generating procedures is 89.2%, while 59.7% in the non-aerosol-generating procedures group. A significant difference was found between emergency procedures in achieving the continuation of the treatment (p=0.001) and the success of delaying endodontic treatment by relieving pain (p=0.050, marginal significance).

There was no significant difference between the success rates of non-aerosol-generating procedures' subgroups (p>0.999); since the temporary restoration success rate was 68.0% and 71.4% in the prescribed group.

Besides, endodontic treatment of 37 teeth belonging to 34 patients (10 male, 24 female patients; mean age: 36.4±17 years, age range: 15-88 years) was initiated in our clinic before the pandemic and was prolonged in this period. Complications experienced during this period can be listed as follows: 3 patients had a temporary filling fracture, one patient came with a sinus tract, one patient had a flare-up, two patients were referred to a surgeon for extraction by fracture of teeth, and four patients did not want to complete their treatment due to the pandemic (Table

Table 1. Patient gender, age, tooth type, intervention, follow-up, duration to actual treatment, vitality and outcome/success rates for each patient received non-aerosol generating emergency procedures

Gender	
Female	37
Male	28
Age	Mean: 35±12.2 years Range: 11-68 years
Tooth type (n=67)	
Maxillary molar	22
Mandibular molar	20
Maxillary premolar	10
Mandibular premolar	6
Maxillary anterior	7
Mandibular anterior	2
Intervention	
Prescription	27
Temporary restoration	39
Prescription + temporary restoration	1
Follow-up	
Yes	40 (59.70%)
No	27 (40.29%)
Duration to actual treatment (days)	78.38±73.370 days Range: 2-271 days
Vitality	
Vital pulp	48 (71.64%)
Non-vital pulp	17 (35.41%)
Root-filled teeth	2 (2.98%)
Outcome/Success rates	
Overall	70%
Vital pulp	67.74%
Non-vital pulp and root filled teeth	77.77%

Table 2. Patient gender, age, tooth type, follow-up, duration to actual treatment, vitality and untimely presence of pain for each patient received aerosol generating emergency procedures

Gender	
Female	36
Male	29
Age	Mean: 35.7±13 years Range: 11-66 years
Tooth type (n=65)	
Maxillary molar	22
Mandibular molar	16
Maxillary premolar	12
Mandibular premolar	8
Maxillary anterior	4
Mandibular anterior	3
Follow-up	
Yes	58 (89.23%)
No	7 (10.76%)
Duration to actual treatment (days)	57.47±34.15 days Range: 24-148 days
Vitality	
Vital pulp	43 (66.15%)
Non-vital pulp	21 (32.30%)
Root-filled teeth	1 (1.53%)
Untimely presence of pain	
Overall	86.2%
Vital pulp	89.18%
Non-vital pulp and root filled teeth	80.95%

3). As a result, the treatment of 31 teeth (83.7%) of 28 patients (82.4%) whose endodontic treatment had been prolonged was completed in an average of 116.6±58 days.

Discussion

With the COVID-19 pandemic, our standard treatment concept will never be the same in dentistry as in other areas. While COVID-19 spread rapidly all over the world, dental practices were restricted as a caution. So, we performed only emergency interventions as recommended during the restriction period in different countries worldwide.

We primarily aimed to compare the efficacy of different endodontic emergency interventions in pain relief and delaying treatment. Aerosolized emergency procedures were significantly more successful than aerosol-free ones in relieving the pain and gaining time for endodontic treatment. As expected, a high success rate of 89.19% was achieved in pain relief by removing the pulp completely with aerosol-generating procedures in vital cases. In comparison, only a 67.75% success rate was achieved with non-aerosol-generating procedures. Also, the motivation of patients who underwent aerosol-generating procedures to continue their treatment was significantly higher. This result may be related to patients' fear of pain recurrence with the involved teeth. Patients experiencing severe pain during the pandemic lockdown for any dental treatment may worry about a possible lockdown again and request their treatment to be completed as soon as possible (16). Patel et al. (17) reported a follow-up rate of 96% and a success rate of 83% for aerosol-free endodontic interventions in a total of 21 patients. However, we observed a 70% success and 59.7% follow-up rate with non-aerosol-generating endodontic emergency procedures. The difference between follow-up rates may be due to methodological differences. Patel et al. (17) followed their patients by telephone questionnaire, while we ranked the continued endodontic treatment of the relevant tooth in our clinic as a follow-up. The difference between success rates may be related to many factors, such as differences in clinicians' diagnostic experience and communities' health-seeking behaviour.

Starting from the normalization period, we could complete endodontic treatment and restoration of 31 teeth (an 83.78% success rate) in a total of 37 teeth, similar to Patel et al.'s (17) study reporting an 83.87% success rate. Although some complications occurred, like temporary restoration fracture, presence of a sinus tract and flare-up, these cases were rated as successful since they were managed appropriately. Two extraction and four uncomplete treatment cases were ranked as failures. In one extraction case, the patient came with swelling five months later and was cleaned and dressed with calcium hydroxide again; however, the tooth had to be extracted after three months due to fracture. In the other case, the patient came with a restorable fracture three months after the root canal treatment was started; however, the patient resorted to extraction with her willingness since she did not want the process to be longer. Endodontically treated teeth might fracture due to several factors, such as weakening the tooth structure

Table 3. Patient gender, age, tooth type, presence of adverse event, time elapsed to treatment completion, vitality and outcome/success rates for each patient received prolonged endodontic treatment

Gender	
Female	24
Male	10
Age	Mean: 36.4±17 years Range: 15-88 years
Tooth type (n=37)	
Maxillary molar	14
Mandibular molar	11
Maxillary premolar	2
Mandibular premolar	1
Maxillary anterior	8
Mandibular anterior	1
Presence of adverse event	
Fractured temporary restoration	3
Refused to complete treatment	4
Pain-flare up	1
Extraction	2
Came with sinus tract	1
No adverse event	26
Time elapsed to treatment completion (days)	Mean: 116.68±58.01 days Range: 29-319 days
Vitality	
Vital pulp	15 (40.54%)
Non-vital pulp	16 (43.24%)
Root-filled teeth	6 (16.21%)
Outcome/success rates	
Overall	83.78%
Vital pulp	86.66%
Non-vital pulp and root filled teeth	81.81%

by endodontic access cavity and chemomechanical preparation of the root canal (18-20).

Additionally, the risk of re-contamination of the root canal system via the temporary restoration can be considered another important adverse event for prolonged endodontic treatment (21). Temporary restorations can allow microorganisms into the root canal system since they cannot provide sufficient sealing for a long time and lead to the formation of microcracks (22,23). Our findings showed that exposure to calcium hydroxide and staying with a temporary filling averagely of 16 weeks in prolonged treatments and eight weeks in aerosolized interventions did not significantly raise these detrimental events, with success rates of 83.78% and 86.2%, respectively. Similarly, a recent study suggested that calcium hydroxide could be considered a suitable interappointment medicament which also may be associated with a predictable outcome (24).

In our clinic, decision-making in endodontic emergency procedures was based on the symptoms of the teeth. In cases with mild symptoms, non-aerosol generating procedures were preferred. In vital cases with cavitation, a temporary filling was preferred to sedatize the tooth and rule out food impaction pain. Analgesics were prescribed for vital teeth that cannot be filled temporarily without aerosolized procedures. In non-vital cases, if the pain was predicted to be due to food impaction, a temporary filling was placed, whereas if the pain was suspected to be caused by infection, analgesics and/or antibiotics were prescribed following the published guidelines. Our clinical records about the pulpal and periapical health status of the teeth was described with vitality, which can be considered a limitation.

Conclusions

Within this retrospective study's limitations, aerosolized endodontic emergency procedures presented more success in pain relief, gaining time for actual treatment with a significantly higher follow-up rate than non-aerosolized procedures. Even so, non-aerosolized endodontic procedures have reduced symptoms to an acceptable level for 70% of patients and postponed the actual treatment for approximately 11 weeks. Additionally, prolonging endodontic treatment with long-term calcium hydroxide dressing does not seem to affect the short-term survival of teeth. Our results may aid clinicians in treatment planning and patient triage in a possible restriction in dental procedures.

Considering the success rates of the treatments reported in the literature, evaluating emergency patients case-by-case with clinical judgment and decision-making is recommended.

Ethics

Ethics Committee Approval: This study was approved by Medical Ethics Committee of Başkent University (project no: D-KA20/42, date: 22.12.2020) and the Turkish Ministry of Health's approval were taken following the Declaration of Helsinki.

Informed Consent: Before treatment, all patients read and signed the informed consent forms.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

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

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Original Article / Özgün Araştırma

A New Method for the Evaluation of pH Changes in Root Dentine by Phenolphthalein Colorimetric Indicator

Kök Dentinindeki pH Değişikliklerinin Yeni Bir Yöntem Olan Fenolftalein Kolorimetrik İndikatörü ile Değerlendirilmesi

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Abstract

Objective: To analyze the effect of two different vehicles and smear layer on the diffusion of hydroxyl ions into the root dentin using phenolphthalein colorimetric indicator.

Materials and Methods: Eighty-eight human mandibular premolars were used. Four randomly selected roots were used as the negative control group. The remaining roots were chemomechanical prepared and randomly divided into 2 main groups according to the final irrigation process: Group 1 (smear-): 4 mL 17% EDTA followed by 4 mL 2.5% NaOCl, group 2 (smear+): 8 mL 2.5% NaOCl. Next, 4 randomly selected roots from group 1 and group 2 were divided into 2 positive control groups. The main groups (n=38) were further divided into 2 subgroups according to the type of vehicle group 1a: CH + glycerin, group 1b: CH + distilled water, group 2a: CH + glycerin, group 2b: CH + distilled water. One week later, root canals were sectioned horizontally. Then, dentin sections were kept in 3% phenolphthalein solution for one day. The stained area, total area and root canal area were measured. The data were statistically analyzed (α=0.05).

Results: The staining pattern was more prominent in the absence of the smear layer ($p<0.001$). In the presence of the smear layer, the CH-glycerin group had more stained areas than the CH-distilled water group ($p<0.01$). In the absence of the smear layer, there was no difference between the vehicles ($p>0.05$).

Conclusion: Both tested vehicles were effective at increasing dentinal pH. Smear layer removal may facilitate the diffusion of hydroxyl ions.

Keywords
Calcium hydroxide, diffusion, intracanal medicament, pH, phenolphthalein

Anahtar Kelimeler
Kalsiyum hidroksit, difüzyon, kök kanal medikamenti, pH, fenolftalein

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Öz

Amaç: Bu çalışmanın amacı, hidroksil iyonlarının kök dentinine difüzyonu üzerinde iki farklı taşıyıcının ve smear tabakasının etkisini fenolftalein kolorimetrik indikatörü kullanarak analiz etmektir.

Gereç ve Yöntemler: Çalışmada 88 adet insan mandibular küçük azı dişi kullanıldı. Negatif kontrol grubu olarak rastgele seçilen 4 örnek kullanıldı. Kalan örnekler kemomekanik olarak hazırlandı ve son irigasyon işlemine göre rastgele 2 ana gruba ayrıldı: Grup 1 (smear-): 4 mL %17 EDTA ardından 4 mL %2,5 NaOCl, Grup 2 (smear+): 8 mL %2,5 NaOCl. Sonrasında, grup 1 ve grup 2'den rastgele seçilen 4 örnek 2 farklı pozitif kontrol grubuna ayrıldı. Ana gruplar (n=38) ayrıca kalsiyum hidroksit taşıyıcısına göre 2 alt gruba ayrıldı; grup 1a: CH + gliserin, grup 1b: CH

+ saf su, grup 2a: CH + gliserin, grup 2b: CH + saf su. Bir hafta sonra kök kanalları yatay olarak kesildi. Daha sonra dentin kesitleri bir gün boyunca %3'lük fenolftalein solüsyonunda bekletildi. Boyalı alan, toplam alan ve kök kanal alanı ölçüldü. Daha sonra veriler istatistiksel olarak analiz edildi (α=0,05).

Bulgular: Örneklerde, smear tabakasının yokluğunda boyalı alan anlamlı olarak daha fazla izlendi (p<0,001). Smear tabakasının varlığında, CH-gliserin grubunda, CH-saf su grubuna göre daha fazla boyalı alan izlendi (p<0,01). Smear tabakasının yokluğunda taşıyıcılar arasında anlamlı fark bulunmadı (p>0,05).

Sonuç: Test edilen her iki taşıyıcı da dentinin pH'ını artırmada etkiliydi. Ayrıca, smear tabakasının uzaklaştırılması, hidroksil iyonlarının difüzyonunu kolaylaştırabilir.

Introduction

The success of endodontic therapy depends on the complete removal of microorganisms and their toxic by-products from root canal system (1). However, it is difficult to eliminate all bacteria only by means of chemo-mechanical preparation (2). Therefore, intracanal medicaments have been widely used to eradicate the bacteria potentially surviving in the root canal system (3). The most popular intracanal medicament, calcium hydroxide (CH), has been widely used since the 1930s (4). CH exerts a high alkaline pH (~12.5) environment in which most of the endopathogens can't manage to survive (5). However, it is also known that CH has a limited antimicrobial spectrum (6). Although CH is not a panacea (7), it is still the most preferred root canal medicament today.

Diffusion of CH into the root dentine has been widely investigated since the 1980s (8-13). In addition to other techniques (10,11), colorimetric indicators had been used in several studies to observe pH changes in dentine following CH use (9,14). Phenolphthalein is a well-known acid-base colorimetric indicator, in aqueous solutions, it is colorless when the pH is below 8.3 and rapidly turns raspberry-purple at higher pH values. In a very strong basic medium (pH≥14), the indicator becomes colorless again (15). To date, phenolphthalein has never been used as a colorimetric indicator. Therefore, the aims of the present study were to investigate:

- The diffusion depth of hydroxyl ions into the root dentine using phenolphthalein colorimetric indicator,
- The effects of two different vehicles (glycerin and distilled water) on the diffusion of hydroxyl ions through root canal dentine.

Materials and Methods

The data from previous research (16) were used to determine the effect size of this study by using G*Power v.3.1 software. An alpha type error of 0.05 and a beta power of 0.80 were specified and calculated based on these parameters. Thus, the minimum estimated sample size for each group was found to be 19.

Single-rooted human mandibular premolars extracted for orthodontic reasons were collected under the permission obtained from the Human Ethics Committee of Adnan Menderes University (number: 98318678-020, date: 04.04.2018). The teeth were stored in 0.2% thymol solution in 37 °C. Thereafter, two periapical radiographs were taken in mesiodistal and buccolingual directions to confirm the presence of a single canal. Specimen selection was made based on relative dimensions, such as similarity in root morphology. Additionally, teeth with curvatures greater than 10° determined by the Schneider criteria (17) were excluded. As described above, a total of 88 teeth were included in the present study. The teeth were randomly divided into 4 experimental and 3 control groups. Seventy-six teeth were divided into 4 experimental groups (n=19) and 12 teeth were divided into 3 control groups (n=4). The negative control group received no preparation or medication (n=4). Samples in the positive control group were prepared chemomechanically similarly to the samples in the smear layer-negative experimental groups (as group 1) (n=4). Samples in the second positive control group were prepared chemomechanically similarly to the samples in the smear layer-positive experimental group (as group 2) (n=4). CH medication was not applied in any of the control groups.

Then, samples were decoronated at the cemento-enamel junction. The roots were cut further to standardize the root canals to a uniform length of 16 mm. Working length was confirmed by inserting a #10 K-file into the root canal until its tip was visible at the apical foramen.

All the root canals were prepared with ProTaper Universal system (Dentsply, Maillefer) according to the

manufacturer's instructions. All canals were shaped up to F5 in full clockwise rotation and files were renewed after every 5 root canals. During the instrumentation, 2 mL of 2.5% NaOCl irrigation was performed with a 27-gauge notched tip irrigation needle (Endo-Eze; Ultradent, South Jordan, UT) between each file. At the end of the instrumentation procedures, the apex of the samples was sealed with a sticky wax to create a closed-end channel according to Tay et al. (18). Then, all the samples were randomly divided into 2 main groups (n=38 in each group) according to the final irrigation procedure as follows:

Group 1 (smear layer negative): 4 mL 17% EDTA followed by 4 mL 2.5% NaOCl; each continued for 1 minute.

Group 2 (smear layer positive): with only 8 mL 2.5% NaOCl in 2 minutes.

Next, all the samples in both groups were irrigated with 10 mL of distilled water and dried with paper point cones. Then, the main groups (group 1 and group 2) were further divided 2 two sub-groups (n=19 in each), according to the type of the vehicles (distilled water or glycerin) to transport CH as follows:

Group 1a: CH + glycerin [(smear (-)]

Group 1b: CH + distilled water [(smear (-)]

Group 2a: CH + glycerin [(smear (+)]

Group 2b: CH + distilled water [(smear (+)]

Both CH pastes were prepared in a creamy consistency in a powder/liquid ratio of 1/1.5 (w/v) and applied into the root canals using a lentulo spiral. After CH pastes were placed into the root canals, the canal orifice was sealed with cotton pellets and temporary filling material (Cavit; 3M ESPE, USA). All the experimental and control groups were kept in 37 °C and 100% relative humidity for 1 week.

One week later, each root canal was irrigated with 10 mL distilled water. Initially, 1 mm apical parts of all the samples and the sticky wax (both control and experimental groups) were removed to obtain a clear vision in the stereo-microscope visualizations. Then, the remaining 15 mm samples were sectioned horizontally at 3, 6, 9 and 12 mm away from the apex under water cooling to have 4 pieces of dentine sections with a thickness of 3 mm each. After dentine sections were rinsed with distilled water, they were finally kept in 3% phenolphthalein solution (Merck, Darmstadt, Germany) at 37 °C for 24 h. Phenolphthalein solution stained dentine in purple shades because of the alkaline pH of CH (Figure 1). Stained samples were rinsed in distilled water to remove phenolphthalein remnants and then dried with air. The stained area around the root canal was evaluated using a stereomicroscope at x20 magnification (Leica S8 APO, Leica Microsystems, Heerbrugg, Switzerland) and photographed with a microscope attached camera. Images were recorded using the computer program LAS (Leica Application Suite Software, version 2.4.0.R1, Leica Microsystems CMS, Germany). The total area, stained area, and the root canal area of each sample were measured and recorded. Measurements were repeated 3 times and the average of these measurements was taken into consideration. The percentage of stained area in all cross- sectional levels was formulated and calculated as follows:

The percentage of the stained area (mm^2) = [Stained area (mm^2) / Total root area & times; Root canal area (mm^2)] × 100

The control groups were not included in the statistical analysis because no staining or penetration was observed in all of them.

Statistical Analysis

Statistical analysis was performed using the R software. This kind of data does not represent a Gaussian process and it does not meet the assumptions of typical parametric linear models. A widespread solution for analyzing data that do not

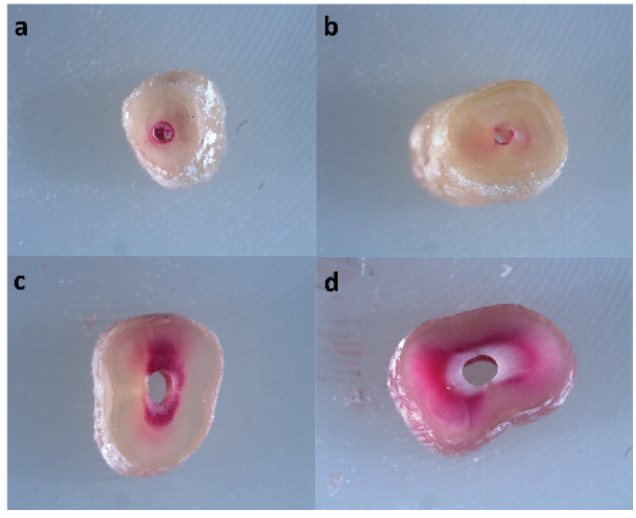

Figure 1. The staining of the same sample in the group 2a by the phenolphthalein colorimetric indicator
(a. 3 mm - cross section, b. 6 mm - cross section, c. 9 mm - cross section, d. 12 mm - cross section)

meet parametric assumptions is to use non-parametric models. However, a common issue of non-parametric models is that they cannot test for interactions among groups. In order to circumvent this issue, data was transformed using a method called align rank transformation (19). ANOVA can be applied to aligned rank transformed data, making it possible to test for both main group effects and their interactions. Align rank transformation and subsequent ANOVA was performed using the R package ARTool (20). Statistical significance was determined at α=0.05. A chi-square test was performed in evaluating the categorical data regarding the presence or absence of staining (α=0.05).

Results

In the statistical analysis, the percentages of staining area, the presence/absence of the smear layer, the type of vehicles (distilled water or glycerin), and the cross-sectional levels were taken into consideration as parameters.

The percentages of the staining area at all cross-sectional levels in the groups are presented in Table 1. The staining pattern was more prominent in absence of the smear layer (p<0.001). However, an interaction was found between the smear layer and the vehicle type (p<0.01). Accordingly, the type of vehicle had a significant effect on staining pattern only when there was the smear layer (p<0.01). In presence of the smear layer, CH-glycerin group had more stained areas than the CH-distilled water group had. However, there was no statistically significant difference by means of the vehicle in absence of the smear layer (p>0.05).

When the cross-sectional levels were evaluated, the coronal sections had more stained areas and there were significantly more non-stained samples in the 3 mm group (p<0.001). Stained area percentages significantly decreased in the apical sections of the groups (Table 1). The numbers of the stained and non-stained samples at all cross-sectional levels are presented in Table 2.

Discussion

CH, the key intracanal therapeutic agent helping in resolution of periradicular periodontitis (2,5-7). In addition to the research on therapeutic and antibacterial activity of CH, numerous studies were conducted on diffusion dynamics of this medicament (9-12, 21). The first study investigating the pH changes in root canal dentine following the placement of CH was performed by Tronstad et al. (9). They found that pH values ranged between 8 to ~11 in the circumpulpal dentine and 7.4 to 9.6 in the peripheral dentine by utilizing 18 different pH indicators (9).

Table 1. The percentages of the staining areas at all cross-sectional levels

Smear & vehicle		Cross-sectional levels				Mean ± SD
		3 mm	6 mm	9 mm	12 mm	
Smear (+)	Glycerin	10.87±11.62	16.17±20.21	32.79±24.07	42.16±16.20	25.50±14.51
	Distilled water	3.39±10.16	23.08±24.14	31.34±20.91	31.16±10.66	22.00±13.15
Smear (-)	Glycerin	2.80±4.10	27.77±21.70	40.07±24.25	39.47±22.68	27.53±17.43
	Distilled water	15.71±11.00	31.72±19.15	45.34±17.01	55.02±17.74	36.95±17.08
Mean ± SD		8.19±10.94	24.69±21.75	37.39±22.07	42.25±19.16	

SD: Standard deviation

Table 2. The numbers of the stained and non-stained samples at all cross-sectional levels

Smear and vehicle	Cross-sectional levels							
	3 mm		6 mm		9 mm		12 mm	
	Stained (+)	Stained (-)	Stained (+)	Stained (-)	Stained (+)	Stained (-)	Stained (+)	Stained (-)
Smear (+) glycerin	11	8	13	6	15	4	19	0
Smear (+) distilled water	2	17	14	5	18	1	17	2
Smear (-) glycerin	7	12	16	3	19	0	17	2
Smear (-) distilled water	18	1	19	0	19	0	19	0

Then, researchers developed a new method by preparing external cavities on the root canal wall where pH changes and Ca^{+2} ion concentration would be observed simultaneously (10). This relatively direct pH measurement method designed by Nerwich et al. (10) was used in various studies (10,13,21). Although a totally different approach was used, Nerwich et al. (10) declared that their dentinal pH measurements were in agreement with those of Tronstand et al. (9).

Although the method used in the present study was somewhat similar to the study of Tronstad et al. (9), a much simpler method was performed by using a single (phenolphthalein) colorimeter indicator to provide visual information. In this method, there are no limitations such as regional changes of dentinal tubule density and calcification that impede phenolphthalein staining. Thus, the method used in the present study offers a simple analysis model with remarkable visual information, which provides accuracy and precision of the pH measurements. The pH changes in root dentine could be marked by phenolphthalein staining, which enables the observer to be able to trace the pH changes within the root dentine.

Phenolphthalein, an acid base indicator, turns pink between pH 8.3 and 14, and colorless if below pH 8.3 or above 14 (15). It has been declared many times in previous research that the pH range of root canal dentine is 8-11.1 after placement of CH (9,10,21,22). Thus, phenolphthalein presented to be an appropriate colorimetric indicator for determining the pH of the root canal dentine after placement of CH.

The effect of the vehicle used to transport CH has also been previously reported that distilled water or camphorated paramonochlorophenol resulted in better diffusion capacity compared to phosphate-buffered saline or propylene glycol (23). According to Staehle et al. (14), an aqueous suspension allows a more efficient release of hydroxyl ions. Safavi and Nakayama (24), reported that the use of high concentrations of glycerin or propylene glycol as a CH carrier reduces the conductivity of CH, and thus may also reduce its effectiveness. According to the results of this study, in the absence of the smear layer, although there was no statistically significant difference between the two tested vehicles, the percentage of the staining in the distilled water group was higher compared to the glycerin group in all cross-sectional levels. This may be due to the fact that an aqueous suspension allows more effective and rapid release of hydroxyl ions compared to the viscous vehicles, especially in the absence of the smear layer. This result was similar to that of the above-mentioned studies (14,23,24).

One of the limitations of this study was that any direct pH-value could not be given because phenolphthalein colorimetric indicators can only determine pH in a range between 8.3-14.0. Its color changes from pink to fuchsia at a more basic pH, but this does not provide an accurate measurement. In order to overcome this limitation, the total diffusion areas were calculated, and the numerical data were obtained. The results of the present study are consistent with most of the previously published studies on pH changes in the root dentine associated with CH delivery (9-11,21). Therefore, the use of phenolphthalein as a colorimetric indicator can be presented as a promising method when analyzing pH changes in root dentine.

Conclusions

Under the experimental conditions of this study, it can be concluded that both tested vehicles used to transport CH were effective in increasing dentinal ph. Additionally, the phenolphthalein colorimetric indicator, which was used for the first time in evaluating the pH changes of root dentine, appears to be a practical and simple method.

Ethics

Ethics Committee Approval: Single-rooted human mandibular premolars extracted for orthodontic reasons were collected under the permission obtained from the Human Ethics Committee of Adnan Menderes University (number: 98318678-020, date: 04.04.2018).

Informed Consent: Informed consent is not required.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: H.D.Ö., B.H.Ş., B.Ş., Design: H.D.Ö., B.H.Ş., Data Collection or Processing: H.D.Ö., Analysis or Interpretation: B.Ş., Literature Search: H.D.Ö., B.H.Ş., B.Ş., Writing: H.D.Ö., B.H.Ş.

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Original Article / Özgün Araştırma

Effect of Low-thermal Degradation on the Flexural Strength of Y-TZP Ceramics

Düşük Isı Bozunmasının Y-TZP Seramiklerinin Eğilme Dayanımına Etkisi

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Abstract

Objective: This research aimed to look into the aging characteristics of three distinct yttria-stabilized tetragonal (t) zirconia polycrystalline, as well as the impact of low-thermal degradation on flexural strength.

Materials and Methods: One hundred twenty disc-shaped specimens were obtained from three different brands (Supra Zr, Zirkonzahn Prettau and CopraPretty). For each brand, specimens in the control groups were left in distilled water at 37 °C for 5 h whereas those in experimental groups were artificially aged under three different conditions. In the thermal cycle group, specimens were fatigued for 10,000 between baths held at 5 °C and 55 °C; left in 4% acetic acid at 80 °C for 168 h in acetic acid groups, and aged at 134 °C, 0.2 MPa for 5 h in autoclave aging groups. T to monoclinic phase transformation (Xm) quantified using X-ray diffraction for all groups. One randomly selected specimen surface was analyzed by scanning electron microscopy from each subgroup. The biaxial flexural strength test was used to assess the flexural strength of the specimens. The data were statistically analyzed.

Results: The highest relative amount of the Xm content was obtained in the autoclave aging groups (Supra Zr 0.36±0.01, Zirkonzahn Prettau 0.41±0.02, CopraPretty 0.58±0.04). There was no significant difference between Zirconzahn Prettau and Supra Zr materials ($p>0.05$), the lowest biaxial flexural strength values were recorded in the CopraPretty material.

Conclusion: Despite aggressive aging conditions, the materials showed high flexural strength values that could withstand intraoral forces. Therefore, clinical use of the materials is anticipated to be possible.

Keywords
Flexural strength, low-thermal degradation, monolithic zirconia

Anahtar Kelimeler
Eğilme dayanımı, düşük ısı bozunması, monolitik zirkonya

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Öz

Amaç: Bu çalışmanın amacı, üç farklı yittria ile stabilize edilmiş tetragonal (t) zirkonya polikristalinin yaşlanma özelliklerini ve ayrıca düşük ısı bozunmasının materyallerin eğilme dayanımına etkisini incelemekti.

Gereç ve Yöntemler: Üç farklı markadan (Supra Zr, Zirkonzahn Prettau ve CopraPretty) 120 adet disk şeklinde örnek elde edildi. Her marka için kontrol gruplarındaki örnekler 5 saat boyunca 37 °C'de distile suda bekletildi. Deney gruplarındaki örnekler, yapay olarak üç farklı koşulda yaşlandırıldı. Örnekler termal döngü grubunda 5 °C ile 55 °C arasında 10.000 döngüye kadar yoruldu; Asetik Asit gruplarında %4 asetik asitte 80 °C'de 168 saat bekletildi ve otoklav yaşlandırma gruplarında 134 °C, 0,2 MPa'da 5 saat yaşlandırıldı. T monoklinik faz dönüşümü (Xm), tüm örneklerde X-ışını kırınımı kullanılarak ölçüldü. Her alt gruptan bir örnek yüzeyi taramalı elektron mikroskobu ile incelendi. Örneklerin eğilme dayanımı değerleri, tüm örnekler için çift yönlü eğilme dayanımı testi ile ölçüldü. Veriler istatistiksel olarak analiz edildi.

Bulgular: En yüksek göreceli Xm miktarı otoklavda yaşlandırma gruplarında elde edildi (Supra Zr 0,36±0,01; Zirkonzahn Prettau 0,41±0,02; CopraPretty 0,58±0,04). İstatistiksel olarak Zirconzahn Prettau ve Supra Zr materyalleri arasında anlamlı bir fark yoktu (p>0,05), en düşük eğilme dayanımı değerleri CopraPretty materyalinde kaydedildi.
Sonuç: Agresif yaşlandırma koşullarına rağmen, materyaller ağız içi kuvvetlere dayanabilecek yüksek eğilme dayanımı değerleri göstermiştir. Bu nedenle materyallerin klinik kullanımlarının mümkün olması beklenmektedir.

Introduction

Monolithic zirconia ceramics are now recognized as promising materials with increased aesthetic and mechanical qualities in dental restorative processes (1).

Polymorphic zirconia crystals can exist in 3 different phases: monoclinic (m), tetragonal (t) and cubic. ZrO_2 can be mixed with other metallic oxides to achieve high molecular stability (2). Stabilized zirconia contains a metastable tetragonal phase that enables the usage of zirconium in bulk at room temperature. When material is subjected to mechanical stress, the metastable t phase which is resistant to crack propagation transforms into the m phase. Phase transformation from volume increases in localized areas around microcracks creates compressive stresses which prevents crack propagation - a phenomenon referred as transformation toughening (3). The metastable t phase transforms into the m phase starting from the material surface and into the bulk in a humid environment without mechanical stress. This causes microcracks and reduces the material strength. This process is called low-thermal degradation (LTD) or aging (4).

Despite its superior mechanical properties, zirconia is susceptible to aging, phase transformation in the presence of moisture, and undergoes LTD (5). Further knowledge is needed on the susceptibility of zirconia to LTD in order to increase clinical success (6).

Monolithic zirconia material must be addressed since the strength of dental restorative materials is an important foundation for appropriate restoration. This study centered on the impact of LTD on the biaxial flexural strength (BFS) of 3 monolithic yttria-stabilized tetragonal zirconia polycrystal (Y-TZP) ceramics and crystallographic phase changes of the materials' surfaces by analyzing X-ray diffraction, and surface characteristics by scanning electron microscope observation.

Consequently, the null hypotheses of the present study were that no significant difference among the monolithic zirconia materials and aging conditions have had no effect on the BFS of the materials.

Materials and Methods

Ethics committee approval for the study was obtained from the meeting of Selçuk University Faculty of Dentistry, Non-Interventional Clinical Research Evaluation Commission (protocol no: 2014/03; date: 25.03.2014).

Specimen Preparation

120 disc-shaped (12 mm Ø and 1.2±0.2 mm thickness) specimens were fabricated (Yenamak D40 milling machine, Yenadent Ltd., İstanbul, Turkey) from three different brands of monolithic zirconia: Supra Zr (Turkuaz Dental, İzmir, Turkey); Zirkonzahn Prettau (Zirkonzahn GmbH, Bruneck, Italy) and CopraPretty (Whitepeaks Dental, Wesel, Germany). At the pre-sintered stage, all specimens were wet-polished with silicon carbide papers (English Abrasives & Chemicals, London, UK) up to #1200 grit to achieve consistent smooth surfaces followed by the specimens were sintered according to the manufacturer's recommendations. Specimens were sintered at 1500 °C and 1600 °C (Supra Zr, 1500 °C; Prettau and CopraPretty, 1600 °C). Specimens were divided into four subgroups in each brand: Control, thermal cyclus, acetic acid ageing, and autoclave ageing. The specimens without any ageing conditions were designated as a control group. The test-groups designated are given in Table 1 together with the group abbreviations.

Artificial Aging

Control: Specimens were maintained in distilled water for five hours at 37°C in an incubator (EN 1200 model; Nuve, Ankara, Turkey).

Thermal cyclus: At thermal cycling machine (Nova, Konya, Turkey), specimens underwent 10.000 thermal

Table 1. Experimental groups with group abbreviations

	Condition			
Brands	**Control**	**Thermal cyclus**	**Acetic acid ageing**	**Autoclave aging**
Supra Zr	SC	ST	SA	SO
Prettau	PC	PT	PA	PO
CopraPretty	CC	CT	CA	CO

cycles between 5 and 55 °C with dwell time of 30 seconds and transfer time of 10 seconds.

Acetic acid aging: Specimens were immersed in 4% acetic acid solution (Puriss, Prolabo; Merck Group, Darmstadt, Germany) and kept in an incubator (EN 1200 model; Nuve, Ankara, Turkey) at 80±5°C for 168 hours.

Autoclave aging: Specimens were kept in a steam autoclave (DAC Professional Autoclave, Sirona Dental, NY, USA) at 134 °C, under two bars of pressure for five hours according to ISO 13356 standard.

X-ray Diffraction Analysis

The crystal structure was identified using an X-ray diffractometer (Bruker D8 Advance, Bruker AXS, Karlsruhe, Germany with monochromatic CuK radiation). The voltage and current were both set to 40 kV and 40 mA, respectively. With a step size of 0.0369, the specimen surfaces were scanned between 20 and 40 2Θ degrees (Lynxeye detector, Bruker AXS, Karlsruhe, Germany). Equation (1), based on the approach of Garvie and Nicholson (3), was used to compute the relative amount of m phase (Xm):

1) $$X_m = \frac{I_{m(111^-)} + I_{m(111)}}{I_{m(111^-)} + I_{m(111)} + I_{t(101)}}$$

$I_{t(101)}$ represents the intensity of a tetragonal peak whereas $I_{m(111-)}$ and $I_{m(111)}$ reflect the intensities of m peaks.

Biaxial Flexural Strength Test

According to the ISO 687274 standard, the piston-on-three-balls method was used on a universal testing machine (ELISTA, TSTM02500, Elista Corp, İstanbul, Turkey) to estimate each specimen's BFS. Each specimen was put on three 3.2 mm diameter supporting steel balls that were equidistant from each other on a 10 mm diameter circle. A flat-end circular loading piston with a 1.4 mm diameter applied the load until the specimens fractured at a cross-head speed of 1 mm/min. The following formulae were used to calculate BFS (MPa) values [Equations (2-4)] according to the ISO 6872 standard:

2) $S = - 0{,}2387\ P(X- Y)/d^2$

3) $X = (1+ v)\ \ln(r_2/r_3)^2 + [\ (1-v)/2]\ (r_2/r_3)^2$

4) $Y = (1+ v)\ [1 + \ln(r_1/r_3)^2] + (1-v)\ (r_1/r_3)^2$

where S is the BFS (MPa), P is the fracture load (N), v is the Poisson's ratio (0.25), r_1 is the support circle radius (mm), r_2 is the loaded area radius (mm), r_3 is the specimen radius (mm), and d is the specimen thickness at fracture origin (mm).

Scanning Electron Microscopy (SEM) Analysis

One specimen from each group was chosen randomly to reveal the surface topography by using SEM analysis. The specimen surfaces were cleaned in 96 per cent ethanol and allowed to air dry gently. The airborne-particle abraded surface of the specimens was coated with gold with a gold plating device (Sputter Coater 108 Auto, Cressington Scientific Instruments Ltd., Watford, England). The surfaces were examined at X1000 and X5000 magnifications (SEM-ZEİSS LS-10, England).

Statistical Analyses

The evaluation of the normal distribution and homogeneity of variances was done using the Kolmogorov-Smirnov and Levene tests, respectively. One-way ANOVA and Welch tests were used to analyze the data. Tukey HSD and Tamhane tests were used to detect the differences among subgroups. In order to clarify the relationship between the phase transformation and BFS, Pearson's correlation was used. The variability of the flexural strength was calculated using the Weibull distribution function. Each group's Weibull modulus (m) and characteristic strength (σ0) values were calculated. The Weibull modulus was calculated using the following formula (7):

5) $$P_f(\sigma) = 1 - \exp[-(\sigma/\sigma_0)^m]$$

where m is the Weibull modulus, Pf(σ) is the probability of failure, σ is the fracture strength, σ0 is the characteristic value that corresponds to 63.21 probability of failure, and. All statistical analyses were performed at a significance level of α=0.05.

RESULTS

Phase Transformation

The intensity of the tetragonal peak was recorded at 30°, whereas the intensities of m peaks were observed around 28 and 31 degrees, respectively.

The mean (± standard deviation) relative Xm of the subgroups are given in Table 2.

According to X-ray diffractometry (XRD) analysis, autoclave aging significantly affected the phase transformation in all groups. The highest amount of m phase transformation was detected in the CO group among the subgroups. There was no significant difference in phase transformation between SC and ST groups.

Biaxial Flexural Strength

Statistical results of BFS were shown in Table 3. The BFS values were affected by artificial aging. Control groups of Supra Zr and Prettau exhibited higher BFS values. Conversely, the BFS values of the CC, CT and CA groups were among the lowest of all the groups, and aging improved the BFS values of the autoclave and acetic acid groups of CopraPretty.

SEM Analysis

Representative SEM images are given in Figure 1. The results of the Xm values were consistent with the SEM images. When compared to the control groups, deep irregularities, pits, and grooves were observed on the surfaces of artificially aged groups. The CopraPretty material shows a different surface structure than other materials.

Weibull Analysis

Weibull analysis was used to analyze the variability of the BFS values of the materials. Characteristic strengths (σo), Weibull modulus (m), and R^2 values are given in Table 4.

According to the results of the Weibull analysis, it was observed that the m values of the materials differ according to the aging procedures. The low m value indicates the low reliability of these groups. The highest m value was found in the specimens in the SC group. This indicates that these specimens have similar BFS values and a low standard deviation, indicating that this material is more trustworthy.

Table 2. Mean (± SD) relative monoclinic phase (Xm) of subgroups (%)

	Supra Zr	Prettau	CopraPretty
Control	0.28±0.03^{A,a}	0.29±0.02^{A,a}	0.11±0.01^{B,a}
Thermal cyclus	0.26±0.02^{A,a}	0.29±0.02^{B,a}	0.12±0.01^{C,a}
Acetic acid	0.29±0.03^{A,ab}	0.31±0.01^{B,ab}	0.18±0.02^{C,b}
Autoclave aging	0.36±0.01^{A,c}	0.41±0.02^{B,c}	0.58±0.04^{C,c}

Subgroups that are not significantly different have the same superscript uppercase letters in the same column and the same superscript lowercase letters in the same row (Tukey's HSD and Tamhane tests, p>0.05)

DISCUSSION

This study purposed to elucidate the LTD properties of 3 types of monolithic Y-TZP ceramics by exposing specimens to various accelerated aging processes. To investigate the impact of accelerated aging treatment among materials the BFS, SEM analysis and XRD characteristics were examined. In light of the findings of the study, there was a significant difference among the monolithic zirconia materials and aging conditions have an effect on the BFS of the materials. As a result, the null hypotheses of the present study were rejected.

Three conditions were used for accelerated aging treatment to compare the effects of LTD in this study. Specimens in the control group were kept in distilled water in an incubator at 37 °C for 5 hours. The first condition was thermal cycling. Although the ISO TR 11450 standard (8) states that a thermo-cycling regimen comprising 500 cycles in water between 5 and 55 °C is a suitable artificial aging test, Gale and Darvell (9) came to the conclusion that 10,000 cycles equate to roughly one year of *in vivo* function. Hence the thermal cycling test used in this study was according to ISO TR 11450 standard (8) modified by Gale and Darvell (9). Acetic acid is the acid used for chemical stability testing, according to ISO standard 6872 (10). Kukiattrakoon et al. (11) used a modified method of ISO 6872 (10) in their study and immersed the specimens in 4% acetic acid at 80 °C for 168 h. The same condition was used for the acetic acid aging group in our study. Chevalier et al. (4) used a proportion of the m phase to compute the longevity of TZP and reported that 134 °C/5 h/0.2 MPa equates to 15 to 20 years in 37 °C water. The last condition was carried out in accordance with the ISO 13356

Table 3. Mean values ± standard deviations for biaxial flexural strength (MPa)

	Supra Zr	Prettau	CopraPretty
Control	1178.116±35.022^{A,a}	1178.663±65.607^{A,a}	834.409±89.539^{B,a}
Thermal cyclus	1095.861±50.166^{A,b}	1109.320±112.012^{A,ab}	806.512±67.343^{B,a}
Acetic acid	984.863±168.067^{A,bc}	1045.040±61.091^{AB,b}	842.452±152.749^{AC,ab}
Autoclave aging	925.829±121.661^{A,c}	1014.606±107.040^{A,b}	963.102±96.573^{A,b}

Same lowercase letters in the same column (Tukey's HSD, Tamhane's test; p>0.05) and same uppercase letters in the same row denote subgroups that were not significantly different

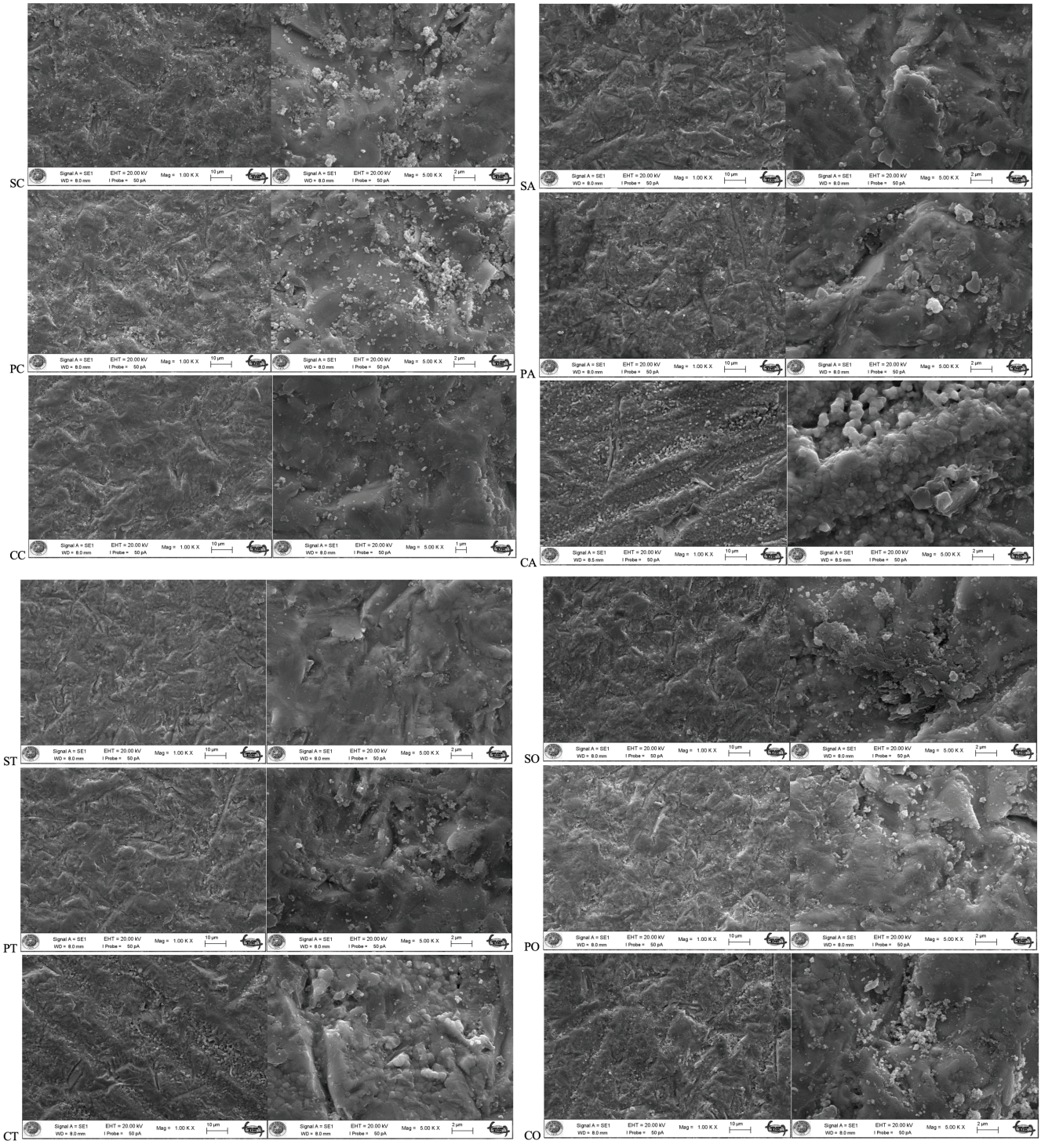

Figure 1. SEM images of experimental groups
SEM: Scanning electron misroscopy

(12) required accelerated aging test, as modified by Chevalier et al. (4).

The first conventional non-destructive technique to evaluate the zirconia transformation kinetics quantitatively was the XRD. Because the X-ray is several millimetres wide, it usually describes the behaviour of a section of the sample surface. However, the signal-to-noise ratio is quite low, available from

Table 4. Characteristic strengths (MPa), Weibull modulus (m) and R^2 values of groups

	Supra Zr			Prettau			CopraPretty		
	σ_o	m	R^2	σ_o	m	R^2	σ_o	m	R^2
Control	1196.00	35.62	0.734	1035.23	21.05	0.921	873.46	10.88	0.897
Thermal cyclus	1118.74	25.60	0.929	1159.75	11.22	0.896	836.62	13.91	0.854
Acetic acid	1054.24	6.72	0.913	1074.80	18.59	0.744	904.81	6.41	0.855
Autoclave aging	977.28	8.81	0.961	1061.29	10.93	0.973	1006.56	11.22	0.898

transformed regions where the converted zirconia fraction is less than 5% (13). XRD measurements have shown a sizable difference in m phase content according to aging conditions and zirconia materials in this study. The highest Xm values were found in the CO (0.58%±0.04) group while the lowest values were in CT (0.11%±0.01).

The biaxial flexural test which is the international standard for dental ceramics (14) was used in this study. The mean BFS values of the materials were 1046,16 MPa (Supra Zr), 1086,90 MPa (Prettau) and 861,61 MPa (CopraPretty) respectively. The BFS values of the control and thermal cycle groups of Supra Zr and Prettau materials were found to be higher than the acetic acid and autoclave aging groups. These results were consistent with a study done by Flinn et al., (15) which found that the flexural strength of the specimens decreased following accelerated aging treatment. However, the highest BFS values were obtained in the acetic acid and autoclave groups in CopraPretty material. These results support those of Pereira et al., (16) who found that volume expansion owing to phase transformation from t to m increase compressive residual stress and improves strength.

There was a negative correlation between phase transformation and BFS in Supra Zr and Prettau materials while a positive correlation occurred in CopraPretty material. While the highest Xm and the lowest BFS values were observed in the acetic acid and autoclave aging groups of Supra Zr and Prettau materials, both Xm and BFS values of acetic acid and autoclave aging groups of CopraPretty material were higher than the material's thermal cyclus and control groups. These results for CopraPretty material may be related to the transformation toughening phenomenon. Even though chemically similar materials were chosen, this apparent significant difference in sensitivity to LTD amongst identical ceramics could be ascribed to differences in manufacturing conditions, microstructure, and flaw distribution. This was also supported by the fact that changes in the surface layers of the materials were observed after accelerated aging treatment by SEM observation.

Two often used statistical measures to describe one aspect of structural reliability are the Weibull modulus (m) and characteristic strength. Assuming equivalent levels of mean strength between ceramics, higher Weibull modulus values correspond to materials with stronger structural reliability. The majority of ceramics are reported to have m values in the range of 5-15 (17). When compared to the other groups in the current study, the SC group displayed a higher Weibull modulus, demonstrating the material's higher clinical dependability. However, according to the m values, the materials have a tolerably low failure probability up to a bending stress level of around 500 MPa, which is higher than the stress caused by occlusal loads routinely measured intraorally (18).

Conclusion

Both hydrolysis and compressive residual stress can impact phase transformation to the m phase, and the major cause cannot be determined with certainty. The study was carried out with disc-shaped specimens in *in-vitro* conditions. Within the limitations of the present study in-vivo conditions with crown-shaped specimens may produce different results.

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Ethics

Ethics Committee Approval: Ethics committee approval for the study was obtained from the

meeting of Selçuk University Faculty of Dentistry, Non-Interventional Clinical Research Evaluation Commission (protocol no: 2014/03; date: 25.03.2014).

Informed Consent: Informed consent is not required.

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Authorship Contributions

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Original Article / Özgün Araştırma

The Role of Fetal Transcerebellar Diameter in Determining Gestational Age in the Second Trimester

İkinci Trimesterde Gebelik Yaşının Belirlenmesinde Fetal Transserebellar Çapın Rolü

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Abstract

Objective: To evaluate the role of transcerebellar diameter (TCD) as an independent parameter for estimation of gestational age (GA) in the second-trimester and its diagnostic performance compared with other fetal biometric parameters (FBP) such as biparietal diameter (BPD), femur length (FL), abdominal circumference (AC), and head circumference (HC).

Materials and Methods: This retrospectively-designed cross-sectional study included recorded data of 120 healthy women between 19 and 24 weeks of gestation with normal singleton pregnancies who applied to our department for second-trimester anomaly scan between December 2020 and 2021. GA was calculated using nomograms on TCD and other FBP and compared with GA determined by last menstrual period (LMP). The relationship between GA based on LMP and FBP was evaluated. The correlation between parameters was evaluated with the Pearson correlation test.

Results: The mean BPD, HC, AC, FL, TCD weeks and TCD values (mm) were 21.39±0.99, 21.36±0.98, 21.28±0.95, 21.24±1, 20.11±0.73, 21.21±1.09, respectively. The highest correlation for GA estimation was shown with FL (r=0.858), followed by AC (r=0.843), TCD values (mm) (r=0.834), and the TCD (week) (r=0.822), respectively. All "r" values indicated a strong correlation and ranged from 0.794 to 0.858. All parameters used in the detection of GA in the second-trimester were statistically significant (p<0.001).

Conclusion: TCD provides successful results in the accurate estimation of GA in the second-trimester. Therefore, we recommend routine TCD measurements for anomaly screening

Öz

Amaç: Bu çalışmanın amacı transserebellar çapın (TSÇ) ikinci trimesterde gebelik yaşının (GY) tahmininde bağımsız bir parametre olarak rolünü ve biparietal çap (BPÇ), femur uzunluğu (FU), karın çevresi (KÇ) ve baş çevresi (BÇ) gibi diğer fetal biyometrik parametrelerle karşılaştırıldığında tanısal performansını değerlendirmektir.

Gereç ve Yöntemler: Retrospektif olarak tasarlanmış bu kesitsel çalışmaya Aralık 2020 ile 2021 arasında bölümümüze ikinci trimester anomali taraması için başvuran, normal tekil gebeliğe sahip 19-24 gebelik haftalar arasında 120 sağlıklı kadına ait veriler dahil edildi. GY, TSÇ ve diğer fetal biyometrik parametrelerin nomogramları

kullanılarak hesaplandı ve son adet tarihi (SAT) ile belirlenen GY ile karşılaştırıldı. SAT'a göre belirlenen GY ile fetal biyometrik parametreler arasındaki ilişki değerlendirildi. Parametreler arasındaki korelasyon için Pearson korelasyon testi kullanıldı.
Bulgular: Ortalama BPÇ, BÇ, KÇ, FU, TSÇ haftaları ve TSÇ ölçümleri (mm) sırasıyla 21,39±0,99, 21,36±0,98, 21,28±0,95, 21,24±1, 20,11±0,73 ve 21,21±1,09 idi. GY tahmini için en yüksek korelasyon FU (r=0,858), ardından KÇ (r=0,843), TSÇ ölçümleri (mm) (r=0,834) ve ortalama TSÇ haftası (r=0,822) ile bulundu. Tüm "r" değerleri güçlü korelasyona işaret etmekte ve 0,794 ile 0,858 arasında değişmekteydi. İkinci trimesterde GY tespitinde kullanılan tüm parametreler istatistiksel olarak anlamlıydı (p<0,001).
Sonuç: TSÇ, ikinci trimesterde GY'nin doğru tahmininde başarılı sonuçlar vermektedir. Bu nedenle anomali taraması için rutin TSÇ ölçümlerini öneriyoruz.

Introduction

Gestational age (GA) is the most used parameter as a standardization tool in the detection and evaluation of fetal development in pregnancy follow-up. Although the last menstrual period (LMP) is the most widely used modality for estimating GA, it can be a wrong guide (1-3). The reliability of LMP is low due to reasons such as inability to remember correctly, irregular menstrual cycle and differences in ovulation time, and first-trimester bleeding. It has been emphasized that only half of pregnant women can remember their LMPs correctly in the literature (1,2).

Fetal development is a dynamic process and no single biometric parameter exists completely accurate or reliable throughout pregnancy. Estimation of GA in the detailed ultrasonographic examination performed in the second-trimester is routinely made with fetal biometric parameters (FBP) such as femur length (FL), biparietal diameter (BPD), head circumference (HC), and abdomen circumference (AC) (4-8). These FBP can be affected by fetal development disorders and skeletal anomalies (9-11).

Transcerebellar diameter (TCD) is considered one of the new and reliable ultrasound parameters that attract the attention of researchers, especially for the estimation of GA in early pregnancy. This is due to the progressive growth of the cerebellum throughout the entire pregnancy period and to little effect from growth restrictions (4,5).

A considerable number of pregnant women who can't remember their LMPs are admitted to the hospital for the first time in the second-trimester. For this reason, accurately determining the GA of the fetus in this period is a challenge encountered in routine practice. Additively, ultrasonography is performed for anomaly scan between 18-24 weeks according to LMP in the second-trimester in Turkey.

In the light of all of these, we evaluated the role of TCD as an independent parameter for estimation of GA in the second-trimester and its diagnostic performance compared to routine FBP.

Materials and Methods

This cross-sectional retrospective study was conducted by the principles of the Declaration of Helsinki. Procedures were thoroughly explained to all participants and their informed consent was obtained. The ethics committee of Muğla Sıtkı Koçman University approved this study (protocol no: 210049, date: 18.01.2022).

In this study, we evaluated 120 healthy women between 19 to 24 weeks of gestation with normal singleton pregnancies without any risk factors who applied to our radiology department (Menteşe State Hospital) for the second-trimester anomaly scan during the one year between December 2020-2021. Only women aged 18-40 years, who were sure of their LMP, or who had a gestational dating scan up to 14 weeks, were included. We excluded women who have medical comorbidities such as heart disease, diabetes mellitus, chronic hypertension, gestational hypertension, and anti-phospholipid antibody syndrome, pregnancy-related pathologies such as oligohydramnios, polyhydramnios, congenital malformations and intrauterine growth restriction (IUGR). Also, pregnancies with a difference of more than 2 weeks between early pregnancy gestational dating screening and LMP (if performed) were excluded. All images and other information about the pregnancy were accessed from the hospital information system.

Ultrasound examinations were obtained using the Mindray DC-8 Expert (Shenzhen Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, China) ultrasound system with 3.2 MHz curvilinear probe (SC5-1E). After the pregnant women were placed on the examination table in the supine position, the second-trimester

anomaly scan was performed by one radiologist with 4 years of experience. Routine FBP were measured using the standard method (12).

From the plane of the BPD, the probe was moved slightly below the transventricular plane to show the septum pellucidum anteriorly, defining the thalamus centrally, while the cerebellum and cisterna magna were viewed posteriorly in the transcerebellar plane (13,14). This plane gives the widest TCD. The measurement of TCD was done by placing the on-screen caliper at the outer margins of the cerebellum (Figure 1) (15). GA was obtained within weeks for measured routine FBP, and TCD, as determined by the embedded software in the Mindray ultrasound scanner based on Hadlock et al.'s (16) and Snijders and Nicolaides et al.'s (17) nomograms. In addition, the measured TCD value (mm) was also noted.

Statistical Analyses

Data were analyzed using the SPSS (v.22 software for windows). Data normality was evaluated with the Kolmogorov-Smirnov test. While, Student t-test and One-Way ANOVA test were used for the parametric data, Kruskal-Wallis test was used for the non-parametric data. TCD, BPD, HC, AC, and FL values were compared according to GA using Pearson correlation. The coefficient of determination (R^2) was calculated. Univariate linear regression was used to identify significant predictors of GA and its relationship with TCD. P<0.05 was considered statistically significant.

Results

The mean age of the women was found 28.35±4.66 years (between 18-40). GAs of them were between 19-24 weeks according to their LMPs. The mean week of gestation was 21.04±2.1. Six groups were obtained according to gestational week. There was no significant relationship between GA and the age of the women.

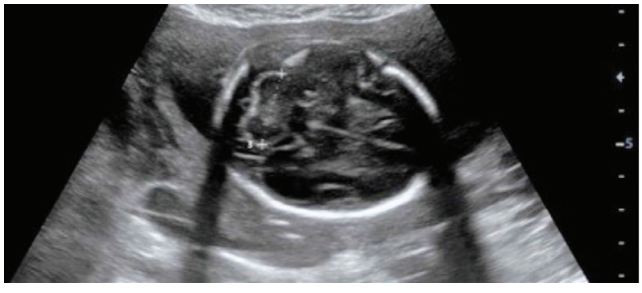

Figure 1. TCD measurement method
TCD: Transcerebellar diameter

The mean BPD, HC, AC, FL, and TCD weeks which were calculated with nomogram and TCD values (mm) were 21.39±0.99, 21.36±0.98, 21.28±0.95, 21.24±1, 20.11±0.73, 21.21±1.09, respectively. The mean values of BPD, HC, AC, FL, TCD value (mm) and the estimated TCD week divided into weeks according to GA calculated with LMP were given and Kruskal-Wallis analysis showed that all FBP increased with advancing GA as the fetus grows (p<0.05) (Table 1).

The GA and all parameters showed a strong correlation. FL showed the strongest correlation with GA (p<0.05) (Table 2). Strong correlations were detected between both TCD values (mm) and weeks with other FBP (p<0.05). TCD value (mm) showed the strongest correlation with BPD (Table 3). Scatter diagrams were obtained to show the relationship between GA and other parameters (Figure 2). We found a formulation using linear regression analysis to estimate GA: TCD(mm)x(0.783+4.947).

Discussion

Accurate estimation of GA is a crucial point for clinicians for the management of pregnancies, and for planning a normal delivery or elective cesarean section. Since a significant number of pregnant women don't have any information about their LMPs and therefore the gestational week cannot be determined in the second-trimester, it becomes difficult to comment on conditions such as pregnancy progression, and fetal macrosomia or IUGR. Some inconsistencies may arise for each of the FBP in the determination of GA in the second-trimester. In the third-trimester, the differences may increase for more than 2-3 weeks (5,18). It is also known that routine FBP can be affected by fetal development disorders and skeletal anomalies (9-11). Therefore, a simple, reliable, independent parameter is needed for the accurate estimation of GA.

The cerebellum, consisting of a median part called the vermis and two hemispheres are surrounded by temporal and occipital bone in the posterior fossa. This anatomical position provides that it is less affected by external pressure (4,19,20). It is stated that the formation of the cerebellum, which can be seen ultrasonographically in the earliest 10-11th weeks

Table 1. Mean values and statistical analysis of BPD, HC, AC, FL, TCD (weeks) and TCD (mm) divided by weeks according to GA

GA*	n	BPD*			HC*			AC*			FL*			TCD (mm)			TCD*		
		Mean	SD	Mean-rank	Mean	SD	Mean-rank	Mean	SD	Mean-rank	Mean	SD	Mean-rank	Mean	SD	Mean-rank	Mean	SD	Mean-rank
19	5	19.66	0.54	8	19.57	0.53	6.9	19.71	0.49	9.4	19.6	0.34	8.1	19.4	0.44	7.6	18.89	0.33	8.1
20	26	20.54	0.87	29.4	20.48	0.82	28.42	20.42	0.81	28.4	20.30	0.80	27.96	20.23	0.73	28.44	19.47	0.52	28.73
21	46	21.37	0.56	58.98	21.31	0.57	56.12	21.16	0.50	53.98	21.17	0.53	55.41	21.10	0.50	55.41	20.04	0.35	55.74
22	33	21.92	0.62	81.55	21.98	0.51	86.77	21.92	0.47	88.59	21.87	0.49	87.86	21.87	0.71	86.56	20.52	0.48	85.68
23	7	22.67	0.75	103.21	22.76	0.75	105.14	22.65	0.59	108.64	22.51	0.70	103.21	22.73	0.99	103.5	21.14	0.67	104.14
24	3	23.14	1.08	109.67	22.67	0.97	101.83	22.91	1.33	102.5	23.33	1.41	107.17	23.9	0.79	117.5	21.81	0.46	117.33
Kruskal-Wallis Test		x^2:60.07			x^2:69.65			x^2:74.11			x^2:71.77			x^2:70.89			x^2:72.08		
		p<0.001			p<0.001			p<0.001			p<0.001			p<0.001			p<0.001		

SD: Standard deviation, GA: Gestational age, BPD: Biparietal diameter, HC: Head circumference, AC: Abdomen circumference, FL: Femur length, TCD: Transcerebellar diameter, *week

of pregnancy, is completed in the 15th week (4,21,22). However, some studies evaluating the closure of the vermis, have shown that the vermis is open at a rate of 13% at 16 weeks of gestation and even the posterior-inferior surface of the vermis can be found to be open until half of the 17th gestational week. For this reason, these studies suggest that cerebellum examinations should be performed after the 16th week (23). In the literature, sonographic examination of TCD reveals a linear relationship with GA in the second-trimester. Measurements in millimeters are approximately equal or close to GA in weeks. The cerebellar growth curve tends to flatten in the later stages of pregnancy (5,20).

In our study, the TCD values (mm) of 120 fetuses (between 19-24 weeks) ranged from 19.40 to 23.90 mm (mean 21.21±1.09 mm). Desdicioglu et al. (24) reported that the average TCD values according to the weeks ranged from 19.89 to 26.42 mm (1,124 pregnant cases between 19-24 weeks were evaluated). Göynümer et al. (22) found that TCD ranged between 18.86 and 25.29 mm in 586 pregnant women between 19-24 weeks of gestation. Although the TCD values in these two studies were almost similar to the current study, numerical differences are striking, especially towards the end of the second-trimester. Considering that only 8.3% of the cases in our study included pregnant women at 23rd and 24th weeks, this was thought to be the reason for the difference from other studies. When our three cases at 24 weeks of gestation were examined in detail, it is seen that TCD values were 23.0 mm, 24.2 mm, and 24.5 mm, respectively. We found that the mean TCD week was 20.11±0.73, and similar to other studies we showed that the mean TCD week increased in parallel with the increase in gestational weeks. In a study performed by Reddy et al. (4), with 50 pregnant women between 15 and 28 weeks, the mean TCD week in the second-trimester was found to be 21.12±4.45.

In our study, the highest correlation for GA estimation was shown with FL (r=0.858), followed by AC (r=0.843), TCD values (mm) (r=0.834), and TCD week (r=0.822), respectively. All "r" values indicated strong correlation and ranged from 0.794 to 0.858. All parameters used in the detection of GA in the second-trimester were statistically significant (p<0.001) and were compatible with the literature in this respect. Also, when the correlation of TCD values with routine FBP was evaluated, we found that the

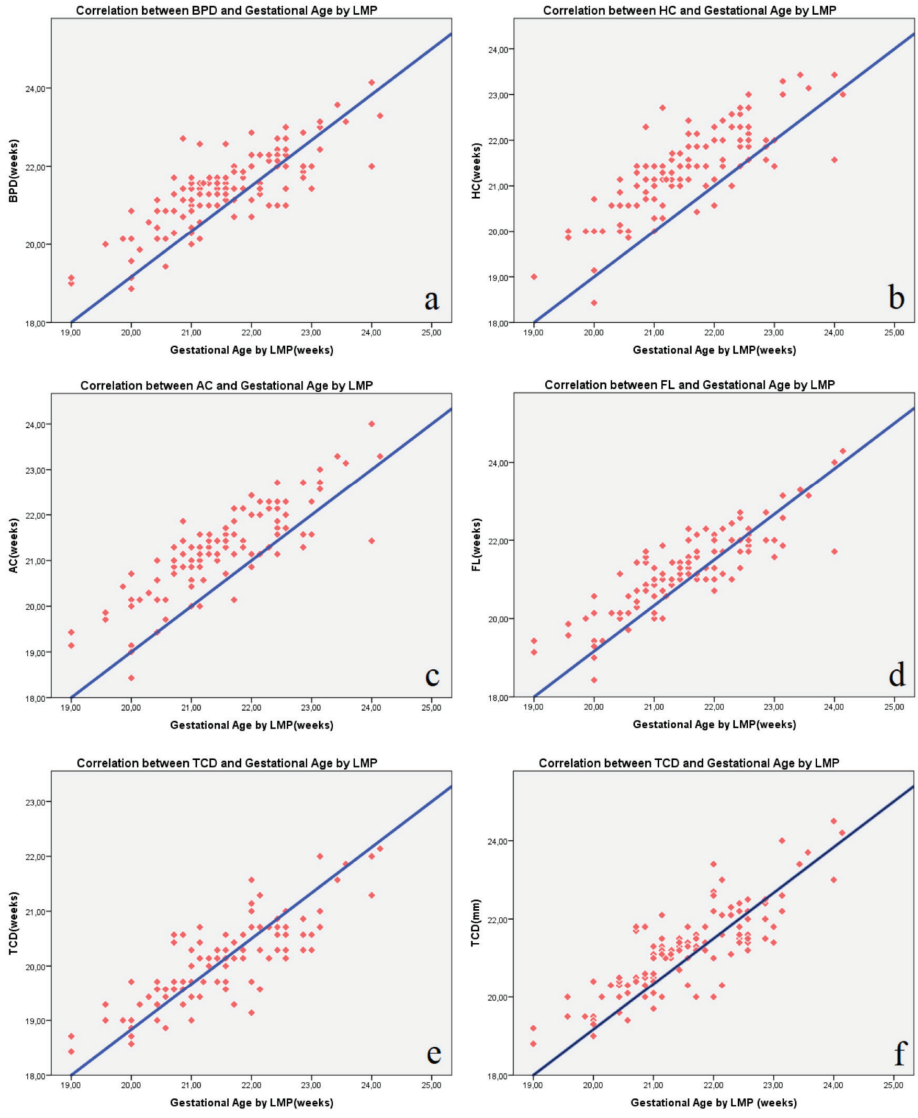

Figure 2. Scatter diagrams showing a linear correlation between the GA and the estimated GA by the BPD (a), HC (b), AC (c), FL (d), and TCD week (e). There is also a linear correlation between GA and measured TCD values (mm) (f)
GA: Gestational age, BPD: Biparietal diameter, HC: Head circumference, AC: Abdomen circumference, FL: Femur length, TCD: Transcerebellar diameter

best correlation was with BPD (r=0.884; p<0.001). Göynümer et al. (22) defined that the TCD values high correlated with GA and HC, similar to ours. Desdicioglu et al. (24) found that the TCD values was strong correlated with FL (r=0.881; p<0.01) and GA (r=0.802; p<0.01). In the study of Reddy et al. (4), the highest correlation for GA prediction in the second-trimester (between 15-28 weeks) was shown with the TCD week (r=0.998), followed by FL (r=0.997) and HC (r=0.997), respectively. In many studies conducted in the third-trimester, it was found that the TCD week and TCD values (mm) showed a higher correlation for GA prediction than other FBP (4-6,20). The highest correlation between GA and FL in current study may be explained by the lower rate of inconsistencies that occur in measurements made with routine FBP in the second-trimester compared to third-trimester. The fact that 91.6% of the pregnant women in our research population are in the early weeks of the second-trimester (weeks 19-22), supports this situation. Many second-trimester studies stated the correlation between GA and TCD were higher than our results

(4,22,24). We think that this was because other studies were nomogram studies, and their sample size was higher and more homogenous than the present study. Also, a strong correlation was found between TCD and other FBP [BPD had the highest correlation (R^2=0.78, p<0,001)].

The correlation between the TCD value (mm) and the TCD week was found to be very strong (R^2=0.98, p<0.001). The TCD values correlated more strongly with GA than with the TCD week (r=0.834 vs. r=0.822, p<0.001). Göynümer et al. (22) showed that TCD values have different percentile values over 22 weeks compared to other parameters in the Turkish population, and the variation increased when compared with other nomograms in the literature. We think that the correlation differences between the device-dependent algorithm in calculating the GA depending on TCD week and TCD values, may be related to this in our study. This finding suggests that nomograms may need to be updated based on countries' populations. In a nomogram analysis study by Chavez et al. (25), the agreement between the TCD values and the TCD week calculated with the nomograms was found to be quite high (R^2=0.94; p<.001). It has been shown that this correlation is superior in the second-trimester compared to the third trimester. This shows TCD values (mm) have a high success rate at the second-trimester level in estimating the gestational week directly. In the light of the literature and our data, we can say that TCD values measured in millimeters are almost equal to the week of gestation in the second-trimester.

Our study had some limitations. Firstly, the heterogeneity of the case distribution and the presence of a small sample size, especially at weeks over 23th. Therefore, further studies with large samples may be needed to support our findings. Secondly, since fetuses with normal development without gender discrimination were included in our study, no comparison was made regarding gender. The inclusion of only normal pregnancies in the study should be kept in mind as another limitation. Finally, a small proportion of pregnant women had not an age-confirming ultrasound during the first-trimester.

Table 2. Correlations of different ultrasonographic FBP with GA in the second-trimester

FBP	Compared with GA*		
	r	R^2	p-value
BPD*	0.794	0.630	<0.001
HC*	0.822	0.676	<0.001
AC*	0.843	0.711	<0.001
FL*	0.858	0.736	<0.001
TCD*	0.822	0.676	<0.001
TCD (mm)	0.834	0.696	<0.001

GA: Gestational age, BPD: Biparietal diameter, HC: Head circumference, AC: Abdomen circumference, FL: Femur length, TCD: Transcerebellar diameter, FBP:Fetal biometric parameters, *week

Table 3. Correlations of different ultrasonographic FBP with TCD values (mm) in the second-trimester

FBP (week)	Compared with TCD (mm)		
	r	R^2	p-value
BPD	0.884	0.781	<0.001
HC	0.881	0.776	<0.001
AC	0.876	0.767	<0.001
FL	0.869	0.755	<0.001
TCD	0.993	0.986	<0.001

TCD: Transcerebellar diameter, BPD: Biparietal diameter, HC: Head circumference, AC: Abdomen circumference, FL: Femur length, FBP: Fetal biometric parameters

Conclusion

TCD provides useful and consistent results in the detection of GA, especially in the second-trimester. Therefore, TCD measurements in anomaly screening will increase the success in estimating GA. Large-scale data obtained by multicenter evaluation of more fetuses and comparing sex, ethnic group, normal and abnormal fetuses will provide more precise results.

Ethics

Ethics Committee Approval: The ethics committee of Muğla Sıtkı Koçman University approved this study (protocol no: 210049, date: 18.01.2022).

Informed Consent: Procedures were thoroughly explained to all participants and their informed consent was obtained.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

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

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Original Article / Özgün Araştırma

The Effect of Paracetamol on Postoperative Nausea and Vomiting in Patients Undergoing Maxillofacial Surgery Under General Anesthesia

Genel Anestezi Altında Maksillofasiyal Cerrahi Geçiren Hastalardaki Postoperatif Bulantı ve Kusma Üzerine Parasetamolün Etkisi

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Keywords
Postoperative nausea and vomiting, paracetamol, maxillofacial surgery, antiemetics

Anahtar Kelimeler
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Abstract

Objective: The management of postoperative nausea and vomiting (PONV), a frequent issue, has included various medications. Maxillofacial surgeries are in the high-risk surgical group for PONV. This study evaluated the efficacy of paracetamol on PONV in adults undergoing maxillofacial surgery.

Materials and Methods: All patient files who underwent elective-maxillofacial-surgery under standard general anesthesia procedures between January 2016 and September 2021 were reviewed. The patients who received paracetamol infusion (i.v. paracetamol 1.5 mL/kg) as an additional analgesics in the recovery room were defined as the paracetamol group; patients who did not use additional analgesics were defined as the control group. The postoperative 0-4 and 4-24 h were defined as the early and late postoperative period, respectively. All episodes of PONV occurring within 24 h after general anesthesia were recorded in the patient files. Antiemetic drug use, postoperative pain and analgesic needs in the early and late postoperative periods were recorded in the first 24 h after surgery.

Results: The incidence of PONV during 0-4 h postoperatively was significantly higher in the control group compared with the paracetamol group (p=0.034 for nausea; p=0.030 for vomiting). The need for rescue antiemetic drug during 0-4 h postoperatively was significantly higher in the control group compared with the paracetamol group (p=0.013). There were no differences among the groups in terms of pain levels during the 24 h postoperatively.

Conclusion: Early period after maxillofacial surgery, the incidence of PONV is decreased by the use of intravenously paracetamol. Paracetamol may help prevent PONV.

Öz

Amaç: Sık görülen bir sorun olan postoperatif bulantı kusmanın (PONV) önlenmesinde hem yüksek etkili hem de yan etkisi düşük ilaçlar tercih edilmektedir. Maksillofasiyal cerrahiler PONV açısından yüksek riskli cerrahi grubundadır. Bu retrospektif çalışmayla, maksillofasiyal cerrahi geçiren erişkinlerde parasetamolün PONV üzerindeki etkinliğini değerlendirebilmek amaçlandı.

Gereç ve Yöntemler: Ocak 2016-Eylül 2021 tarihleri arasında standart genel anestezi altında elektif maksillofasiyal cerrahi uygulanan tüm hasta dosyaları incelendi. Derlenme odasında ek analjezik olarak parasetamol infüzyonu (i.v. parasetamol 1,5 mL/kg) alan hastalar parasetamol grubunu oluştururken, herhangi bir ek analjezik kullanmayan hastalar kontrol grubu olarak tanımlandı. Ameliyat sonrası 0-4 saat ve 4-24 saat sırasıyla erken ve geç postoperatif dönem olarak

belirlendi. Genel anestezi sonrası 24 saat içinde ortaya çıkan tüm bulantı kusma atakları hasta dosyalarından bakılarak kaydedildi. Ayrıca ameliyat sonrası ilk 24 saatte antiemetik ilaç kullanımı, postoperatif ağrı ve postoperatif erken ve geç dönemdeki analjezik ihtiyaçları kaydedildi.

Bulgular: Erken postoperatif dönem (0-4 saat) PONV insidansı, parasetamol grubu ile karşılaştırıldığında kontrol grubunda anlamlı olarak daha yüksekti (bulantı için p=0,034; kusma için p=0,030). Yine 0-4 saat arasındaki kurtarıcı antiemetik ilaç ihtiyacı kontrol grubunda parasetamol grubuna göre daha yüksek bulundu (p=0,013). Postoperatif 24 saat boyunca ağrı düzeyleri açısından gruplar arasında fark yoktu.

Sonuç: IV parasetamol kullanımı, maksillofasiyal cerrahi sonrası erken postoperatif dönemde PONV insidansını azaltır. Parasetamol PONV'yi önlemede yardımcı olabilir.

Introduction

General anesthesia patients frequently have postoperative nausea and vomiting (PONV) (1). The length of the patient's hospital stay is increased by PONV, which also causes oral intake to be delayed. It is also the leading cause for unanticipated hospital admissions (2). It may cause patient discomfort, electrolyte abnormalities due to dehydration, aspiration pneumonia, and intracranial pressure elevation (3). As a result, efforts have been made to use medications with an antiemetic effect to lower the frequency of PONV in patients following surgery (4).

Since more than a century ago, paracetamol has been used extensively as an efficient painkiller and as an antipyretic. In contrast to other analgesics, it has a favorable safety profile and well-established efficacy and tolerance (5). Single or repeated intravenous paracetamol doses generally provided efficient postoperative analgesia following a variety of surgical procedures in several large, well-designed trials including adult patients (6). Although paracetamol promotes descending serotonergic inhibitory pathways and inhibits the cyclooxygenase enzyme, the precise mechanism behind its analgesic actions is yet understood (5,6). According to certain research (7), paracetamol may also reduce the risk of PONV by affecting a few serotonergic pathways in the central nervous system.

We thought that intraoperative intravenously (IV) paracetamol, which is used as a pain reliever in adults in maxillofacial surgeries, may be useful in the prevention of PONV. Therefore, we aimed to retrospectively evaluate the risk of PONV in adult patients who underwent maxillofacial surgery with paracetamol used during the recovery period compared to those who did not use paracetamol.

Materials and Methods

This retrospective clinical study was approved by the Aydın Adnan Menderes University Institute of Health Sciences Clinical Research Ethics Committee (protocol no: 2021/038, date: 27.10.2021). In accordance with the Declaration of Helsinki, American Society of Anesthesiologists classification (ASA) physical status I-II eighty patients between the ages of 18-50, who underwent elective maxillofacial surgery (orthognathic surgery with maxillary and/or mandibular osteotomies) under general anesthesia between January 2016 and September 2021 were included in the study.

Patients with a history of opioid use, liver or kidney disease, coronary, psychotic and neurological diseases, those with a history of antiemetic, antihistamine, analgesic or corticosteroid use in the last 24 hours (h) before surgery, those who did not receive standard general anesthesia, those who were administered antiemetics at the end of surgery, those who received opioids in the postoperative period and those with missing information in their files was excluded.

A standard general anesthesia procedure was applied to all patients included in the study. As a standard general anesthesia procedure; after performing routine monitoring in the operating room, such as pulse oximetry, non-invasive arterial pressure monitoring, and electrocardiography, anesthesia was induced using propofol 2 mg/kg, remifentanil 1 g/kg, and rocuronium bromide 0.6 mg/kg. A nasotracheal tube was used to intubate all of the patients. 1.5-2% sevoflurane, 50% oxygen in the air, and an infusion of remifentanyl at 0.5-1 g/kg/min were used to maintain anesthesia. All patients received tenoxicam 0.3 mg/kg at the conclusion of surgery for analgesic and anti-inflammatory effects. The patients were extubated after the procedure, taken to the post-anesthesia care unit (PACU), and then released to the ward after being

under monitoring there. At the end of the follow-up in PACU, 50 mg IV dexketoprofen trometamol was used as rescue analgesia for postoperative pain control at visual analogue scale (VAS) 3 and above.

All patient files were reviewed. Patients who received paracetamol infusion as an additional analgesic in the recovery room were in the paracetamol group (i.v. paracetamol 1.5 mL/kg); Patients who did not use additional analgesics were divided into two groups as the control group.

Age, gender, weight, ASA, duration of anesthesia, recovery time data related to the patient and the surgical procedure were recorded from the patient files.

The primary goal of this study was to assess the effect of paracetamol on the incidence of nausea and vomiting in the first 24 h after surgery, while also investigating postoperative pain during the first 24 h. Early and late postoperative periods were defined as 0-4 h and 4-24 h, respectively. PONV was identified when there was a recording of vomiting or when actions suggestive of nausea, such as repeated gagging or spitting, were observed within 24 h of general anaesthesia. All episodes of nausea and vomiting occurring within 24 h of anesthesia were noted from the patient files. Antiemetic drug use in the early and late postoperative period was recorded in the first 24 h after surgery. In the event of two or more vomiting episodes, metoclopramide (0.2 mg/kg) was injected IV as a rescue antiemetic.

During the first 24 h after surgery, postoperative pain at rest was assessed using a standard 10 cm VAS (0 cm = no pain, 10 cm = worst pain imaginable). Hourly VAS values in patient files were recorded by taking the mean of the early and late postoperative period. The presence of analgesic need was recorded.

Statistical Analysis

The incidence of nausea and vomiting within the first 24 h postoperatively was the study's main finding. The IBM-SPSS Statistics program was used to conduct the statistical analyses. A 0.05 p-value was regarded as statistically significant. Depending on the parameter distribution, either parametric or non-parametric tests were used to assess the values of the two groups. Kolmogorov-Smirnov and Shapiro-Wilk tests were used to determine whether the data were normal. For quantitative variables, the results are presented as mean standard deviation, and for categorical variables, as absolute frequency and %. Quantitative variables were compared using the independent sample t-test, while categorical variables were compared using the chi-square test or Fisher's Exact test.

Results

A total of 80 patients were analyzed (paracetamol group, n=40; control group, n=40) (Figure 1). The patients' characteristics including gender, age, weight, duration of anaesthesia and ASA status were similar in both groups (Table 1).

The total incidence of nausea and vomiting in all patients in the first 4 h after surgery was 35% and 20% respectively, and the total incidence of nausea and vomiting in postoperative 4-24 h was 21.3% and 6.3%, respectively.

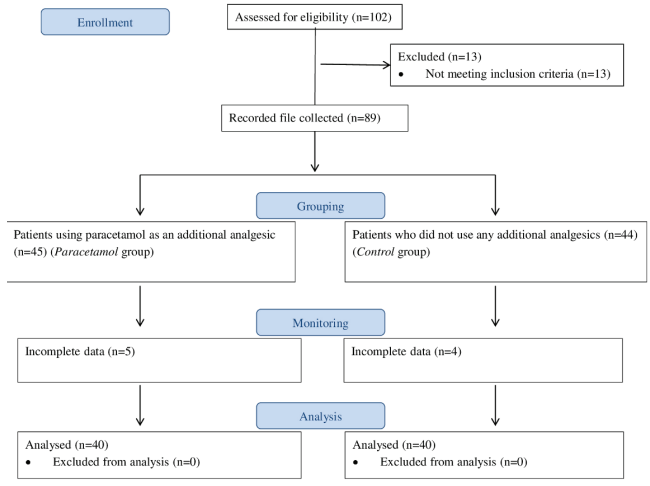

Figure 1. CONSORT flow diagram. The course of patients through this study was shown
CONSORT: Consolidated Standards of Reporting Trials

Table 1. General and clinical data of the patients

	Paracetamol group (n=40)	Control group (n=40)	p-value
Age (years)	32.62±10.66	36.85±11.43	0.091
Weight (kg)	70.97±12.06	69.72±10.34	0.620
Sex (M/F)	19/21	24/16	0.370
ASA (I/II)	27/13	22/18	0.359
Duration of anaesthesia (min)	195.87±84.00	185.12±75.23	0.548

Data are mean ± SD and numbers; F: Female, M: Male, p<0.05. ASA: American Society of Anesthesiologists classification, SD: Standard deviation

PONV was significantly more common in the control group than in the paracetamol group during the first 4 h postoperatively (p=0.034 for nausea; p=0.030 for vomiting). The frequency of nausea and vomiting was lower in the paracetamol group than in the control group during the first 4 h postoperatively (p=0.034 for nausea frequency; p=0.030 for vomiting frequency) (Table 2). The need for rescue antiemetic medication was significantly higher in the control group compared to the paracetamol group (p=0.013) (Table 3).

There are no differences in pain levels between groups during the first 24 h after surgery (Table 4). No patient in either group experienced a delay in receiving their hospital release, and no patient was ever readmitted because of PONV.

Discussion

In this retrospective study, the efficacy of IV paracetamol administrations for the prevention of PONV was investigated in patients undergoing maxillofacial surgery. Our study is the first to evaluate the effect of IV paracetamol on PONV in patients undergoing maxillofacial surgery. This study was demonstrated that IV paracetamol was effective in preventing PONV in the early postoperative period (0-4 h).

The reported incidence of PONV is generally reported to be around 20% to 30% for all surgical types. However, in high-risk surgeries for PONV, the incidence of PONV is reported to be up to 80% (8-11). Maxillofacial surgeries are among the predisposing surgeries to PONV. Oropharynx iritation, blood in the stomach, a changed diet, and hypotension throughout the recovery phase can all be used to explain this (8,11,12). However, only a small number of studies (8,12,13) have shown a greater prevalence of PONV following oral and maxillofacial surgery. The most frequent reason for postoperative complications in oral and maxillofacial operations, according to Perrott

Table 2. Postoperative nausea and vomiting during 24-h postoperatively

		Paracetamol group (n=40)	Control group (n=40)	p-value
0-4 h	Number of patients with nausea	9 (22.5)	19 (47.5)	0.034*
	Frequency of nausea	0.37±0.74	0.95±1.25	0.015*
	Number of patients with vomiting	5 (12.5)	11 (27.5)	0.030*
	Frequency of vomiting	0.17±0.54	0.52±0.87	0.036*
4-24 h	Number of patients with nausea	7 (17.5)	10 (25.0)	0.586
	Frequency of nausea	0.22±0.53	0.40±0.81	0.257
	Number of patients with vomiting	3 (7.5)	2 (5.0)	0.644
	Frequency of vomiting	0.07±0.26	0.07±0.34	0.952

Data are numbers (percentages); *p<0.05

Table 3. The need for rescue antiemetics during 24-h postoperatively

	Paracetamol group (n=40)		Control group (n=40)	p-value
Need for rescue antiemetics	0-4 h (+/-)	2 (5.0)	11 (27.5)	0.013*
	4-24 h (+/-)	3 (0)	4 (2.2)	0.692

Data are numbers (percentages); *p<0.05

Table 4. Postoperative pain during 24-h postoperatively

		Paracetamol group (n=40)	Control group (n=40)	p-value
Postoperative pain (VAS) score	0-4 h	2.47 (±1.75)	2.30 (±1.57)	0.640
	4-24 h	1.12 (±1.41)	1.27 (±1.43)	0.639
Need for rescue analgesics	0-4 h	17 (42.5)	17 (42.5)	1.000
	4-24 h	14 (35)	18 (45)	0.494

Data are mean ± SD; VAS: Visual analog scale, SD: Standard deviation, *p<0.05

et al., (12) was PONV. Dobbeleir et al. (8) found a PONV incidence as high as 46.1% after maxillofacial surgery in postoperative 3-day follow-up. Apipan et al. (11) reported that the overall incidence of PONV was 25.3% in patients of all ages who had undergone oral and maxillofacial surgery. Similarly, Alexander et al. (14) reported a PONV incidence of 11.3% in patients who have undergone maxillofacial surgery in all age groups during the postoperative 6 h, while Silva et al. (13) recounted a 40.08% incidence of PONV in patients over 14 years of age during the first 24 h postoperatively. We found the incidence of nausea and vomiting in all patients in the first 4 h postoperatively to be 35% and 20%, respectively, while the incidence of nausea and vomiting in postoperative 4-24 h was 21.3% and 6.3%, respectively. This high variability of PONV incidence may be due to differences in age and follow-up time.

It is generally known that paracetamol is a secure analgesic. It has been observed that paracetamol administered IV is efficient and secure for postoperative analgesia. Recently, paracetamol has been mentioned in its antiemetic activity as well as its analgesic effect (15). Many studies in head and neck surgeries such as thyroidectomy and tonsillectomy have reported preventive effects of paracetamol on nausea and vomiting (7,16). However, some studies have reported that paracetamol does not contribute to the prevention of PONV (17-19). It can be due to variations in treatment timing. Only when given prophylactically, either before surgery or while it is being performed, has intravenous acetaminophen been demonstrated to be beneficial for treating both nausea and vomiting. Studies have demonstrated that paracetamol inhibits the cyclooxygenase enzyme and affects various serotonergic pathways in the central nervous system, despite the fact that the analgesic activities of paracetamol have unknown mechanisms (7). According to Cok et al., (7) intraoperative IV paracetamol treatment reduces the likelihood of PONV in children for the first 24 h following strabismus surgery. The brainstem vomiting center contains serotonin. Anandamide reuptake is inhibited by AM404, a byproduct of paracetamol metabolism in the brain (20). In humans, it was discovered that decreased anandamide levels were linked to a high prevalence of nausea and vomiting (21). This may be yet additional explanation for paracetamol's antiemetic properties. Although paracetamol has not yet been shown to bind to receptors, recent research suggests that it indirectly modifies the serotonergic system (22). Paracetamol may interfere with the serotonergic system through the EP3 receptor, a crucial prostaglandin E2 receptor found in the majority of serotonergic cells in the medulla oblongata (23). Another putative mechanism of action is an increase in cannabinoid tone, which may assist in lowering vomiting brought on by the chemoreceptor trigger zone (22).

High dose antiemetics may cause minor side effects such as restlessness, dizziness, drowsiness, headache, constipation and diarrhoea. The therapeutic use of antiemetics in the prevention of PONV is, however, constrained by concern over extrapyramidal side effects. As an example, tardive dyskinesia and dystonic responses are uncommon side effects that are typically observed in individuals who are taking high doses of antiemetics (24). Analgesics are routinely used in all surgical procedures for postoperative patient comfort. When paracetamol was thought to demonstrate both analgesic and antiemetic activity, the use of paracetamol is of great importance, especially for surgical procedures with PONV risk, in avoiding side effects of antiemetic drugs, unnecessary drug use and cost effectiveness.

Previous research has shown that using intraoperative paracetamol reduces postoperative pain in a variety of surgeries (15,25). Some studies have also found that IV paracetamol administration after surgery has an antiemetic effect (7,15,25). This is the first study to look at the effect of IV paracetamol on PONV in patients undergoing maxillofacial surgery.

The antiemetic effect of non-steroidal anti-inflammatory drugs is widely thought to result from a dose-dependent reduction in opioid intake. However, Apfel et al. (15) associated the decrease in PONV with the reduction in pain intensity, regardless of the decrease in opioid consumption and showed that pain itself is a risk factor for PONV. We included patients who followed an opioid-free protocol for postoperative pain control. Thus, we were able to clearly evaluate the effectiveness of paracetamol by excluding the negative effect of opioid on PONV.

The pathophysiology of nausea and vomiting is extremely complicated and involves numerous central nervous system regions. Vomiting is a

brainstem reaction, whereas nausea is controlled in the cortex (9,10). According to several studies, postoperative analgesia that is effective may stop the stimulus for PONV from starting, and sufficient pain treatment eliminates 80% of these symptoms (7). Postoperative pain is a risk factor for PONV (15). However, according to our findings, while there was no difference between the groups in terms of pain in the early postoperative period, a significant difference was found in terms of PONV. Therefore, we think that the decrease in PONV incidence in the first 0-4 h after surgery in the paracetamol group may be due to the specific antiemetic effect of paracetamol rather than it's pain reduction.

We conducted a retrospective analysis on a modest sample size. Larger sample sizes are required for prospective studies to assess paracetamol's impact on the prevalence of PONV.

Conclusion

PONV is reduced in the early postoperative period after maxillofacial surgery under general anaesthesia when IV paracetamol is used. Furthermore, paracetamol significantly reduced the need for rescue antiemetic drugs. Paracetamol, a safe and effective analgesic, may be helpful in preventing PONV as well as postoperative pain. More research is needed to demonstrate the effectiveness of paracetamol in reducing the incidence of PONV in patients undergoing maxillofacial surgery.

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Ethics

Ethics Committee Approval: This retrospective clinical study was approved by the Aydın Adnan Menderes University Institute of Health Sciences Clinical Research Ethics Committee (protocol no: 2021/038, date: 27.10.2021).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

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Original Article / Özgün Araştırma

Molecular Response Assessment in Patients with Chronic Myeloid Leukemia; Clinicopathological Retrospective Research

Kronik Miyeloid Lösemili Hastalarda Moleküler Yanıt Değerlendirmesi: Klinikopatolojik Retrospektif Bir Araştırma

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Keywords
Chronic myeloid leukemia, major molecular response, molecular follow-up, prognosis

Anahtar Kelimeler
Kronik miyeloid lösemi, majör moleküler yanıt, moleküler takip, prognoz

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Abstract

Objective: Chronic myeloid leukemia (CML) is a stem cell disease caused by clonal increase of precursor cells. In the studies conducted, it is stated that follow-up of patients has positive effects on the prognosis. In this study, it is aimed to review the molecular response assessment used in the follow-up of CML patients by sharing the clinical-pathology experience and to review the literature.
Materials and Methods: Seventy-six cases who underwent bone marrow biopsy samples assessment in Adnan Menderes University Faculty of Medicine Department of Pathology in 2018-2019 and clinically diagnosed as myeloproliferative neoplasia/CML, followed by *BCR-ABL* analysis at the 3rd, 6th and 9th months.
Results: Seventy-one (93.4%) of our cases were in chronic phase, 4 were in accelerated phase (5.3%) and 1 (1.3%) was in blastic phase. Major molecular response (MMR) was observed in 31 patients in the 3rd month (40.8%), 42 patients in the 6th month (55.3%) and 51 patients (67.1%) in the 9th month. The mean follow-up period of the patients was 20.5 months. During this period, uneventful survival was observed in 65 patients according to ELN criteria, death in 5 patients (6.6%) and relapse in 7 patients (7.9%). While the MMR observed in the early period was observed to be related to the patient's life span ($p \leq 0.05$), it was not associated with relapse ($p \geq 0.05$).
Conclusions: Achieving the MMR is important for prognosis. The importance of molecular monitoring, which is a more sensitive method for evaluating treatment effectiveness and monitoring the response, is increasing.

Öz

Amaç: Kronik miyeloid lösemi (KML), öncü hücrelerin klonal artışından kaynaklanan bir kök hücre hastalığıdır. Yapılan çalışmalarda hastaların takibinin prognoz üzerinde olumlu etkileri olduğu belirtilmektedir. Bu çalışmada klinik ve patoloji deneyimi paylaşılarak KML hastalarının takibinde kullanılan moleküler yanıt değerlendirmesinin gözden geçirilmesi ve literatürün gözden geçirilmesi amaçlanmıştır.
Gereç ve Yöntemler: 2018-2019 yıllarında Adnan Menderes Üniversitesi Tıp Fakültesi Tıbbi Patoloji Anabilim Dalı'nda kemik iliği biyopsi örneği değerlendirmesi yapılan ve klinik olarak miyeloproliferatif neoplazi/KML tanısı alan, ardından 3., 6. ve 9. aylarda *BCR-ABL* analizi yapılan 76 olgunun retrospektif olarak dosya taraması yapıldı.
Bulgular: Olgularımızın 71'i (%93,4) kronik, 4'ü akselere (%5,3) ve 1'i (%1,3) blastik fazdaydı. Otuz bir hastada (%40,8) 3. ayda, 42 hastada (%55,3) 6. ayda ve 51 hastada (%67,1) 9. ayda majör moleküler yanıt görüldü. Hastaların ortalama takip süresi 20,5

aydı. Bu dönemde European Leukemia Network kriterlerine göre 65 hastada sorunsuz sağkalım, 5 hastada (%6,6) ölüm ve 7 hastada (%7,9) nüks görüldü. Erken dönemde gözlenen majör moleküler yanıt hastanın yaşam süresi ile ilişkili iken ($p \leq 0,05$), nüks ile ilişkili değildi ($p \geq 0,05$).

Sonuç: Majör moleküler yanıt prognoz değerlendirilmesinde önemlidir. Majör moleküler yanıtın alınamadığı durumlarda ek kromozomal mutasyonlar veya direnç oluşabilir. Bu durumlarda tedavi değişiklikleri yapılarak hastanın takibine devam edilmesi gerekir. Tedavi etkinliğinin değerlendirilmesinde ve yanıtın izlenmesinde daha duyarlı bir yöntem olan moleküler izlemenin önemi giderek artmaktadır.

Introduction

Chronic myeloid leukemia (CML) is a stem cell disease caused by clonal proliferation of myeloid precursor cells. 15% of leukemias seen in adult are CML. It is more common in males (M) than females (F) (M/F: 2/1.2) (1-5).

CML was first described by Virchow and Bennetin in 1845. The discovery of the Philadelphia (Ph) chromosome by Nowel and Hungerford in 1960 provided a better understanding of the pathogenesis of the disease (6). In 1973, the broken regions t(9; 22) (q34; q11) of the chromosome were identified by Rowley (7). In 1980, this translocation has been found to cause the formation of the *BCR/ABL* fusion gene. This fusion results in the *BCR/ABL1* chimeric gene form encoding the P190 *BCR/ABL1* and P210 *BCR/ABL1* proteins, depending on the breakpoints in the *BCR*. In most of the CML cases, weights 210-kDa have increased tyrosin kinase activity p210, an oncogenic protein, is synthesized (8-11). Ph chromosome can also be detected in acute lymphoblastic leukemia (ALL) and acute myeloblastic leukemia (AML). Ph chromosome is a diagnostic factor for CML and a prognostic indicator for ALL and AML (12,13).

With the introduction of imatinib mesylate, a tyrosine kinase inhibitor (TKI), in 1998, the lifespan of these patients increased (13,14). In the follow-up of their treatment, hematological, cytogenetic and molecular responses are evaluated (13,15). Patients who develop treatment unresponsiveness or loss of response during follow-up should be detected early and effective treatment changes should be made (15). Hematological follow-up includes leukocyte, platelet count, basophil myeloblast, promyelocyte, myelocyte examination and splenic evaluation in environmental blood. Cytogenetic monitoring is done by evaluating Ph positive metaphase phases as percentage. Molecular monitoring is done by real-time polymerase chain reaction (RT-PCR). Molecular follow-up should be performed every 3 months until the major molecular response (MMR) is obtained and confirmed, then repeated in every 3-6 months (15-17).

In this study, pathology experience was shared in order to evaluate molecular follow-up in CML patients and to observe their contribution to the treatment.

Materials and Methods

Seventy-six patients with a diagnosis of myeloproliferative neoplasia (MPN)/CML were included in this study. The study protocol was approved by the Ethics Committee from Aydın Adnan Menderes University (protocol number: 2019/195, date: 23.01.2020). Bone marrow biopsy specimen evaluated and patients with who had molecular response at the consecutive 3rd, 6th and 9th month follow up were included in the study. In addition, patients with follow-up between 9-24 months and with whom we could reach clinical information were included in the study. Patients who were excluded from follow-up and could not obtain sufficient clinical information were excluded from the study. Follow-up was performed with clinical information in the form of uneventful survival, death and relapse according to the European Leukemia Network (ELN) criteria. Bone marrow biopsies were evaluated with 2 mm thick HE sections after decalcification processing and routine tissue processing. The sections were applied CD34, MPO, Glycophorin, CD117 antibodies immunohistochemically and Reticulin stain histochemically. Additional immunohistochemical stains were requested when the differential diagnosis was suspected. Sections were evaluated under a light microscope.

Total RNA isolation was performed from the peripheral blood sample of the cases. Samples were studied within 24 hours to ensure that the RNA copies were not degraded. cDNA synthesis was performed with reverse transcriptase from the total RNA obtained. BCR-ABL amplification was performed with quantitative RT-PCR method using specific primers

and probes (Ipsogen BCR-ABL1 MBcr IS-MMR kit). MMR was performed according to international scale (IS) 0.05. According to the instructions for use of the kit, IS≤0.5 MMR is present, 0.05 0,0 IS≤0.15 gray zone response is uncertain, IS≥0.15 MMR is evaluated as no response.

The demographic features, treatment and lifetimes of the cases were achieved by scanning files and sometimes by contacting clinical physicians.

Statistical Analyses

Descriptive statistics were performed in the SPSS statistics program. Data was expressed as number, percentage and mean. Kaplan-Meier method and log-rank test were used in survival analyzes. P<0.05 was considered as statistically significant.

Results

Forty four of our patients were female (57.9%) and 32 of them were male (42.1%). The patients were between the ages of 28-81 (mean 53.29). Bone marrow biopsy sample of all these cases were also evaluated. Sixty-nine patients were diagnosed with CML (90.8%) and 7 (9.2%) were diagnosed with MPN (subtype undetermined) (Figure 1). Seventy-one (93.4%) of our cases were in chronic phase, 4 were in accelerated phase (5.3%) and 1 (1.3%) was in blastic phase.

BCR-ABL was studied in the 3rd, 6th and 9th months of the cases. In the third month, MMR was observed in 31 patients (40.8%), MMR was not observed in 32 patients (42.1%), and treatment response in 13 patients (17.1%) was evaluated as gray zone uncertain. In the sixth month, 42 patients (55.3%) had MMR, 26 patients (34.2%) had no MMR, and at 8 patients (10.5%) the gray zone was reported as uncertain. In the 9th month, MMR was observed in 51 patients (67.1%), while MMR was not observed in 15 patients (19.7%), and the response was uncertain (13.2%) in 10 patients (Figure 2, 3). In 58 (76.3%) of 76 patients, 1st generation TKI were used, while in 19 (23.7%) 2nd generation TKI have been used. Due to the development of side effects in 3 of these patients, 2nd generation TKI, 15 of them were treated depending on the resistance to treatment or the desired response 2nd generation TKI was used.

The cases were followed up between 9-24 months. The mean follow-up period of the patients was 20.5 months. During this period, uneventful survival was observed in 65 patients according to ELN criteria, death in 5 patients (6.6%) and relapse in 7 patients (7.9%). Total survival rate is 93.42%.

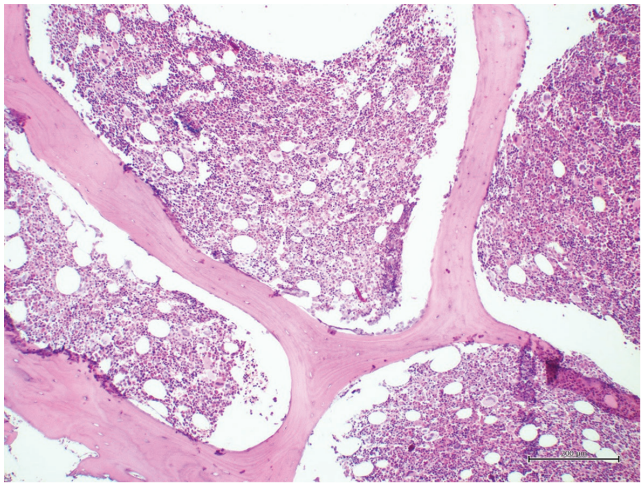


Figure 1. CML in bone marrow biopsy H&EX200
CML: Choronic myeloid leukemia

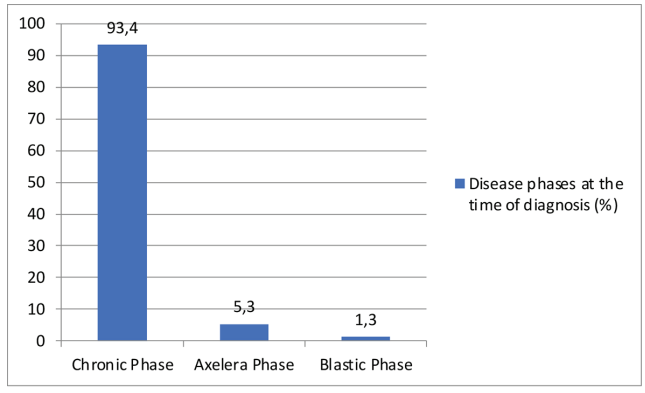


Figure 2. Disease phases at the time of diagnosis of cases

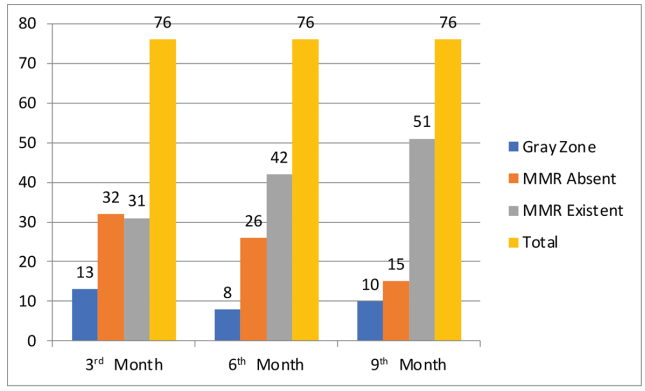


Figure 3. MMR distribution of cases
MMR: Major molecular response

None of the 5 cases who died in the third and 6th month follow-up were found to have MMR in the ninth month follow-up, 3 of 5 death cases were not followed by MMR, 1 was followed by MMR, and 1 was evaluated as an uncertain gray zone. The survival times of those with and without MMR during the 3rd, 6th and 9th month follow-up are shown in the Kaplan-Meier graphs (Figure 4, 5). In the 3rd month follow up of 7 cases who relapsed, 3 cases had no MMR, 2 cases had MMR, and 2 cases were reported as uncertain gray zone. In the 6th and 9-month follow-ups, 3 cases had MMR, 3 cases had no MMR and 1 was reported as gray zone.

Discussion

Analysis by molecular methods is an important part of pathology laboratories (18). In our country, molecular units are spreading rapidly in pathology laboratories in recent years. In this article, we wanted to present MMR follow-up as a pathology experience and emphasize the importance of the clinicopathological approach in the diagnosis as well as in the follow-up of the patient.

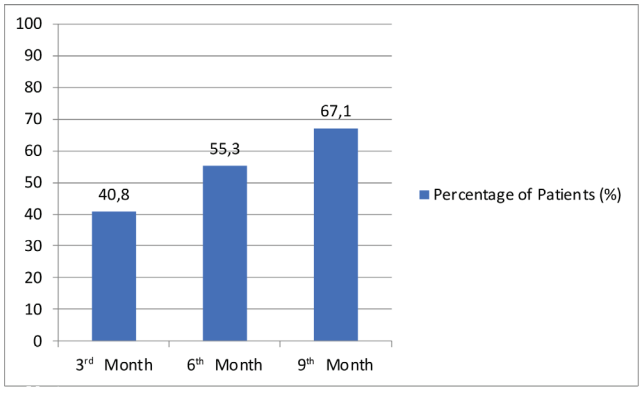
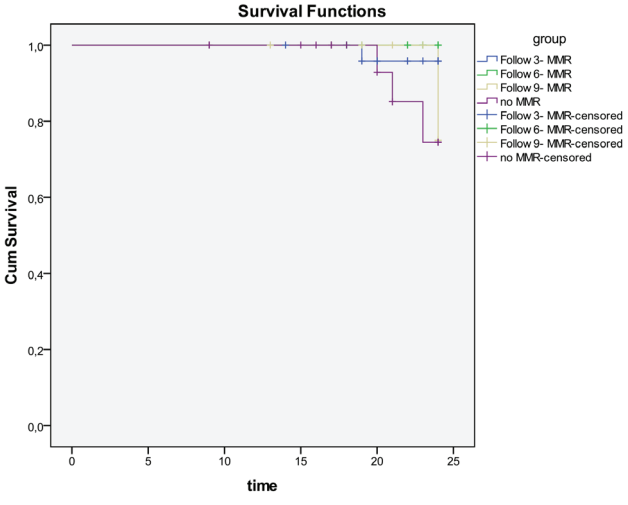


Figure 4. MMR rates of cases by months
MMR: Major molecular response



Figures 5. Evaluation of uneventful survival according to MMR (p≤0.05)
MMR: Major molecular response

In addition to the diagnosis and treatment of CML, the Ph chromosome also provided a better understanding of the pathophysiology and molecular biology of the disease (3,4,6,19). Ph chromosome arises as a result of reciprocal translocation. This translocation occurs as a result of the fusion of the ABL1 (Abelson) protooncogene in the 9th chromosome and the *BCR* gene located in the 22nd chromosome. Compared to the normal *ABL* gene, the *BCR-ABL1* hybrid gene synthesizes a chimeric fusion protein with high tyrosine kinase activity. As a result of the fusion of these two genes, the c-ABL protooncogene is activated (4,14,15). Oncogenic *BCR-ABL1* proteins affect cell proliferation, adhesion, migration and DNA repair mechanisms by altering various signal pathways (4,14,19,20).

It is more common in males than females (M/F: 2/1.2) (1,3,4,14,20). However, in this series, this ratio was reversed, it was observed more in women (M/F: 1/1.3). This rate may have been due to regional characteristics. Since patients without clinical follow-up are excluded from the study, it may be because the female patients are more compatible with the follow-up.

CML progresses in chronic, accelerated and blastic stages. Most of the patients (about 85%) are in chronic stage at the time of diagnosis. Seventy-one (93.4%) of our cases were in chronic phase, 4 were in accelerated phase (5.3%) and 1 (1.3%) was in blastic phase. The findings are consistent with the literature (1,3,4,14,15,19,20).

During the treatment, it is necessary to improve the quality of life of patients, to better treat patients who do not get a response or have a loss of response over time, and to prevent further stages.

CML has been the first and the most successful example of targeted therapies in hemato-oncological diseases with the use of TKI (3,4,15,21). The treatment response criteria recommended by the ELN, the National Comprehensive Cancer Network and the Turkish Society of Hematology (THD) should be applied for the follow-up of treatment. THD treatment

recommendations are also applied in the hematology clinic (15,22).

Molecular response tracking is done by RT-PCR. Molecular follow-up is important in the treatment follow-up of the CML patient since a patient with a complete cytogenetic response may still have leukemia cells (15,16,17,23). It should be repeated every 3 months until MMR is obtained, then it should be repeated every 6 months unless there is a loss of response during treatment (15,16). It is practical and easy to follow the molecular margins from bone marrow sampling. In the follow-up of the disease, it is more sensitive than cytogenetic follow-up in order to evaluate the minimal level of disease. Furthermore, molecular follow-up according to IS has ensured standardization by eliminating differences between laboratory evaluations (15,16,23). MMR (Ipsogen *BCR-ABL1* Mbcr IS-MMR kit) kit is used in department of pathology. There are publications about the positive effects of early detection of MMR on prognosis in cases (17,23,24). In the third month, MMR was observed in 31 patients (40.8%), in the sixth month; MMR was observed in 42 patients (55.3%), and MMR was observed in 51 patients (67.1%) in the 9th month. None of the 5 cases who died in the third month follow-up were found to have MMR. MMR could not be obtained in any of the patients who died during the sixth month follow-up. In the third and sixth months, the statistical are significant in terms of survival between the group with MMR and the group without MMR ($p \leq 0.05$, $p \leq 0.05$). In the 9th month follow-up, the difference found between the MMR and non-MMR groups in terms of survival in the log-rank test is not significant ($p \geq 0.05$). Our findings are consistent with publications emphasizing the importance of early detection of MMR in terms of prognosis (17,23).

Molecular follow-up does not provide information about bone marrow morphology or chromosomal changes. Developing mutations can lead to resistance to treatment. Many mutations that can cause resistance have been identified today. Cytogenetic monitoring should be performed to detect mutations (4,15,25). In other words, in cases of resistance or non-response to treatment, only molecular follow-up may not be sufficient, cytogenetic follow-up should be performed.

Despite the importance of molecular monitoring in predicting long-term results and evaluating treatment success, minor fluctuations in patients' *BCR-ABL1* transcript levels should not be over-interpreted (25). In our cases, the values reported as gray zone continued to be monitored if there were no side effects or mutations without treatment changes.

In histopathological examination, an increase in megakaryocytes is observed in hypercellular bone marrow, myeloid hyperplasia and small megakaryocyte morphology (1,2). There were similar bone marrow findings in our cases. There may be reticulin fibrosis detected in the bone marrow with a reticulin stain and gradually increases during the disease (1,2,15). In our cases, increased reticulin fiber is evident.

Conclusion

Molecular follow-up, which is a sensitive method for evaluating treatment effectiveness and monitoring its response, is increasing. Getting MMR in the early period suggests that it will be good in its prognosis. Patients who cannot obtain MMR in the early period should be monitored more carefully and treatment changes should be made if necessary.

Ethics

Ethics Committee Approval: The study protocol was approved by the Ethics Committee from Aydın Adnan Menderes University (protocol number: 2019/195, date: 23.01.2020).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: İ.H.E., M.Ç.B., A.Z.B., İ.Y., Concept: İ.H.E., A.Z.B., İ.Y., Design: F.K.D., Data Collection or Processing: İ.H.E., Analysis or Interpretation: F.K.D., Literature Search: İ.H.E., M.Ç.B., Writing: F.K.D., M.Ç.B.

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

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Original Article / Özgün Araştırma

Comparison of Microleakage and Root Canal Dentin Adaptation After Single Visit Apexification Treatment with Retrograde Filling Materials in Single Rooted Young Permanent Teeth

Tek Köklü Genç Daimi Dişlerde Retrograt Dolgu Materyalleri ile Tek Seans Apeksifikasyon Tedavisi Sonrası Mikrosızıntı ve Kök Kanal Dentinine Adaptasyonun Karşılaştırılması

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Keywords
Young permanent teeth, microleakage, MTA, Biodentine

Anahtar Kelimeler
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Abstract

Objective: The aim of this study was to evaluate coronal and apical microleakage and dentin adaptation of three different types of mineral trioxide aggregate (MTA) and Biodentine using scanning electron microscope (SEM) in single visit apexification procedures carried out *in vitro*.

Materials and Methods: A single visit apexification treatment was applied to the 200 single-rooted permanent teeth with the use of Angelus MTA, Ortho MTA, Retro MTA and Biodentine as 20 samples in each group. Coronal and apical leakage was assessed for each group; positive and negative control groups. For the microleakage test, samples were incubated in methylene blue, and then measured under a stereomicroscope. Sixteen specimens were prepared for evaluating the dentin adaptation. SEM examinations were performed by measuring micrometric measurements of the material and dentin spaces on the images of the apical and coronal regions. The obtained data were analysed statistically.

Results: According to the microleakage evaluation, apical and coronal measurements indicated significantly more leakage than Biodentine Angelus MTA and Retro MTA, but no significant difference was found to be between MTA groups ($p<0.05$). While there was no significant difference in the apical region between the groups dentin adaptation; in the coronal region, Biodentine was found to have more gap dimensions at the significant level than the other groups ($p<0.05$).

Conclusion: Biodentine was not as successful as the other groups in the microleakage and marginal adaptation.

Öz

Amaç: *İn vitro* olarak 3 farklı mineral trioksit agregat (MTA) materyali ve Biodentine'in tek seans apeksifikasyon uygulamalarında koronal, apikal mikrosızıntı ve taramalı elektron mikroskobu (SEM) ile dentin adaptasyonu değerlendirmesi hedeflenmiştir.

Gereç ve Yöntemler: Her grupta 20 örnek olacak şekilde toplam 200 tek köklü daimi dişte Angelus MTA, Ortho MTA, Retro MTA ve Biodentine kullanılarak tek seans

apeksifikasyon tedavisi uygulanmıştır. Her grup ve pozitif, negatif kontrol grupları için koronal ve apikal sızıntı değerlendirilmiştir. Mikrosızıntı testi için örnekler metilen mavisi içerisinde bekletildikten sonra stereomikroskop altında ölçümler yapılmıştır. Dentin adaptasyonunun değerlendirilmesi için 16 örnek hazırlanmıştır. SEM incelemesi ile apikal ve koronal bölgelerdeki görüntüler üzerinde materyal ve dentin arası boşluklar bilgisayar ortamında mikrometrik olarak ölçülerek yapılmıştır. Elde edilen veriler istatistiksel olarak analiz edilmiştir.

Bulgular: Mikrosızıntı değerlendirmesi sonuçlarına göre apikal ve koronal ölçümlerde Biodentine Angelus MTA ve Retro MTA'ya göre anlamlı derecede daha fazla sızıntı gösterirken, MTA grupları arasında anlamlı bir fark bulunmamıştır ($p<0,05$). Dentin adaptasyonu açısından apikal bölgede gruplar arası anlamlı bir fark görülmezken, koronal bölgede Biodentine diğer gruplara göre anlamlı derecede daha fazla boşluk boyutuna sahip grup olarak saptanmıştır.

Sonuç: Biodentine'nin mikrosızıntı ve marjinal adaptasyon açısından diğer gruplar kadar başarılı sonuçlar veremediği görülmüştür.

Introduction

Young permanent teeth, traumas, dental anomalies, and deep caries that damaged the pulp tissue may lose its vitality, and pulp necrosis causes the cessation of root development. Endodontic treatments are needed during this stage; however, thin and fragile dentin walls, wide canals, and apex morphology make the endodontic treatment difficult. In the treatment of young permanent teeth, a root canal filling should be made by closing the apex with natural or artificial barriers. Some treatment procedures also aimed at maintaining the root development and regeneration of pulp tissues.

Traditional apexification treatments have yielded successful results recently. However, long treatment sessions and repeated visits led to the search for alternative treatments. One of these alternatives, regenerative endodontic therapy, comes to the forefront with practical application and the use of easy-to-reach materials. In addition, retaining the tooth with tissues free from immune system reactions and that does not cause pathological responses is preferable (1).

The single visit apexification method is a treatment based on the principle that the open apex is covered with a biocompatible material to fill the canal (2). Unnecessary prolonged treatment processes are regarded as advantages in this method (3). Single visit apexification treatment was first described using a mineral trioxide aggregate (MTA). Various MTA preparations have been developed to overcome the adverse effects, such as long curing time and discoloration over time, which are the advantages of MTA in endodontic treatment (4).

One of the goals of root canal filling is to obtain a hermetic seal in the root canal system to support healing after endodontic treatment. Inadequate filling and failure to provide the apical plug may cause fluid penetration due to the periapical chronic inflammatory reaction and may prevent the success of the treatment (5). As young permanent teeth morphologically differ, leakage after endodontic treatment also poorly affects the prognosis.

This study aimed to evaluate the dentin adaptation with three different MTA and Biodentine materials *in vitro* with coronal, apical microleakage, and scanning electron microscopy images (SEM).

Materials and Method

This study was approved by the Aydın Adnan Menderes University Medical Faculty Non-Invasive Clinical Research Ethics Committee on 14 June 2016 with the decision number 25.

A total of 20 teeth were selected in each experimental group, with a total of 160 teeth, and 8 and 16 teeth in the positive and negative control groups, respectively (Table 1). A total of 16 dental SEM examinations were planned, with four materials applied for each SEM.

The study included permanent teeth indicated for extraction due to orthodontic reasons in patients aged between 12 and 18 years who visited the Department of Dentistry Faculty in Aydın Adnan Menderes University. Decayed, fractured, cracked, restored, open apex and root resorbed permanent teeth were not included in the study. The teeth were stored in 5.25% NaOCl for 1 hour (h) after removal from the soft tissue using curettes. Samples were stored in the sterile saline solution at room temperature for approximately 8 weeks until being used in the study.

The root lengths of the teeth used in the study were shortened to 12 mm. The traditional canal cavity was shaped on the teeth. The apical portions of the

Table 1. Experimental and control groups

	N	Investigated region	Material
Group 1	20	Apical	Angelus MTA tricalcium silicate, dicalcium silicate, tricalcium aluminate, calcium oxide, calcium tungsten, bismuth oxide
	20	Coronal	
Group 2	20	Apical	Retro MTA calcium carbonate, silicon dioxide, aluminium oxide, calcium zirconia mixture
	20	Coronal	
Group 3	20	Apical	Ortho MTA tricalcium silicate, dicalcium silicate, tricalcium aluminate, tetracalcium aluminoferrite, calcium oxide, bismuth oxide
	20	Coronal	
Group 4	20	Apical	Biodentin tricalcium silicate, dicalcium silicate, calcium carbonate, calcium oxide, iron dioxide, zirconium dioxide
	20	Coronal	
Positive control	4	Apical	--
	4	Coronal	
Negative control	8	Apical	Angelus MTA, Retro MTA, Ortho MTA, Biodentin
	8	Coronal	

MTA: Mineral trioxide aggregate

samples were expanded using 1-6 numbered Gates-Glidden drills (JS Dental, Ridgefield, USA), and the root morphology of open apex teeth was simulated. In the canal, preparations were made with files (Golden Star Medical Co., Shenzhen, China). The coronal and middle sections were enlarged with circumferential inclination until the #80 file easily reached the apex. The root canals were irrigated with 2 mL of 2.5% NaOCl (Kim San Medicine, İstanbul, Turkey). After preparing the root canal, irrigation was performed with 5 mL of 2.5% NaOCl and 5 mL of sterile saline solution. Root canals were dried using paper points (Diadent, Diadent Group International, Burnaby, BC, Canada).

The prepared samples were filled with paperpoint so as not to overflow from the apical part, and the top of the apical part was covered with modeling wax. Subsequently, the paper points were removed and the canals were filled with the test materials prepared in accordance with the manufacturer's instructions. The canals were filled by condensation. However, no canal filling was applied in the positive control group; the same canal filling procedure with two coronal and apical evaluation of each material was performed in the negative control group. Radiographs were taken from the canal filling samples and checked.

The samples in the experimental and positive control samples, which will be used to evaluate apical leakage, were embedded in acrylic blocks with apical 2 mm sections exposed. The exposed root surface was covered with a nail polish to leave a 1 mm apical opening. In samples that should be evaluated for coronal leakage, the apical part was embedded and the exposed part of the coronal region was covered. Unlike those in the negative control groups, the area where the leakage was to be evaluated was completely covered with nail polish.

All samples were incubated for 72 h in the dye solution at 37 °C, with 2% methylene blue (Kim San Medicine, İstanbul, Turkey). After 72 h, the samples were washed with tap water for 5 minute and dried. The specimens were divided into two with a water-cooled diamond disc in the bucco-lingual direction (Moddental Medical Testing Devices, Ankara, Turkey).

The prepared sections were examined with X150 magnification under a stereomicroscope (Olympus SZ61, Tokyo, Japan). The amount of progress in the dye was measured micrometrically using the Olympus Stream Image Analysis Software (Olympus, Tokyo, Japan) program. The highest leakage value observed in each sample was considered. Images and numerical values transferred from the microscope to the computer were recorded.

The SEM study for the evaluation of dentin adaptation was conducted at the Erciyes University Technology Research and Application Centre laboratory. A total of 16 teeth were prepared with four canals filled with each material. The investigations were conducted under the ×1000 magnification with

Leo 440 computer-controlled Digital SEM (Zeiss, Oberkochen, Germany). Using the Image-Pro Plus Software program, the distance between the dentin and the material was measured at four points, and the average of 16 values obtained from each group was calculated. The results were evaluated statistically.

Statistical Analysis

All statistical analyses were performed using the "Statistical Package for the Social Sciences" (SPSS 24, SPSS Inc., Chicago, Illinois, USA). The Shapiro-Wilk and Kolmogorov-Smirnov tests were used to determine the normal distribution fit of the data and the homogeneity of the variances. The Mann-Whitney U test was used to compare the difference between the two independent samples according to the normality results of the data; the Kruskal-Wallis test and ANOVA tests were used to compare the differences between the two groups. The level of significance in tests was <0.05. The two groups had statistically significant differences according to the level of significance.

Results

The average microleakage values of the experimental and control groups are shown in Table 2.

Statistical analysis revealed that the Biodentine group in the apical and coronal regions showed more microleakage at the statistically significant level than the Angelus MTA and Retro MTA group. The difference between the Ortho MTA group and all other groups was not statistically significant, those between Angelus MTA and Retro MTA were significant (p>0.05).

When examining the relationship between coronal and apical microleakage values of the same material, no statistically significant difference was observed between the two regions in all materials (p>0.05).

The mean values obtained from the measurements from the SEM images and between groups distributions are shown in Table 3. The difference between the groups in the apical region was not statistically significant. A statistical difference was found between Biodentine and other groups in the coronal region (p<0.05). No statistically significant difference was found between the MTA groups (p>0.05). The images examined are shown in Figures 1-4.

Discussion

In young permanent teeth that differ from teeth that have developed morphologically, leakage after endodontic treatment negatively affects the prognosis.

MTA is widely used in single visit apexification treatments; however, new materials are being developed to eliminate its disadvantages. The easiest is the Angelus MTA, which shortened the setting time. *In vitro* conditions, a single visit apexification applied in the teeth has been successful in preventing microleakage and promoting fracture resistance (6,7). Moore et al. (8) found that Angelus MTA was successful in a single visit apexification treatment in an average

Table 2. Linear microleakage values of the experimental and control groups (µm)

Group		N	Mean	Median	SD
Angelus MTA	Apical	20	1833.50*	1747.50	804.15
	Coronal	20	1800.15*	1731.00	847.85
Ortho MTA	Apical	20	2109.55*†	1686.50	1469.86
	Coronal	20	2087.80*†	2061.50	907.53
Retro MTA	Apical	20	1829.00*	1787.00	689.34
	Coronal	20	1647.25*	1263.00	1221.38
Bio dentine	Apical	20	4018.95†	2890.00	3098.25
	Coronal	20	3347.85†	2442.00	2505.03
Positive control	4	Apical	12000.00	12000.00	0.00
	4	Coronal			
Negative control	Apical	8	0.00	0.00	0.00
	Coronal	8	0.00	0.00	0.00

*Statistically significant values in the same column were shown with different symbols (p<0.05). MTA: Mineral trioxide aggregate, SD: Standard deviation

Table 3. SEM dentin-material spacing measurement averages and inter-group distribution

Group		N	Mean	SD
Angelus MTA	Apical	2	2.84*	3.92
	Coronal	2	2.54*	4.05
Orhto MTA	Apical	2	2.13*	3.10
	Coronal	2	2.30*	2.79
Retro MTA	Apical	2	2.63*	4.07
	Coronal	2	2.66*	3.77
Biodentine	Apical	2	4.38*	3.02
	Coronal	2	5.18†	1.31

*Statistically significant values in the same column are shown with different symbols (p<0.05). MTA: Mineral trioxide aggregate, SD: Standard deviation

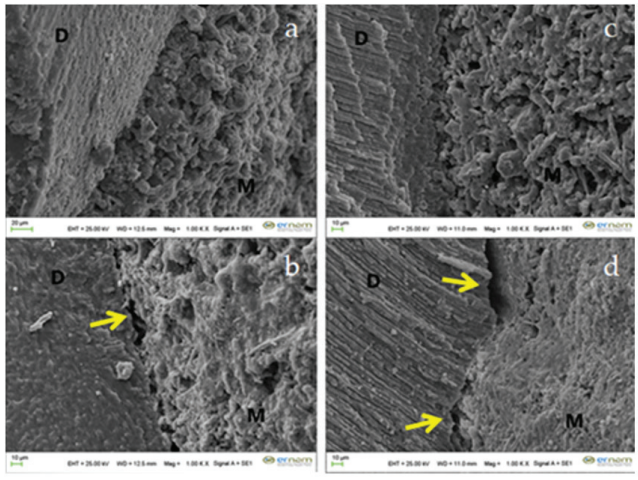

Figure 1. Images of the samples filled with Angelus MTA. a.b. Apical region images c.d. Coronal region images
MTA: Mineral trioxide aggregate, D: Dentin, M: Material (The spaces between the material and dentin are indicated by arrows.)

of 23.4 months following the clinical trial. Ortho MTA, which has been recently developed, has been shown to be an alternative to conventional canal treatment methods after the microleakage assessment of single rooted permanent teeth (9). It was also found to be marginally adaptive in retrograde cavities (10). Retro MTA, defined as zirconium oxide-containing calcium aluminate, is in the foreground for short setting time (11). Biodentine appears to be an alternative to single visit apexification treatments with short setting time and no discoloration. Biodentine has also been reported to show low microleakage and high dentin adaptation. It has been shown as a successful material

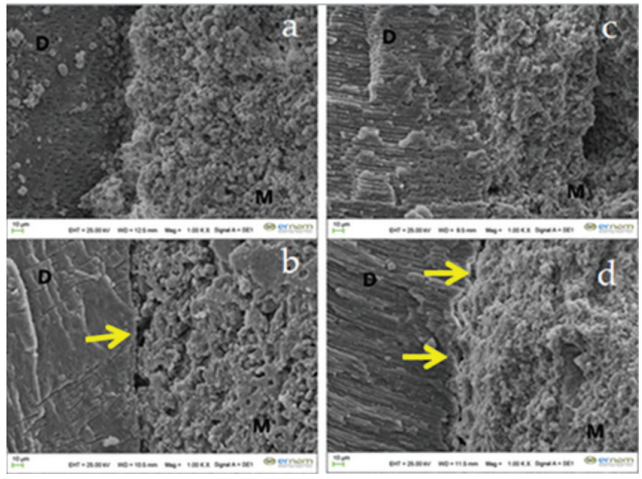

Figure 2. Images of the samples filled with Ortho MTA. a.b. Apical region images c.d. Coronal region images
MTA: Mineral trioxide aggregate, D: Dentin, M: Material (The spaces between the material and dentin are indicated by arrows.)

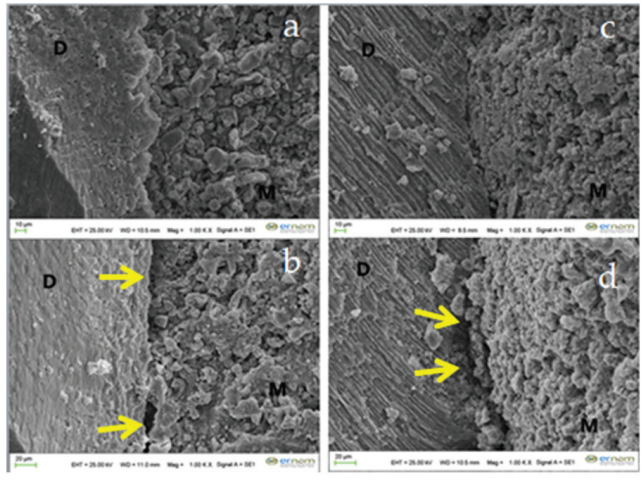

Figure 3. Images of the samples filled with retro MTA. a.b. Apical region images c.d. Coronal region images
MTA: Mineral trioxide aggregate, D: Dentin, M: Material (The spaces between the material and dentin are indicated by arrows.)

in single session apexification treatments in many case reports (12-14).

Nur et al. (15) evaluated the microleakage using dye penetration method, MTA had lower leakage values compared to Biodentine. These findings are consistent with the results of our study. No statistically significant difference was found between Biodentine and MTA in other microleakage studies in which teeth with open apex roots were replicated (16,17). It was thought that the difference between the MTA and

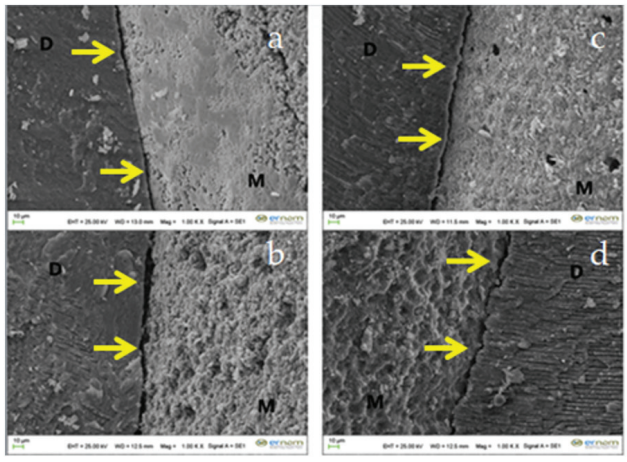

Figure 4. Images of the samples filled with Biodentine. a.b. Apical region images c.d. Coronal region images
MTA: Mineral trioxide aggregate, D: Dentin, M: Material (The spaces between the material and dentin are indicated by arrows.)

microleakage evaluation method used in our study from other studies may be related to the results.

In order to prevent failures in endodontic treatments, not only apical but also coronal leakage should be tested (18,19). Therefore, coronal leakage evaluation was performed in our study. The lowest coronal leakage according to the obtained data was shown by Retro MTA, followed by Angelus MTA and Ortho MTA. The largest coronal microleakage value was observed in the Biodentine group. Statistically, Biodentine showed more leakage at a significantly higher level than the Angelus and Retro MTA groups, but no significant difference was found among the other groups.

The relationship between microleakage and clinical success of the root canal system is controversial. Some researchers argue that clinical success rates do not correlate with the microleakage values and that these studies may provide questionable results without assessing the quality of care (20,21). On the contrary, studies that evaluate microleakage in the root canal system continued. Some authors refer to the consideration of treatment methods and criteria in understanding the characteristics and mechanism of the materials (5,22). More *in vitro* and long-term clinical trials are needed to compare the extent of microleakage and clinical success.

SEM plays an important role in assessing the marginal compatibility of materials used in the root canal system, the adaptation of apical barriers in the incomplete development of teeth, and the microstructure of materials. Marginal adaptation correlates with the sealing ability of dental materials and therefore influences the clinical success rate. Brenes-Valverde et al. (17) underwent a single visit apexification treatment to provide a 4 mm apical plug with Biodentine and MTA on the teeth with open apex form. According to their result, the two materials showed similar success rates in marginal adaptation. In our study, although Biodentine had a higher mean of gap size between dentin-material than MTA groups, the difference between them was not statistically significant.

Other materials for microleakage and marginal adaptation showed more successful results than Biodentine. This condition may be due to the possible manipulation of Biodentine in terms of placement in the root canal system via the orthograde and the consideration of short setting time. The other materials did not show a significant difference between their results, because they exhibited more similar characteristics in terms of structure.

Conclusion

The microleakage data and SEM evaluation results obtained in this study are parallel and support each other. Biodentine gives more negative results in both evaluations compared to other groups. Other materials did not show a significant difference in their results. However, *in vitro* and *in vivo* studies are needed to evaluate the use of these biomaterials in apexification therapies.

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Ethics

Ethics Committee Approval: This study was approved by the Aydın Adnan Menderes University Medical Faculty Non-Invasive Clinical Research Ethics Committee on 14 June 2016 with the decision number 25.

Informed Consent: Informed consent is not required.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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Original Article / Özgün Araştırma

Evaluation of the Relationship between Diabetes and Smoking and IL-6, IL-17 and IL-23 Levels in Individuals with Stage II, IV Periodontitis

Evre II, IV Periodontitis Olan Bireylerde Diyabet ve Sigara ile IL-6, IL-17 ve IL-23 Düzeyleri Arasındaki İlişkinin Değerlendirilmesi

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Keywords
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Abstract

Objective: The purpose of the present investigation was to examine the relationship between diabetes mellitus (DM) and smoking on gingival crevicular fluid (GCF) levels of interleukin (IL)-6, IL-17, and IL-23 in patients with stage II and stage IV periodontitis.

Materials and Methods: Individuals were divided into nine groups according to their periodontal diagnosis, smoking, and DM status: periodontally and systemically healthy individuals (PH-H, n=14), periodontally healthy smokers (PH-S, n=14), periodontally healthy diabetics (PH-D, n=14), systemically healthy individuals with stage II periodontitis (SII-H n=14), smokers with stage II periodontitis (SII-S, n=14), diabetics with stage II periodontitis (SII-D, n=14), systemically healthy individuals with stage IV periodontitis (SIV-H, n=14), smokers with stage IV periodontitis (SIV-S, n=14), and diabetics with stage IV periodontitis (SIV-D, n=14). GCF samples were collected and analyzed.

Results: The mean GCF levels of IL-6, IL-17, and IL-23 in patients with stage IV periodontitis were significantly higher than those in patients with stage II periodontitis and in periodontally healthy individuals.

Conclusion: As the stage of periodontitis increases, the levels of IL-6, IL-17, and IL-23 increase.

Öz

Amaç: Bu araştırmanın amacı, evre II ve IV periodontitisli hastalarda diyabetes mellitus (DM) ve sigara ile dişeti oluğu sıvısındaki (DOS) interlökin (IL)-6, IL-17 ve IL-23'ün seviyeleri arasındaki ilişkiyi incelemektir.

Gereç ve Yöntemler: Bireyler periodontal tanı, sigara ve DM durumlarına göre periodontal ve sistemik olarak sağlıklı (PH-H, n=14), periodontal sağlıklı sigara içen (PH-S, n=14), periodontal sağlıklı diyabetik (PH-D, n=14), evre II periodontitisli sistemik sağlıklı (SII-H n=14), evre II periodontitisli sigara içen (SII-S, n=14), evre II periodontitisli diyabetik (SII-D, n=14), evre IV periodontitisli sistemik sağlıklı (SIV-H, n=14), evre IV periodontitisli sigara içen (SIV-S, n=14) ve evre IV periodontitisli diyabetik (SIV-D, n=14) olarak dokuz gruba ayrılarak DOS örnekleri analiz edildi.

Bulgular: evre IV periodontitisli hastalarda IL-6, IL-17 ve IL-23'ün ortalama DOS seviyeleri, evre II periodontitisli hastalardan ve periodontal olarak sağlıklı bireylerden önemli ölçüde daha yüksekti.

Sonuç: Periodontitis evresi arttıkça IL-6, IL-17 ve IL-23 seviyeleri yükselir.

Introduction

Periodontitis is a multifactorial inflammatory disease characterized by loss of tooth-supporting tissues, and clinical expression is determined by genetic, environmental, and behavioral factors (1).

Smoking and having diabetes mellitus (DM) are major risk factors for periodontitis. In the most recent classification of periodontal diseases, smoking and DM were identified as modifying risk factors in determining the progression rate of periodontitis (2). Smoking alters the host's defense by causing changes in vascular function, neutrophil/monocyte activity, and cytokine and inflammatory mediator release. DM increases the risk of the initiation and progression of periodontitis by contributing to inflammation in the periodontal supporting tissues (3). Although the effects of DM and smoking on gingival crevicular fluid (GCF) cytokine levels have been investigated, most of these studies were conducted according to the 1999 classification system, and the effects of smoking and DM were not evaluated comparatively (4). In the 2017 world workshop on the classification of periodontal and peri-implant diseases and conditions, periodontitis was categorized in 4 different stages and 3 different grades. Stage II periodontitis (SII) individuals reflect the clinical findings of periodontitis more than stage I periodontitis individuals. As a result, they apply to our clinic more frequently with complaints such as pain, swelling and bleeding. Stage IV periodontitis (SIV) is the terminal phase of the periodontitis (2). Interleukin (IL)-6 is a cytokines associated with inflammatory periodontal diseases. Increased IL-6 level is associated with osteoclastic activity (5). IL-17 is a proinflammatory cytokine. It has been reported that IL-17 supports osteoclastogenesis by increasing receptor activator of nuclear factor-B ligand production in osteoblastic cells (6). IL-23 is a member of the IL-12 family. IL-23 increases IL-17 release in T cells (7). In this context, the aim of this study is to examine the effects of DM and smoking on GCF levels of IL-6, IL-17, and IL-23 in patients with Stage II and SIV.

Materials and Methods

This study was conducted between May 2019 and January 2020 at Uşak University in the Faculty of Dentistry of the Department of Periodontology. The individuals were informed about the study, and written consent was obtained. This study was designed according to Helsinki declaration principles and approved by the Uşak University Faculty of Medicine Ethics Committee (decision no: 13-13-14, date: 05.01.2022).

Sample Size

A sufficient sample size was determined for IL-6 using a one-way ANOVA test (8). The f-type effect size (0.42), type 1 error (α=0.05), and test power (1-β=0.95) were determined. According to these calculations, a minimum of 11 individuals per group (total sample size of 99 individuals) would be necessary. Considering the possible setbacks at the clinical stage, the number of people in each group was determined as 14, and the total number of samples was determined as 126.

Participants

Clinically, 526 people over the age of 18 were examined. When the extrusion criteria were applied, 126 people were reached. Smokers (S) had smoked more than five packs of cigarettes during their lifetime and continued to smoke. Diabetics (D) had been diagnosed with type 2 DM by a physician and were on insulin supplementation or oral hypoglycemic agents (8).

Exclusion Criteria

Exclusion criteria included periodontal treatment in the previous six months, use of antibiotics or anti-inflammatory drugs in the previous six months, lactation, pregnancy, or any systemic condition that could affect periodontal disease progression (impaired lipid metabolism or hypercholesterolemia).

Clinical Periodontal Measurements

Individuals were diagnosed according to the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions (2). Plaque index (9), gingival index (10), probing depth (PD), and clinical attachment loss were assessed at six sites of all teeth except third molars using a manual periodontal probe (Williams, Hu-Friedy, Chicago, IL).

Classification of Individuals

Individuals were divided into nine groups according to their periodontal diagnosis, smoking history, and DM status: periodontally (PH) and systemically healthy (PH-H, n=14), PH-S, n=14, PH-D, n=14, systemically healthy individuals with SII-H, n=14, S with SII-S, n=14, D with SII-D, n=14, systemically healthy individuals with SIV-H, n=14, S with SIV-S, n=14, and D with SIV-D, n=14.

GCF Sampling

Clinical examination was performed one week before GCF samples were collected. In patients with Stage II and SIV, four nonadjacent and deep periodontal pockets were selected for GCF sampling. In PH patients, four nonadjacent and non-inflamed sites were selected.

Cytokine Quantification

Enzyme-linked immunosorbent assay was used to analyze the GCF levels of IL-6, IL-17, and IL-23 with commercially available kits. Assays were carried out according to the manufacturer's recommendations.

Statistical Analysis

Data analysis was performed using the IBM SPSS V23 and R program. Shapiro-Wilk ve Kolmogorov-Smirnov tests were used to check the normality of the data. Two-way analysis of variance was used to compare the normally distributed data according to the group and subgroups, and multiple comparisons were examined with the Tukey test. Two-way Robust test was used using the WRS2 1 package of data that were not normally distributed according to the group and subgroups, and multiple comparisons were examined with the Bonferroni test. Pearson's chi-square test was used to compare gender according to group and subgroups. One-way analysis of variance was used to compare normally distributed data according to groups of 3 and above, and multiple comparisons were examined with the Tukey test. Kruskall-Wallis test was used to compare data that were not normally distributed according to groups of 3 and above, and multiple comparisons were examined with Dunn's test. Analysis results were presented as mean ± standard deviation and median (minimum-maximum) for quantitative data. Statistical significance level were set at $p<0.05$.

Results

Demographic Data

A total of 126 individuals (73 male, 53 female) were included in the study. The mean age of the Stage IV group was significantly higher than that of the Stage II and PH groups ($p<0.001$) (Table 1).

There was no significant difference between the PH, Stage II, and Stage IV groups in terms of gender distribution ($p>0.05$) (Table 1).

Smoking and DM Status

In individuals with DM, years of DM and Hemoglobin A1c (HbA1c) levels in the SIV-D group were significantly higher than in the SII-D and PH-D groups ($p<0.001$). In S, years of smoking in the SIV-S group was significantly higher than in the SII-S and PH-S groups ($p<0.001$). The number of cigarettes smoked per day in the SIV-S and SII-S groups was significantly higher than in the PH-S group ($p<0.001$) (Table 2).

Periodontal Clinical Parameters

The mean PD of the Stage IV group were significantly higher than those of the Stage II and PH groups ($p=0.001$) (Table 3).

Table 1. The gender distribution of groups

Groups		Gender n (%)			Age (Mean ± SD)	p-value
		Female	Male	p-value		
PH	PH-H	18 (42.9)	24 (57.1)	0.798	33.64±7.02[c]	<0.001
	PH-S					
	PH-D					
SII	SII-H	17 (40.5)	25 (59.5)		51.31±5.13[b]	
	SII-S					
	SII-D					
SIV	SIV-H	20 (47.6)	22 (52.4)		58.24±7.03[a]	
	SIV-S					
	SIV-D					

Pearson's chi-square test

Analysis of variance test statistic

[a-c]No difference between groups and subgroups with the same letter

PH: Periodontal health, SII: Stage II periodontitis, SIV: Stage IV periodontitis, H: Health, D: Diabetes, S: Smoking

GCF Levels Of IL-6, IL-17, And IL-23

The mean IL-6 level of the Stage IV group was significantly higher than that of the Stage II and PH groups (p<0.001). IL-17 and IL-23 levels of the Stage IV group were significantly higher than those of the Stage II and PH groups (p<0.001). IL 23 level of the SIV-D and SII-D groups were higher than the other subgroups (p<0.001) (Table 3).

Discussion

According to this study, the extent and severity of periodontitis increased as the mean age of the groups increased, which was expected because periodontitis is often seen as a cumulative disease (11). The current study confirmed that gender was not significantly associated with periodontal disease but previous studies have reporting that periodontitis more prevalent in men than in women (12).

Table 2. The smoking and DM status of individuals

Groups		Years of diabetes	p-value	HbA1c level	p-value	Years of smoking	p-value	Number of cigarettes/day	p-value
PH	PH-S		<0.001		<0.001	6±0.96[c]	<0.001	8.21±3.73[b]	<0.001
	PH-D	4.14±0.77[c]		6.88±0.27[c]					
SII	SII-S					13.14±3.46[b]		18.93±4.46[a]	
	SII-D	8.21±1.37[b]		7.89±0.32[b]					
SIV	SIV-S					24.07±5.55[a]		22.5±5.8[a]	
	SIV-D	11±2.48[a]		8.19±0.29[a]					

One-way analysis of variance, [a-c]No difference between groups with the same letter.
PH: Periodontal health, SII: Stage II periodontitis, SIV: Stage IV periodontitis, H: Health, D: Diabetes, S: Smoking

Table 3. Probing depth and GCF cytokine levels of groups

	Alt grup	Grup					
		PH		SII		SIV	
PD	D	1.99±0.18	1.95 (1.77-2.44)[A]	3.07±0.29	3.08 (2.44-3.66)[C]	4.91±0.65	4.97 (3.95-6.13)[D]
	H	1.99±0.17	1.98 (1.74-2.31)[A]	2.68±0.39	2.82 (2.06-3.23)[BC]	4.44±0.9	4.48 (2.99-6.33)[D]
	S	2.27±0.19	2.3 (1.96-2.56)[B]	3.22±0.39	3.17 (2.56-3.88)[C]	5.26±0.82	5.12 (4.13-7.56)[D]
	Total	2.08±0.22	2.01 (1.74-2.56)[c]	2.99±0.42	3 (2.06-3.88)[b]	4.87±0.85	4.96 (2.99-7.56)[a]
IL-6	D	0.52±0.09	0.51 (0.41-0.71)	0.88±0.35	1.04 (0.42-1.38)	1.08±0.14	1.09 (0.83-1.33)
	H	0.69±0.16	0.69 (0.35-0.94)	0.73±0.13	0.68 (0.54-0.94)	0.89±0.41	0.71 (0.43-1.83)
	S	0.49±0.1	0.47 (0.35-0.68)	0.69±0.14	0.7 (0.43-0.91)	0.97±0.42	0.83 (0.45-2.19)
	Total	0.57±0.15[c]	0.55 (0.35-0.94)	0.77±0.24[b]	0.71 (0.42-1.38)	0.98±0.35[a]	1 (0.43-2.19)
IL-17	D	6.14±0.25	6.14 (5.76-6.66)	7.17±0.87	7.31 (5.97-9.12)	8.26±0.56	8.46 (7.24-8.98)
	H	5.84±0.63	5.87 (4.44-6.95)	6.57±0.59	6.49 (5.24-7.8)	7.44±0.83	7.48 (5.97-8.83)
	S	6.07±0.25	6.03 (5.76-6.53)	6.99±0.92	6.65 (6.11-9.12)	7.57±0.71	7.34 (6.53-8.98)
	Total	6.02±0.43[c]	6.04 (4.44-6.95)	6.91±0.83[b]	6.72 (5.24-9.12)	7.75±0.78[a]	7.76 (5.97-8.98)
IL-23	D	4.15±0.59	4 (3.22-5.23)[A]	5.92±0.68	5.92 (4.67-6.99)[C]	6.87±2.43	6.47 (4.31-12.49)[BC]
	H	4.06±0.18	4.03 (3.69-4.4)[A]	5.07±0.67	5.22 (3.52-6.2)[BC]	5.63±1.68	5.65 (3.51-9.26)[ABC]
	S	4.36±0.69	4.18 (3.44-5.8)[AB]	5.24±0.98	5.21 (3.87-7.18)[ABC]	5.83±1.74	5.51 (4.22-11.46)[C]
	Total	4.19±0.54	4.04 (3.22-5.8)[b]	5.41±0.86	5.4 (3.52-7.18)[a]	6.11±2.01	5.7 (3.51-12.49)[a]

PH: Periodontal health, SII: Stage II periodontitis, SIV: Stage IV periodontitis, PD: Probing depth, H: Health; D: Diabetes; S: Smoking, [A-D]No difference between interactions with the same letter, [a-c]No difference between groups and subgroups with the same letter

In diabetic individuals, the stage of periodontitis was associated with DM duration and HbA1c levels. This result is in line with studies reporting that elevated HbA1c levels and longer DM durations increase inflammation in periodontal tissues, which is associated with a higher risk of periodontitis (13). The number of cigarettes smoked per day by the PH-S group was significantly lower than that of the SII-S and SIV-S groups, which is in line with studies demonstrating that increased dosage and intensity of smoking are risk factors for worsened periodontal health (14).

The results of the current study demonstrated that IL-6 was upregulated as the severity of periodontitis increased. The results obtained from this study are in accordance with those reported by Becerik et al. (15) S in the PH, Stage II and SIV groups presented IL-6 levels similar to those of the healthy and diabetic individuals in those groups. In contrast, other studies have shown higher or similar GCF levels of IL-6 in S in comparison with non-S (16). This indicates that smoking seems to modulate IL-6 levels in healthy sites; however, as periodontitis becomes established, the level of IL-6 is regulated by periodontal infection rather than by smoking.

This study addressed the significant increase in the GCF levels of IL-17 with an increase in the amount of periodontal destruction. This can be explained by the role of IL-17 in the functional impairment of polymorphonuclear leukocytes and the activation of fibroblasts to produce inflammatory mediators (17). In accordance with this result, higher GCF levels in periodontitis patients compared to healthy controls and role of IL-17 in the severity of periodontal inflammation were reported (18).

This present finding indicates that S and non-S patients with periodontitis and those who were PH did not exhibit different IL-17 levels. The present IL-17 data showed that smoking did not affect GCF levels of IL-17 in PH and diseased individuals, which is in accordance with Buduneli et al. (19). The fact that smoking did not affect GCF IL-17 levels in this study can be explained as follows: S in the PH, Stage II, and Stage IV groups had deeper PDs than non-S in those groups. According to Johnson et al. (20) GCF levels of IL-17 increase as the PD increases that requires higher IL-17 levels in S than in non-S according to this study. However, considering the suppressing effect of smoking on proinflammatory cytokines, the elevated level of IL-17 caused by higher PD comes to a level similar to that of non-S. In other words, the GCF level of IL-17 that increases with increasing PD decreases with the suppressive effect of smoking.

Although periodontitis patients with diabetes tend to have higher GCF levels of IL-17, PH individuals with and without DM have similar IL-17 levels. Explanation can be offered regarding this result. The glycemic control of the patients with DM may modulate the level of IL-17, as the HbA1c levels of the PH-D group were significantly lower than those of the SII-D and SIV-D groups.

This study implies a strong association between GCF IL-23 level and periodontal health and disease. IL-23 levels increased significantly as the severity of periodontal destruction increased. This result is in accordance with that of Lester et al. (21) and DM and smoking exerted no considerable impact on IL-23 levels in patients with periodontitis or PH individuals. This result is not accordance with previous studies that reported smoking causes cytokine expression downregulation and that diabetes stimulates the production of proinflammatory cytokines (21). The lack of effect of smoking and diabetes on IL-23 levels may be because S and D who are PH or have periodontitis have microbial loads similar to those of non-S and non-D who are PH or have periodontitis. This similar microbial load stimulates macrophages and dendritic cells, thereby coincidently stimulating similar production of IL-23.

A strength of the present study is that it was the first to investigate the impact of DM and smoking on the levels of IL-6, IL-17, and IL-23 in patients with different stages of periodontitis. However, the study has some limitations. First, this is a cross-sectional study that cannot determine causal relationships. Second, smoking status and DM duration were determined by self-report.

Conclusions

This study implies an association between GCF levels of IL-6, IL-17, and IL-23 and periodontal health and disease. As the stage of periodontitis increases, the levels of IL-6, IL-17, and IL-23 increase. Also, periodontal status worsens as the duration and frequency of smoking and years of diabetes and HBa1c level. As the stage of periodontitis increases, the

time and cost spent for the treatment of the disease also increases. To prevent this negative situation, periodontal treatment and follow-up appointments of S and diabetic patients should be increased.

Ethics

Ethics Committee Approval: This study was designed according to Helsinki declaration principles and approved by the Uşak University Faculty of Medicine Ethics Committee (decision no: 13-13-14, date: 05.01.2022).

Informed Consent: The individuals were informed about the study, and written consent was obtained.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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Original Article / Özgün Araştırma

The Investigation of the Marginal Microleakage of Ceramic Veneer Crowns with Different Finish Lines

Farklı Marjinal Bitim Tiplerinin Seramik Kronların Kenar Sızıntısına Etkisi

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Abstract

Objective: This study aimed to evaluate the microleakage of lithium disilicate and zirconium dioxide all ceramic crowns.
Materials and Methods: A total of 80 premolar teeth with two different marginal finish lines were divided into 2 groups to produce zirconia and lithium disilicate crowns. The crowns were cemented by two different cements (n=10). Samples were stained with 0.5% basic fuchsin solution and the dye penetration was scored. Statistical comparisons were made using two-way ANOVA and two-sample t-test.
Results: There were no statistically significant differences in preparation types (p>0.05). However, ceramic systems and cement types for the microleakage test showed significant differences (p<0.05).
Conclusion: The use of self-adhesive cement is recommended for lithium disilicate restoration to minimize marginal microleakage.

Keywords
Microleakage, all-ceramic, marginal finish line

Öz

Amaç: Bu çalışmanın amacı lityum disilikat ve zirkonyumdioksit tam seramik kronların mikrosızıntısını değerlendirmektir.
Gereç ve Yöntemler: İki farklı marjinal bitiş çizgisine sahip toplam 80 premolar diş zirkonya ve lityum disilikat kronların üretilmesi için 2 gruba ayrıldı. Kuronlar iki farklı simanla yapıştırıldı (n=10). Örnekler %0,5'lik bazik fuksinde bekletildi ve boya penetrasyonu skorlandı. İki yönlü ANOVA ve iki örneklem t-testi kullanılarak istatistiksel karşılaştırmalar yapıldı.
Bulgular: Preparasyon tipleri arasında istatistiksel olarak anlamlı fark bulunmazken (p>0,05), seramik sistemler ve siman tipleri mikrosızıntı testi için önemli farklılık gösterdi (p<0,05).
Sonuç: Mikrosızıntıyı en aza indirmek için lityum disilikat restorasyonlar için self-adeziv siman kullanılması önerilmektedir.

Anahtar Kelimeler
Mikrosızıntı, tam seramik, marjinal bitiş çizgisi

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Introduction

Microleakage the flow of bacteria, oral fluids, molecules or ions between the restorative material and the dental tissue has a critical impact on the long-term clinical success of all-ceramic restorations (1). Such leakages cause discoloration, cracks, dentin sensitivity and

secondary caries formation in the crown area of the restorations in the long run; increased leakage can lead to inflammation and necrosis of the vital pulp, which might necessitate endodontic treatment (2).

Adhesive resin cements impede microleakage by filling the micro-gap between the tooth structure and the restoration (3) however, factors like the polymerization shrinkage, solubility in oral fluids, variable thermal expansion coefficient in different dental tissues of resin cement significantly impact adhesion and hence the amount of microleakage (4). Resin and bonding procedures are as effective as degree of bond between tooth and restoration. For this purpose, the application of acid and primer to the tooth surface strengthens the bond. In self-etch systems, acid and primer application is combined in one step and requires an additional step before cementation (5). Although these systems show high bond strength, they require technical precision. The resin matrix of the cement recently introduced in self-adhesive systems that do not require pre-surface treatment, consists of methacrylate monomers modified with phosphoric acid. Adhesion is dependent on micromechanical retention and chemical interaction between monomeric acidic groups and hydroxyapatite (6). Due to a less mineralized structure of dentin, dentinal fluid flow and tubular structure, bonding is more difficult and requires precision compared to collagen and enamel (7). Another factor affecting microleakage is marginal and internal fit between tooth and restoration. An increase in the marginal gap exposes a larger area of cement to the oral environment, this results in higher cement solubility and ultimately microleakage (8).

This study aimed to evaluate the microleakage of full ceramic restorations produced with different techniques and bonded using two different adhesive systems on teeth with chamfer and rounded shoulder marginal finish designs. The null hypothesis of the study is that different marginal finish design, all-ceramic and adhesive systems will not make a difference in terms of microleakage level of restorations.

Materials and Methods

The ethical approval for this study was acquired from the Selçuk University Faculty of Dentistry Non-Invasive Clinical Research Evaluation Commission (meeting number: 2012/05, date: 03.05.2012). Eighty newly extracted second premolar teeth were used for periodontal and orthodontic purposes. Soft tissue residues and dental calculus on the surfaces of the extracted teeth were cleaned with the help of a periodontal curette, before tooth surfaces were polished. The teeth were kept in distilled water at room temperature throughout the study. Before the preparation, the teeth with their occlusal surfaces on top were embedded in acrylic, 2 mm below the enamel-cement junction line in molds with a diameter of 2x2x2 cm, and the samples were randomly divided into 8 groups (n=10). All teeth were prepared using a lathe with a reduction of 2 mm from the incisal edges, 1 mm from the palatal surfaces, 1.2 mm from the vestibule and approximal surfaces, and a taper angle of 12°. Half of the samples were prepared with a 135° angled chamfer and the other half with a rounded shoulder end design with a step thickness of 1 mm.

After the preparation of the 2nd upper premolar teeth, impressions were taken using polyvinyl siloxane impression material (Zetaplus putty, Oranwash L light hydrocompatible, C-silicone, Zhermack Clinical Badia Polesine, Italy). Samples were placed in the mold with finger pressure using a one-stage impression technique. Type IV dental hard plaster (GC Fujirock EP, GC Europe N.V, Leuven, Norway) was poured into the impressions and left for an hour to solidify. The samples obtained were divided into two groups for the construction of two different ceramic restorations (n=40).

Preparation of IPS Empress eMax Press Ceramic Samples

Using a die spacer thin brush (Megadental GmbH Büdingen, Germany), plaster day materials were applied to the walls of the working models to obtain 2 coats with a 15 µ thickness. To prepare the restorations, modeling was made from wax (Bego, Bremer Goldschlägerei Bremen, Germany) developed for eMax Press. Baking and pressing process were done according to the manufacturer's instructions. The samples were sandblasted with 50 µ Al_2O_3 particles at 2 millibar pressure and cleaned in an ultrasonic cleaner for 10 minutes. Each ceramic substructure was placed on the die and eMax Ceram (Ivoclar Vivadent AG, Schaan, Liechtenstein) ceramic was applied to give the upper 2nd premolar tooth form and fired. The prepared IPS Empress eMax all ceramic crowns were placed on the cut teeth and their marginal integrity

and harmony were checked, and the final glaze layer was applied and fired.

Preparation of Zirkonzahn Specimens

Imaging spray (White Peak Systems GmbH&CO. KG Langeheide, German) was sprayed evenly all over the samples. After, the plaster model was fixed on the scanner's table and images were taken with the scanner. On the digital impression obtained, the marginal limits of the restoration were determined with the help of a computer and digital designs were created. The milling process was carried out by transferring the obtained data to the milling unit (Yenamak, İstanbul, Turkey). Samples were treated with 50 µ Al_2O_3 for 15 seconds and sandblasted under 2 bar pressure over time.

Cementation of Specimens

Multilink N (Ivoclar Vivadent AG, Schaan, Liechtenstein) and RelyX U200 (3M-ESPE, USA) resin cements were used for the cementation of IPS Empress eMax, and Zirkonzahn restorations.

The inner surfaces of IPS Empress eMax restorations were treated with hydrofluoric acid (IPS Ceramic Etching Gel, Ivoclar Vivadent, Schaan, Liechtenstein) for 20 s. After washing restorations for 30 s with water and dried, RelyX and Multilink N cements were applied to all samples according to manufacturer's instructions. Then, the samples were placed in a universal testing machine (Elista Co, İstanbul, Turkey) under constant pressure and polymerized with LED (450 mW/cm²) light device (Bluephase, Ivoclar Vivadent, Schaan) for 40 s under a constant load of 30 N., Liechtenstein). The materials used are given in the Table 1.

Thermal Cycle Application and Staining of Samples

All samples were kept in distilled water at 37 °C for 24 hours and placed in the Thermal Cycler Tester (Dental Technic, Konya, Turkey) and treated with 5000 thermal cycles for 30 sat +5 °C and +55 °C. The samples were placed in 0.5% basic fuchsin solution in groups with their crowns down and in this way they were kept in an oven at 37 °C for 24 h. The samples extracted from the basic fuchsin solution were washed under running water until all the dye was removed. To cut the crown, the long axis of the crown was perpendicular to the ground plane at a low speed, under the water cutting device (Buehler Isomet 1000 Low Speed Saw, Buehler Ltd, Lake Bluff, IL, USA) was placed. Each tooth was divided in the bucco-lingual and mesio-distal directions into 4. The roots of the teeth were cut 2 mm below the crown margin, perpendicular to the long axis of the tooth, under water cooling. A total of 8 measuring surfaces were obtained from each sample. The surfaces of all sections to be measured were polished with 600, 800 and 1200 numbered sandpaper respectively. Dye penetration amounts in the sections were examined under a stereomicroscope (Olympus, SZ-PT, Japan) at x20 magnification. Mean of 8 measurement values obtained from each sample was taken.

Microleakage assessment was made according to the following scoring;

0= no leakage

1= leakage extending 1/3 of the margin

2= leakage extending 2/3 of the margin

3= leakage entire

4= leakage extending more than 1/3 of the axial wall

Table 1. Materials used in the study

Material type	Commercial name	Lot	Manufacturer	Type
Resin cement	Multilink N	R05845	Ivoclar Vivadent AG, Schaan, Liechtenstein	Sef-etch cement
Resin cement	RelyX U200	505225	3M ESPE Deutschland, Germany	Self-adhesive cement
Pressable ceramic	IPS Empress eMax press	R54294	Ivoclar Vivadent AG, Schaan, Liechtenstein	Lithium disilicate
CAD/CAM ceramic	ICE Zirkon	ZA9192A	Zirkonzahn GmBh, Bruneck, Italy	Zirconium dioxide
Acid-etch	IPS etching gel	R48184	Ivoclar Vivadent, Schaan, Liechtenstein	Hydrofluoric acid

CAD/CAM: Computer-aided design/computer-aided manufacturing

5= leakage extending more than 2/3 of the axial wall

6= leakage extending on the entire axial wall, including the incisal edge

7= leakage beyond the incisal edge (9).

Statistical Analysis

Statistical analysis of the microleakage results obtained in our study was performed in SPSS (Windows, SPSS 17.0) package program. Two-way analysis of variance was used to determine whether there was an interaction between the preparation, cement and porcelain factors. Independent two-sample t-test was used for pairwise comparisons between the factors with a difference.

Results

According to the two-way ANOVA, there are significant differences in cement and porcelain types ($p<0.05$) but there was no difference in their interactions ($p>0.05$). Results of two independent sample t-tests (Table 2) showed that among the same full ceramic system and the same cement type groups, the step type did not make a statistically significant difference on microleakage ($p>0.05$). Although shoulder samples presented lower microleakage values than chamfer samples, there was no significant difference between the two groups ($p>0.05$). The highest dye penetration in all groups is the score of 3. The lowest dye penetration scores were in the Empress-Shoulder-RelyX group and the highest micro-free scores were seen in the Zircon-Chamfer-Multilink group. In the Empress-Shoulder-RelyX group, there was no dye penetration in 41 sections, while score 1 in 35 sections and score 2 in 4 sections were seen score 2. In the Zircon-Chamfer-Multilink group, dye penetration in 5 sections was not seen, while the score 3-level was observed in 8 sections. The highest score was found at 2 levels in all groups. Microlekage score in specimens are given Table 3.

Among the same full ceramic system and the same step type groups, it was found that the type of cement used had a significant effect on the microleakage ($p<0.01$). RelyX had lower microleakage values in all groups than Multilink cement. Similarly, Empress restorations in all groups exhibited lower microleakage values than zirkonzahn restorations ($p<0.05$). Mean, minimum, maximum and standard deviation value for each group presented Table 4.

Table 2. Result of the ındependent two-sample t-test

	Variant	Mean	SD	p-value
Ceramic	IPS Empress Zirkonzahn	0.893 1.099	0.061 0.076	0.038
Cement	RelyX Multilink	0.615 1.377	0.024 0.046	0.002
Preparation	Chamfer Shoulder	1.039 0.952	0.067 0.074	0.385

SD: Standard deviation

Discussion

This study evaluated the microleakage scores of zirconia and lithium disilicate crowns fabricated on natural teeth, self-adhesive and self-etch cements as well as marginal finish design. The hypothesis was partially rejected because the all-ceramic systems used in the construction of the restorations and the adhesive systems used in the cementation affected the microleakage scores. The part of the hypothesis related to the marginal finish design was accepted. It was observed that the marginal preparation design did not affect the microleakage level.

Table 3. Microleakage scores in specimens

Group	n (section)	0	1	2	3
Empress-Shoulder-Multilink	80	15	38	25	2
Empress-Shoulder-RelyX	80	41	35	4	-
Zirkon-Chamfer-Multilink	80	5	31	36	8
Zirkon-Chamfer-RelyX	80	33	36	11	-
Empress-Chamfer-RelyX	80	38	38	4	-
Empress-Chamfer-Multilink	80	12	36	30	2
Zirkon-Shoulder-Multilink	80	7	33	34	6
Zirkon-Shoulder-RelyX	80	35	38	7	-

Table 4. Mean, minimum, maximum and standart deviation value for each group

Group	n	Mean	Min.	Max.	SD
Empress-Shoulder-Multilink	10	1.177	0.88	1.5	0.0651
Empress-Shoulder-RelyX	10	0.527	0.38	0.75	0.0361
Zirkon-Chamfer-Multilink	10	1.59	1.13	2	0.0873
Zirkon-Chamfer-RelyX	10	0.702	0.5	1.0	0.0562
Empress-Chamfer-RelyX	10	0.577	0.5	0.88	0.0388
Empress-Chamfer-Multilink	10	1.29	0.88	1.63	0.0746
Zirkon-Shoulder-Multilink	10	1.452	1.0	1.88	0.09
Zirkon-Shoulder-RelyX	10	0.653	0.5	1.0	0.0447
Total	80	0.996	0.38	2.0	0.05

Min.: Minimum, Max.: Maximum, SD: Standard deviation

There are studies evaluating this relationship based on the low level of microleakage at the crown-cement interface associated with the small marginal gap (10). Asavapanumas and Leevailoj (11) examined the marginal fit of IPS eMax, Cercon and Lava systems and found the best fit values in the Cercon group and the highest mismatch values in the Lava group. Use of optical scanner in Lava system over use of laser scanner in Cercon system creates a difference which may affect marginal compliance. Mou et al. (12) indicated that the triangular region between the axial wall and the margin cannot be scanned by the camera in the posterior region due to the angulation of the camera in the intraoral scan, and this would negatively affect the marginal alignment. They reported this as the 'distal shadow' phenomenon. Despite advances in computer-aided design/computer-aided manufacturing (CAD/CAM) technology, software and hardware limitations during restoration design adversely affect marginal fit, and access to the incisal and inner regions may be limited during the milling phase. The eMax Press technique is comprised of fewer steps compared to CAD/CAM systems, and the compatibility of the restoration is more related to the technician's sensitivity and experience. Lower microleakage values of the eMax Press in our study is believed to be from the marginal accuracy of the restorations. Pressable ceramics are based on the preparation of a wax sample directly on the die model obtained from the prepared tooth. Since the pressing is done under pressure and vacuum in the molten porcelain ingot, the details, especially on the margins, are accurately obtained. Shrinkage occurs at a very low rate (0.2%) since the thermal shrinkage of the restoration is compensated by the soft porcelain mass in the casting cone during cooling after pressing, and the thermal expansion of the special investment used (13). In this way, restorations with dimensional stability are obtained.

Chamfer and rounded shoulder finish lines are highly recommended for all-ceramic restorations; however, there is no consensus on which design is better. There are different results from studies evaluating marginal fit which may be due to differences in margin preparation style and restoration materials (14). Komine et al. (15) evaluated the effect of shoulder, rounded shoulder and chamfer preparation on internal compliance in zirconium dioxide-based restorations and reported that internal compliance was significantly lower in shoulder ending design. In our study, rounded shoulder and chamfer design were applied; and even though microleakage values were found to be lower in the samples with rounded shoulder group, no significant difference was found between the two. In the rounded shoulder marginal design, defects and openings in the marginal region can be determined more easily than in the chamfer marginal design, accordingly the marginal gap can be reduced and the microleakage level can be minimized (16).

Resin and bonding procedures are as effective as the degree of bond between tooth and restoration. Application of acid and primer to the tooth surface can strengthen the bond. In self-etch systems, acid and primer application care can be combined in one step and requires an additional step before cementation (5). In order to reduce these steps that require clinical sensitivity, self-adhesive systems have been developed; they can simultaneously demineralize

enamel and dentin thanks to the acidic nature of phosphoric methacrylates in functional monomer components. In this way, micromechanical bonding takes place (17). Chemical bonding also occurs as a result of the reaction of phosphate groups in the functional monomer composition and hydroxyapatite in dental hard tissues (18). In our study, RelyX cement also showed lower microleakage values than Multilink N. The multifunctional orthophosphoric acid methacrylates in RelyX cement are believed to interact with the tooth surface and provide an effective seal. This result may be due to the less acidification of the tooth surface by Multilink and a weaker resin infiltration and hybrid layer formation. In addition to the complex structures formed by calcium ions, it is accepted that various physical interactions such as hydrogen bonds or dipole-dipole bonds are effective in self-adhesion (19).

Conclusion

This study demonstrates that ceramic restorations containing lithium disilicate have lower microleakage than zirconia restorations. Self-adhesive cements may be preferred in restorations over self-etch resin cements to reduce microleakage. For clinical application in the marginal design selection both rounded shoulder and chamfer types can be recommended.

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Ethics

Ethics Committee Approval: The ethical approval for this study was acquired from the Selçuk University Faculty of Dentistry Non-invasive Clinical Research Evaluation Commission (meeting number: 2012/05, date: 03.05.2012).

Informed Consent: Informed consent is not required.

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Authorship Contributions

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Original Article / Özgün Araştırma

The Level of Serum Uric Acid as Evidence of Endothelial Dysfunction in Normal Weight and Obese Children with Primary Hypertension

Hipertansiyonu Olan Normal Ağırlıklı ve Obez Çocuklarda Endotel Disfonksiyon Göstergesi Olarak Serum Ürik Asit Düzeyinin Yeri

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Keywords
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Abstract

Objective: Primary hypertension (HT) and obesity with a global increase in prevalence in children has become a critical health problem. HT and obesity are among the well-established cardiovascular risk factors. In this study, we investigated the relationship between arterial stiffness (AS) and serum uric acid (UA) levels in normal-weight and obese children with primary HT.

Materials and Methods: This prospective study was conducted at the university hospital between September 2016 and September 2017. The children aged 6-18 years and recently diagnosed with HT (n=77) were categorized into two groups based on body mass index values as group 1 (HT + obese) (n=46) and group 2 (HT + normal weight) (n=31). The control group (n=35) consisted of age and gender-matched healthy children. The AS of all children included in the study was measured by ossilometric methods using mobil-O-Graph.

Results: A total of 112 children with a mean age of 13.2±3.1 years and most males (61.6%) were included in this study. The values of pulse wave velocity (PWV), central systolic blood pressure (SBP)- diastolic blood pressure (DBP), peripheral SBP-DBP, mean BP, and cardiac output in both HT groups (HT + normal weight and HT + obese) were significantly higher than those in the control group ($p<0.05$). The pulse wave reflection (%) was the lowest in the HT + obese group. When the serum UA levels were compared among the groups, the highest value was found in the HT + obese group.

Conclusions: A higher AS-PWV was detected in the normal weight and the obese children with a recent diagnosis of primary HT compared with the control group.

Öz

Amaç: Çocuklarda primer hipertansiyon (HT) ve obezite, prevalansının tüm dünyada artmasıyla birlikte kritik bir sağlık sorunu haline gelmiştir. HT ve obezite, iyi bilinen kardiyovasküler risk faktörleri arasındadır. Bu çalışmada, primer HT'si olan normal kilolu ve obez çocuklarda arteriyel sertlik (AS) ile serum ürik asit (UA) düzeyleri arasındaki ilişkiyi araştırmayı amaçladık.

Gereç ve Yöntemler: Bu prospektif çalışma Eylül 2016 ve Eylül 2017 tarihleri arasında bir üniversite hastanesinde yapıldı. Altı - on sekiz yaş arası ve yakın zamanda HT tanısı almış (n=77) çocuklar, vücut kitle indeksi değerlerine göre grup 1 (HT + obez) (n=46) ve grup 2 (HT + normal kilo) (n=31) olarak iki gruba ayrıldı. Yaş ve cinsiyet açısından aynı olan sağlıklı çocuklar kontrol grubunu (n=35) oluşturdu. Çalışmaya dahil edilen tüm çocukların arter sertliği Mobil-O-Graph kullanılarak ossilometrik yöntemlerle ölçüldü.
Bulgular: Ortalama yaşları 13,2±3,1 yıl olan ve çoğu erkek (%61,6) olan toplam 112 çocuk bu çalışmaya dahil edildi. Her iki HT grubunda (HT + normal ağırlık ve HT + obez) nabız dalga hızı (PWV), santral sistolik kan basıncı (SKB)- diyastolik kan basıncı (DKB), periferik SKB-DKB, ortalama KB ve kardiyak debi değerleri kontrol grubundan anlamlı derecede yüksekti (p<0,05). Nabız dalga yansıması (%), HT + obez grubunda en düşüktü. Gruplar arasında serum UA düzeyleri karşılaştırıldığında, en yüksek değer HT + obez grubunda bulundu.
Sonuç: Normal kilolu ve yakın zamanda primer HT tanısı alan obez çocuklarda kontrol grubuna göre daha yüksek AS-PWV saptandı.

Introduction

Primary hypertension (HT) with a global increase in prevalence in children has become a critical health problem (1-3). Obesity, which has an increasing frequency in childhood, is primarily an essential factor among the underlying causes of pediatric HT. Obesity seems to be a fundamental problem in deve loped and developing countries like Turkey (4). Several mechanisms have been proposed for the explanation of increased blood pressure (BP) in obese people (5). Along with an earlier age for childhood obesity, which has been reduced to levels of 6-7 years, the age for primary HT has also decreased to the levels of six years (6).

The measurement of arterial BP is a component of physical examination, it only provides information about the peripheral arteries. Therefore, the measurement of central aortic pressure and pulse-wave analysis by non-invasive methods like arterial stiffness (AS) have been suggested for a detailed assessment of vascular structures in childhood (7). However, those methods have not been in routine use yet but are mostly reserved for studies in high-risk patients. Moreover, the research on that subject is too limited (7). The increase in uric acid (UA), the end product of purine metabolism, is a risk factor for HT in children and adults (5). UA is known to cause HT by extreme vasoconstriction via increasing renin release and reducing endothelial nitric oxide production (5,8).

In this study, we aimed to investigate the relationship between AS and serum UA levels in normal-weight and obese children with primary HT.

Materials and Methods

This prospective study was conducted in the Department of Pediatrics, University Hospital between September 2016 and September 2017. The study, funded by the University Scientific Research Projects (#TPF-17018), was approved by the Ethics Committee of Aydın Adnan Menderes University Faculty of Medicine (protocol no: 2016/982, date: 29.09.2016).

The power analysis, according to the study by Tokgöz et al. (3), showed that the sample size for each study group should be a minimum (min) of 24 people for an effect size of 5.3, an alpha of 5%, and a statistical power of 90%.

BP was measured by the same researcher during the outpatient visit via auscultation method using an aneroid manometer and an appropriate-sized cuff. The primary HT group consisted of the patients with BP values ≥95 percentile determined for age, gender, and height. The final BP value of a patient was estimated by using the mean value of two measurements at different times (8). The children with body mass index (BMI) ≥95 were defined as obese and the ones within the 5-84 percentile as normal weight according to the growth charts prepared for age and gender by the Center for Disease Control and Prevention (9).

The children aged 6-18 years and recently diagnosed with HT (n=77) were categorized into two groups based on BMI values as group 1 (HT + obese) (n=46) and group 2 (HT + normal weight) (n=31). The control group (n=35) consisted of age and gender-matched healthy children presented to the outpatient clinics for well-visits and whose BMI values were within 5-84 percentile and BP values were <90 percentile. The BP of children in the control group was measured twice in a 30-minute interval by the same nurse using an aneroid manometer, and the mean of two measurements was recorded as the final value. Informed consent forms were obtained from the patients and their families.

The exclusion criteria of the study included previous or secondary HT diagnosis, secondary obesity, the

use of antihypertensives or any other medications for obesity, the presence of a genetic syndrome, diabetes mellitus, an and active infection in children aged ≤5 and ≥19 years. And also, children with office BP ≥95th percentile but ambulatory BP monitorization (ABPM) BP <95th percentile and BP load <25% were excluded from the study.

Ambulatory Blood Pressure Monitorization (ABPM)

The children with a recent diagnosis of primary HT had been monitored for BP in inpatient clinics. The monitorization of BP was performed for 24 hours using a Welch Allyn-24 hour ABPM (Version 12.0) on the right arm with an appropriate sized cuff in 20- and 30-minute intervals during awake and sleep times, respectively. The same pediatrician measured and evaluated the BP of each patient. The reference values were used to compare the recorded ABPM data, and the values ≥95th percentile and BP load ≥25% were accepted as HT. A load of BP was determined using the number of values that exceeded the 95th percentile for age, height, and the selected days (10).

Pulse Wave Analysis

Many studies reported that AS could accurately be evaluated using an automated oscillometric device (Mobil-O-Graph) (3). The velocity of the pulse wave is calculated by dividing the distance of the wave by the time used for that distance meter/second (m/sec) (1-3). When there is stiffness in the artery or a change in vascular tone, the pulse wave velocity (PWV) increases because of the reduction in the reflection of the produced wave (1).

The AS of all children included in the study (n=112) were measured by ossilometric methods using Mobil-O-Graph (I.E.M, Industrielle Entwicklung Medizintechnik und Vertriebsgesllschaft mbH, Stolberg, Germany). The mean value of three measurements with 15-minute intervals in the sitting position for each child was calculated. The measurements were uploaded to the computer using the I.E.M program and the Bluetooth function of the Mobil-O-Graph device. The central aorta systolic and diastolic pressures were determined. The vascular structures were evaluated by the pulse wave AIx@75, the peripheral resistance, the reflection of the pulse wave, and PWV.

The serum UA levels were measured in the Biochemistry Laboratory of university hospital using the autoanalyzer (C8000 Architect, Abbott, Abbott Park, IL, USA) and the commercial kits provided by the manufacturer.

Statistical Analysis

IBM SPSS (SPSS, Chicago, Illinois, USA) software was used for statistical analyses. Kolmogorov-Smirnov test was used to examine the normal distribution of quantitative variables. The intergroup comparison for normally distributed variables in independent groups was performed using either t-test or one-way variance analysis considering the number of groups, and descriptive statistics were shown as mean ± standard deviation. Mann-Whitney U or Kruskal-Wallis tests were used to comparing groups that did not distribute normally, and descriptive statistics were shown as median (25-75 percentile). Qualitative data were analyzed using the chi-square test, and descriptive statistics were shown as frequency (%). Spearman correlation analysis was used for the associations between variables. Statistical significance was accepted when the p-value was below 0.05.

Results

A total of 112 children with a mean age of 13.2±3.1 years [min-maximum (max): 6-18 years] and most males (61.6%) were included in this study. The number of cases with primary HT was 77, while 35 children were normotensive. The hypertensive patients were classified into two groups as HT + obese (n=46) and HT + normal weight (n=31) when the BMI value was ≥95th and 5-85 percentile, respectively. Table 1 demonstrates the demographics of participants. No statistically significant differences in gender, age, and height were found among the three groups (p>0.05). The weight and BMI values were the highest in the HT + obese group, as expected. The diagnostic SBP and diastolic BP (DBP) measurements in the children with primary HT were statistically significantly higher than those in the control group.

Especially for ABPM evaluation, the nocturnal SBP/DBP load was found to be relatively high in two hypertensive groups. However, no significant difference in ABPM values was found between the two HT groups (p>0.05).

The values of PWV, central SBP-DBP, peripheral SBP-DBP, mean blood pressure and cardiac output in both HT groups (HT + normal weight and HT + obese) were significantly higher than those in the control

group (p<0.05). The pulse wave reflection (%) was the lowest in the HT + obese group (p=0.03) (Table 2).

The median AS-PWV value of participants was 4.8 m/sec (25th-75th percentile: 4.4-5.1). Logistic regression analysis revealed that the PWV value increased (>4.8 m/sec) 9.7, 2.6, and 1.2 times by the presence of HT, female gender, and advanced age, respectively (Table 3).

In hypertensive children, the AS-PWV value showed positive correlations with daytime load of SBP (r=0.287, p=0.011), daytime load of DBP (r=0.239, p=0.036), nocturnal load of SBP (r=0.233, p=0.041) and nocturnal load of DBP (r=0.343, p=0.002).

When the serum UA levels were compared among groups, the highest value was found in the HT + obese group (p<0.001, Figure 1). The serum UA levels in HT + obese, HT + normal weight, and the control groups were 6±1.1, 4.8±1.2, and 4.5±1.1 mg/dL, respectively.

Moreover, the serum UA levels in hypertensive children (n=77) showed positive correlations with PWV (r=0.233, p=0.042) and cardiac output (r=0.455, p=0.001) and negative correlations with (AIx@75) (r=-0.349, p=0.002), augmentation pressure (r=-0.284, p=0.012), and reflection magnitude (r=-0.254, p=0.026). Besides, the serum UA levels in hypertensive children had positive correlations with daily SBP (r=0.351, p=0.002), nocturnal SBP (r=0.315, p=0.005), nocturnal DBP (r=0.257, p=0.024) and nocturnal load of SBP (r=0.275, p=0.016).

Discussion

In this study, the AS-PWV measurements in normal weight and obese children with a recent diagnosis of primary HT were found significantly higher than those in the control group. Additionally, serum UA level, which is a critical indicator for endothelial damage, was the highest in the HT + obese group. Moreover, serum UA levels had shown positive correlations with nocturnal load of SBP and DBP of ABPM.

In our study, which evaluated 77 children aged between 6 and 18 years and with a recent diagnosis of primary HT, the rates of obesity and normal weight

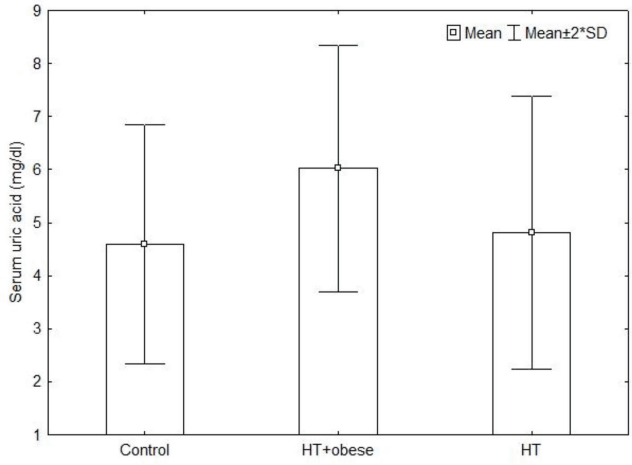

Figure 1. Comparison of serum uric acid levels for three groups
HT: Hypertension, SD: Standard deviation

Table 1. The demographic data of the patients and control group

Variables	HT + Normal weight (n= 31)	HT + Obese (n=46)	Control (n=35)	p-value
Age (year)	14 (10-16)	14 (12-16)	14 (11-15)	0.777
Gender (male/female) (n) (%)	20/11 (65/35)	29/17 (63/37)	20/15 (57/43)	0.800
Height (cm)	164 (157-172)	166 (146-174)	160 (149-170)	0.403
Height SDS	0.57 [(-)0.29-1.19]	0.42 [(-)0.37-1.26]	0.21 [(-)0.69-0.87]	0.156
Body weight (kg)	63 (43.5-71.5)	89 (75.1-103.2)	51,5 (40-64)	<0.001*
Body weight SDS	0.76 (0.3-1.28)	3.07 (2.22-3.76)	-0.04 [(-)0.8-1.07]	<0.001*
BMI (kg/m²)	22.3 (19.7-24.5)	30.8 (28.9-36.6)	20 (17.6-22.9)	<0.001*
BMI SDS	0.66 (0.03-1.07)	2.64 (2.22-3.23)	0.03 [(-)0.63-0.76]	<0.001*
Office auscultatory systolic BP (mmHg)	130 (125-135)	135 (130-140)	110 (100-115)	<0.001**
Office auscultatory diastolic BP (mmHg)	70 (65-80)	75 (70-80)	65 (60-70)	<0.001**

Data are mean ± SD or median (25-75 persantile)
HT: Hypertension, BMI: Body mass index, BP: Blood pressure, SDS: Standard deviation score
*HT + Obese versus HT + Normal weight and control
**Control versus HT + Normal weight and HT + obese

were 59.7% and 40.3%, respectively. In line with the literature, we observed that obesity has an essential role in children with HT.

In our study, both HT groups (normal weight and obese) had oscillometric ABPM. We observed that especially the nocturnal SBP and DBP loads were approximately 33-50% without any significant difference between the two HT groups. Conclusively, a critical nocturnal BP load and end-organ damage risk were demonstrated in both the normal weight and obese groups. In ABPM, the expected reduction in nocturnal BP had not been observed in 50% of the children with obesity (11). Supporting the literature, our study did not show any nocturnal reduction in BP in both HT groups. Thus, we suspect that the risk for end-organ damage would be relatively higher in those recently diagnosed patients in case of an uncontrolled BP.

Studies have found that the main determinant of primary HT is obesity. A significant relationship was determined between increased BMI and the increase in the prevalence of HT, and essential HT was detected in approximately 30% of children with obesity (10). In a study from our country, the prevalence of obesity was estimated as 8.9%, and the prevalence of HT in obese children (11.4%) was found to be approximately twice more than the children with normal weight (5.6%). The mean BMI of obese children in our study was 30.8 kg/m^2. Conclusively, we suspect that the increased BMI observed in obese children would contribute to chronic kidney disease in the future if weight loss in those children could not be achieved (10). Endothelial dysfunction has an important place among factors that cause atherosclerosis. Atherosclerosis usually has a childhood-onset while the related clinical problems

Table 2. The comparison of arterial stiffness among groups

Variables	HT + Normal weight (n=31)	HT + Obese (n=46)	Control (n=35)	p-value
PWV (m/s)	4.9 (4.6-5.1)	5 (4.7-5.2)	4.4 (4.2-4.7)	<0.001*
AIx@75 (%)	25.5±11.6	24±9.4	20.6±11	0.163
Peripheral SBP (mmHg)	122.6±11.5	127±13.9	108.8±10.7	<0.001*
Peripheral DBP (mmHg)	73 (65-76)	70.5 (65.7-78.2)	67 (63-69)	0.002*
MAP (mmHg)	93.6±7.4	96.1±9	84.4±7	<0.001*
Pulse	89.2±18.1	91.8±12.8	83.2±13.5	0.036**
Central SBP (mmHg)	105.4±9.6	106.7±10.4	96±9.6	<0.001*
Central DBP (mmHg)	70 (62-74)	68 (63.7-75)	65 (61-67)	0.003*
Cardiac output (lt/min)	5 (4.5-5.5)	5.5 (5-5.8)**	4.6 (4-5.3)	<0.001*
Pulse wave reflection (%)	59 (49-64)	53 (42.7-60.5)	56 (49-63)	0.03***
Augmentation Pressure (mmHg)	6 (4-8)	5 (3-7)	5 (3-7)	0.171

Data are mean ± SD or median (25-75 percentile)

HT: Hypertension; PWV: Pulse wave velocity, AIx@75: Augmentation index, BP: Blood pressure, MAP: Mean arterial pressure, SBP:Systolic blood pressure, DBP: Diastolic blood pressure

*Control versus HT + Obese and HT + Normal weight

**HT + Obese versus Control

***HT + Obese versus HT + Normal weight and control

Table 3. The logistic regression analysis of risk factors that affect the pulse wave velocity in arterial stiffness measurements (>4.8 m/s)

Dependent variable	Independent variable	OR	95% CI for OR		p-value
			Low	High	
AS PWV	Gender (Female)	2.647	1.049	6.679	0.039
	Age	1.233	1.063	1.429	0.006
	Group (HT)	9.728	3.534	26.782	0.001

AS: Arterial stiffness, PWV: Pulse wave velocity, HT: Hypertension, OR: Odds ratio, CI: Confidence interval

generally appear in middle-to-older ages. Thus, the evaluation of the vascular structure is essential in childhood (10,11).

The measurement of AS is a method that aids identifying the atherosclerosis risk before any clinical symptoms and the prognosis of present atherosclerosis. The endothelial damage and atherosclerosis commonly seen in hypertensive patients are the most important causes of end-organ damage. Recently, the PWV and augmentation index have been frequently used to assess AS (12). In our study, we evaluated the AS measurements using the oscillometric method.

The PWV, which gives information about the vascular structures, is the most commonly used parameter in non-invasive AS evaluation (13). The PWV is low in elastic vessels, and the reflection wave reaches to aortic root in the diastole. In the case of AS, the PWV increases, and the reflection wave which reaches the aorta earlier combines with the forward wave and causes an increase in SBP.

The threshold value for PWV has been stated as 10 m/sec for the adults in the ESH-ESC guideline published in 2013. Also, measurements over 12 m/sec in middle-aged hypertensive patients were reported to indicate vascular dysfunction (14). Although there have been some studies on children to identify the threshold value for PWV, there is still no consensus on a typical value. In our study, the PWV values measured in both hypertensive groups (4.9 and 5 m/sec) were significantly higher than those of the control group (4.4 m/sec). Therefore, we suspect the presence of AS in the children with a recent diagnosis of HT, in terms of their being normal weight or obese, in our study. We also detected that HT, being of the female gender and having advanced age, caused the PWV value >4.8 m/sec with 9.7, 2.6, and 1.2 times more recently. Additionally, we observed that AS measurements correlated with both the ABPM measurements and the serum UA levels.

Hyperuricemia causes HT by endothelial dysfunction, mild tubulointerstitial damage, and inflammation via increasing renin and decreasing plasma nitric oxide levels. In HT evaluation of the pediatric patients, the serum UA level ≥5.5 mg/dL indicates essential HT strongly. In the obese patients, serum UA levels increase three times more than the levels in the normal-weight patients. In our study, we detected the maximum serum UA levels (6±1.1 mg/dL) in the HT + obese group. The serum UA level in the normal weight HT patients (4.8±1.2 mg/dL) was lower than those reported in the literature. We consider that physicians should pay attention to the elevation in UA levels in the obese HT children as an important parameter for end-organ damage during diagnosis. Additionally, we observed that the serum UA tested during the diagnostic studies correlated with most of the AS measurements (PWV, peripheral SBP/DBP, augmentation pressure, cardiac output) in both the normal weight and the obese HT children.

Among the limitations of our study, the most important one was the small size of the study group.

Conclusion

Conclusively, a higher AS-PWV was detected in the normal weight and the obese children with a recent diagnosis of primary HT compared to the control group in this study. Additionally, serum UA, an indicator of endothelial damage, was found to be at maximum levels in the HT + obese group. Therefore, we consider that the HT + obese patients who have both AS and endothelial damage should be attentively taken care of for the development of atherosclerosis. Therefore, more extensive studies are needed.

Ethics

Ethics Committee Approval: The study was approved by the Ethics Committee of Aydın Adnan Menderes University Faculty of Medicine (protocol no: 2016/982, date: 29.09.2016).

Informed Consent: Informed consent forms were obtained from the patients and their families.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

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

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Original Article / Özgün Araştırma

Surgical Treatment of Intracardiac Masses: A Single Center Experience

İntrakardiyak Kitlelerin Cerrahi Tedavisi: Tek Merkez Deneyimi

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Abstract

Objective: Intracardiac masses are highly rare and challenging to diagnose and treat. Surgical resection is typically the only treatment option for most simple primary cardiac masses upon diagnosis. In this retrospective study, we provided our clinical experience, surgical technique, and early and midterm outcomes for patients who underwent surgery for intracardiac mass.

Materials and Methods: Ten patients who underwent surgical treatment in our department because of an intracardiac mass over a three-year period were included in the study. Under general anesthesia, all surgeries were conducted electively by median sternotomy.

Results: The mean age of the patients was 61.8±15.25 years. In eight patients, a left atriotomy was performed, whereas two individuals underwent a right atriotomy. Mitral valve replacement was the most prevalent concomitant procedure (n=3, 30%), followed by coronary artery bypass grafting (n=2, 20%) and patent foramen ovale repair (n=1, 10%). Two patients died from non-cardiac causes during the early postoperative period. The median follow-up duration was 45.57 months and during the follow-up period, no patient experienced a recurrence.

Conclusions: Cardiac masses can present with various clinical symptoms, and when required, successful surgical excision with low morbidity and mortality can be performed with the help of preoperative advanced imaging techniques and careful clinical evaluation.

Keywords
Intracardiac, mass, surgery

Anahtar Kelimeler
İntrakardiyak, kitle, cerrahi

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Öz

Amaç: İntrakardiyak kitleler oldukça nadir olup tanı ve tedavisi zordur. Cerrahi rezeksiyon, tanı konulduktan sonra basit primer kardiyak kitlelerin çoğunluğu için tek tedavi seçeneğidir. Bu retrospektif çalışmada intrakardiyak kitle nedeniyle cerrahi tedavi uygulanan hastalarda klinik deneyimimizi, cerrahi tekniğimizi, erken ve orta dönem sonuçlarımızı sunmayı amaçladık.

Gereç ve Yöntemler: Çalışmaya üç yıllık süre içinde intrakardiyak kitle nedeniyle kliniğimizde cerrahi tedavi uygulanan 10 hasta dahil edildi. Tüm ameliyatlar genel anestezi altında, elektif olarak, medyan sternotomi ile yapıldı.

Bulgular: Hastaların yaş ortalaması 61,8±15,25 yıl idi. Sekiz hastada sol atriyotomi, iki hastada sağ atriyotomi yapıldı. Mitral kapak replasmanı en yaygın eşlik eden prosedürdü (n=3, %30), bunu koroner arter baypas greftleme (n=2, %20) ve patent foramen ovale onarımı (n=1, %10) izledi. Ameliyat sonrası erken dönemde iki hasta kalp dışı nedenlerle kaybedildi. Medyan takip süresi 45,57 aydı ve takip süresi boyunca hiçbir hastada nüks görülmedi.

Sonuç: Kardiyak kitleler çok çeşitli klinik semptomlarla karşımıza çıkabilmekte ve gerektiğinde preoperatif ileri görüntüleme teknikleri ve dikkatli klinik değerlendirme ile düşük morbidite ve mortalite ile başarılı cerrahi eksizyon yapılabilmektedir.

Introduction

Intracardiac masses are remarkably rare and difficult to manage. Cardiac masses are comprised of primary and secondary tumors, thrombotic, infectious, and congenital. Primary cardiac tumors are likewise infrequent, with an estimated incidence of 0.001% to 0.3% in large autopsy series (1). Few of these tumors are malignant, whereas the most are benign. Myxomas account for the majority of benign tumors, followed by papillary fibroelastomas and fibromas. Primary malignant heart tumors are mostly sarcomas with angiosarcoma and undifferentiated sarcomas being the most frequent (2,3). Although most cases of primary cardiac tumors are sporadic, some cases based on genetics, such as myxomas, have been reported. Carney complex, a familial syndrome linked to a mutation in the *PRKAR1A* gene, accounts for 3-10% of cardiac myxomas (4). Sporadic cardiac myxomas develop 2-4 times more frequently in women than in men and are most typically located in the left atrium (5).

Due to its stealthy beginning, the clinical presentation of cardiac masses poses a significant diagnostic problem. Depending on the affected area and size of the lesion, patients present with a variety of symptoms, including dyspnea, heart failure, embolic events, and rhythm problems (5). Medical history, physical examination, echocardiographic data, laboratory results, and radiographic imaging are used to make a diagnosis in patients with intracardiac masses. Transthoracic echocardiography (TTE), transesophageal echocardiography (TEE), magnetic resonance imaging (MRI), and computed tomography (CT) are the four most common imaging modalities used to make differential diagnosis of cardiac masses. Upon diagnosis, surgical resection is frequently the sole therapeutic option for the majority of simple primary cardiac tumors.

In this retrospective study, we aimed to present our clinical experience, surgical strategy and early and mid-term outcomes of patients who were operated on for intracardiac mass over a 3-year period.

Materials and Methods

The study included 10 patients who underwent cardiac surgery in our department due to an intracardiac mass between January 2017 and December 2019. The study was carried out retrospectively by collecting the patients' demographic data and clinical parameters as well as operative details from hospital records. An experienced cardiologist performed TTE on all of the patients. The Cardiology - Cardiovascular Surgery Council decided on the operation based on the echocardiographic recordings and the clinical and comorbid characteristics of the patients.

Patients with angina, impaired left ventricular (LV) systolic function, objective evidence of ischemia, history of coronary artery disease, or coronary risk factors (including males over 40 years old and postmenopausal women) were scheduled for coronary angiography prior to surgery. Coronary angiography is crucial for showing the feeding artery in cardiac masses as well as providing information regarding coronary artery disease. Genetic screening was not performed in patients diagnosed with myxoma.

The study protocol was approved by the Local Ethics Committee of the Selçuk University Medical Faculty (Approval number: 2022/267 and approval date: 24.05.2022). Each patient signed a written informed consent form. The study was performed in conformity with the Declaration of Helsinki's principles.

Echocardiographic Assessment

In this study all patients underwent transthoracic echocardiographic examination by Vivid E9 (Vivid E9, GE Healthcare, Milwaukee, Wisconsin) echocardiography device with a 1.5-4.5 MHz transducer. TEE was performed using GE Vivid i echocardiography device with a 3.0-8.0 MHz transducer (GE Healthcare, Milwaukee, Wisconsin). All echocardiographic examinations were performed by the same experienced operator. Ejection fraction, LV end-systolic, and end-diastolic diameters, valvular regurgitations and specific features of cardiac masses were noted.

Surgical Procedure

All procedures were performed electively via median sternotomy under general anesthesia. Following aorto-bicaval cannulation, caval snares were placed around both vena cava, cardiopulmonary bypass was initiated and moderate hypothermia was chosen. To prevent systemic embolization, the heart was minimally manipulated prior to aortic cross-clamping at this stage of the operation. After the aorta was cross-clamped, cold blood cardioplegia was administered to achieve diastolic cardiac arrest.

Patients with right atrial mass underwent right atriotomy (n=2, 20%) while those with left atrial mass underwent left atriotomy (n=8, 80%) via the interatrial groove (Figure 1). The pedicle of the mass was resected with a wide excision together with the attached endocardial tissue. Efforts were made to remove the masses in a gentle, non-crumbling manner. It was found that the removed masses ranged in size from approximately 2.5 to 8 centimeters (Figure 2). The excised masses were transported under suitable conditions to the Department of Pathology for evaluation. Following mass removal, the heart cavities were irrigated with cold isotonic sodium chloride carefully. The incisions (left/right atriotomy) were then closed with a continuous suturing technique. Three patients underwent concomitant mitral valve replacement, two had coronary artery bypass grafting surgery, and one had patent foramen ovale closure. Following appropriate de-airing techniques, the aortic cross clamp was removed. All surgical procedures were accomplished without any complications.

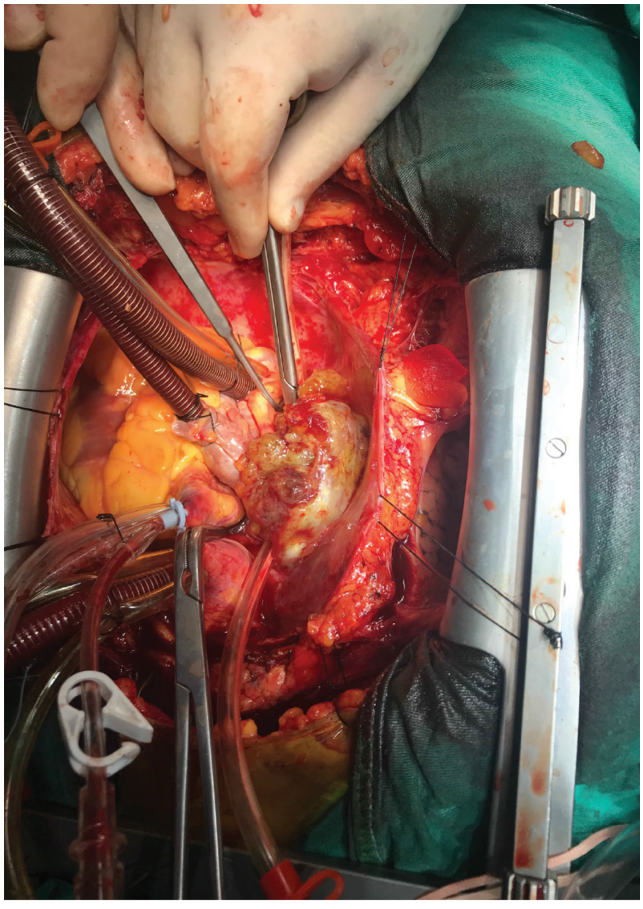

Figure 1. Following left atriotomy, a huge intracardiac mass appeared

Statistical Analysis

IBM SPSS Statistics for Windows, version 22.0 (IBM Corp., Armonk, N.Y., USA) was used to conduct statistical analysis. For normally distributed variables, mean and standard deviation were used, whereas for data with a non-normal distribution, median and interquartile ranges were employed. Nominal variables were expressed as a number and a percentage. Using the Kolmogorov-Smirnov test, a normality analysis was performed.

Results

The demographic, clinical and echocardiologic characteristics of the patients are presented in Table 1. The mean age of the patients included in the study at the time of diagnosis was 61.8±15.25 years. The majority of the patients (n=9, 90%) were female. The most prevalent symptom was dyspnea (n=6, 60%), while angina, palpitation and stroke were reported in 2, 1 and 2 individuals, respectively. TTE was used to evaluate all patients. However, 4 (40%) patients also underwent TEE, and 3 (30%) underwent MRI. Eight (80%) patients who met the criteria outlined in the Material and Methods section had coronary angiography prior to surgery.

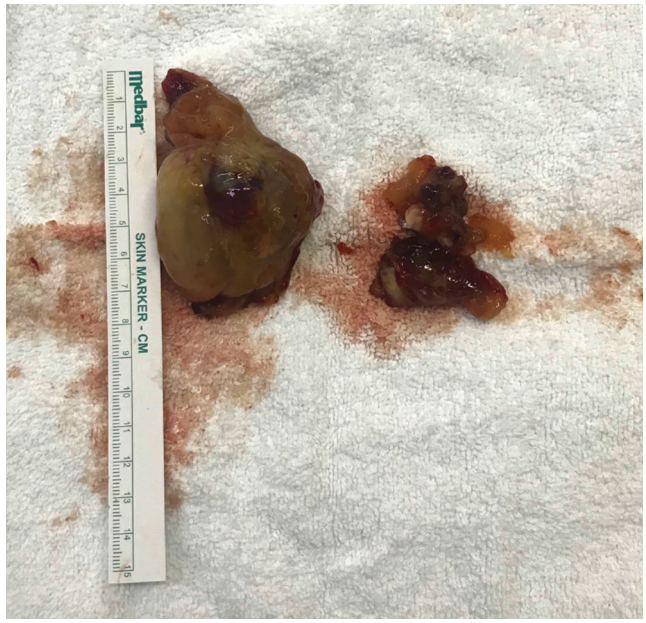

Figure 2. The resected intracardiac mass measured 8 centimeters in length

Table 2 details the surgical and postoperative data of the patients. The most common concomitant procedure was mitral valve replacement (n=3, 30%), followed by coronary artery bypass grafting (n=2, 20%) and patent foramen ovale repair (n=1, 10%). The mean time for X-clamp and cardiopulmonary bypass was 50.9±24.63 and 70.1±31.23 minutes, respectively. Only one patient had a positive inotrope need following surgery. Intensive care unit length of stay was 2 days (1.75-7) [median (25-75 percentile)], whereas hospital length of stay was 9.5 days (7-17.5) [median (25-75 percentile)]. The median follow-up duration was 45.57 months. None of the patients suffered a recurrence over the time of follow-up. Two patients died in the hospital after surgery. One of the patients was 88 years old, had chronic renal failure, and was in poor general condition before the procedure. She died on the 12th postoperative day due to non-cardiac reasons. The other patient who died at the hospital was 75 years old and her general condition was poor before the operation. This patient also underwent mitral valve replacement and coronary artery bypass grafting surgery in addition to the intracardiac mass excision. The patient died on the 14th postoperative day due to respiratory failure. Two patients died during follow-up period. One of the patients had chronic obstructive pulmonary disease and died as a result of it and the other patient died 52 months after surgery from complications related to dialysis.

Table 3 summarizes the related cardiac structure, localization, and histopathologic diagnosis of the resected cardiac masses. The most common

Table 1. The demographic and echocardiologic characteristics of the patients

	Patients (n=10)
Male	1 (10%)
Age (years)	61.8±15.25
Symptoms Dyspnea Angina Palpitation Stroke	 6 (60%) 2 (20%) 1 (10%) 2 (20%)
Hypertension	6 (60%)
Diabetes mellitus	4 (40%)
Chronic renal failure	2 (20%)
Previous systemic embolism	2 (20%)
Body mass index (kg/m^2)	29.75±5.72
Preoperative rhythm Normal sinus rhythm Atrial fibrillation	 9 (90%) 1 (10%)
Imaging modality Transthoracic echocardiography Transesophageal echocardiography Magnetic resonance imaging	 10 (100%) 4 (40%) 3 (30%)
Preoperative coronary angiography	8 (80%)
Left ventricular ejection fraction (%)	57.2±3.12
Systolic pulmonary artery pressure (mmHg)	35.2±10.58
Left ventricular end-diastolic diameter (mm)	47±3.94
Left ventricular end-systolic diameter (mm)	29.7±2.41

Table 2. Operative and postoperative characteristics of the patients

	Patients (n=10)
Concomitant procedures Mitral valve replacement Coronary artery bypass grafting Patent foramen ovale repair	 3 (30%) 2 (20%) 1 (10%)
Surgical approach Left atriotomy Right atriotomy	 8 (80%) 2 (20%)
X-clamp time (minutes)	50.9±24.63
Cardiopulmonary bypass time (minutes)	70.1±31.23
Postoperative positive inotrope need	1 (10%)
Intensive care unit duration (days) [median (25-75 percentile)]	2 (1.75-7)
Hospital stay duration (days) [median (25-75 percentile)]	9.5 (7-17.5)
Follow-up duration (months) [median (25-75 percentile)]	45.57 (9.69-58.25)
Recurrence	0
In-hospital mortality	2 (20%)
Long-term mortality	2 (20%)

Table 3. Related cardiac structure, localization and histopathologic diagnosis of the excised cardiac masses

Related cardiac structure	Localization	n (%)	Pathological identification
Left atrium	Interatrial septum	4 (40)	Myxoma
	Posterior wall	1 (10)	Myxoma
	Posterolateral wall	1 (10)	Thrombus
Right atrium	Lateral wall	2 (20)	Thrombus
Mitral valve	Posterior annulus	2 (20)	Chronic inflammation

histopathologic diagnosis was atrial myxoma (n=5, 50%), followed by thrombus (n=3, 30%) and chronic inflammation (n=2, 20%). Eight (80%) patients had left sided intracardiac mass while 2 (20%) had right-sided.

Discussion

Cardiac masses consist of primary and secondary tumors, as well as thrombotic, infectious, cystic, and congenital pathologies (1). Primary cardiac tumors are uncommon, with 10% of them being malignant and 90% of them benign, according to data from autopsy series. Myxomas account for the vast majority of benign tumors and are most typically located in the left atrium (2,3). Consistent with previous research, the majority of primary intracardiac masses in our study were atrial myxomas. Left atrial septum was the most common location among the five patients diagnosed with atrial myxomas. In fact, thrombi are the most prevalent intracardiac masses, appearing in the atria and ventricles of patients with cardiovascular pathologies or hypercoagulable conditions as malignancy and intracardiac catheters (2). Systolic dysfunction usually with aneurysm formation, is the most common cause of thrombus of left ventricle in patients with ischemic heart disease and dilated cardiomyopathy. The right atrium and left ventricle were the most common locations for thrombi, whereas the left atrium had the fewest. We excluded patients in whom thrombus was definitively diagnosed prior to surgery. However, in our patient group, three patients who underwent surgery with a preliminary diagnosis of primary cardiac mass were unexpectedly diagnosed with thrombus on histopathologic examination. Thrombus is the first entity that comes to mind when considering a differential diagnosis for cardiac masses. Consequently, while non-invasive techniques such as echocardiography and MRI are advantageous in determining the key features of the cardiac mass, such as location and tissue characteristics, definite identification requires surgery and biopsy. Just after a cardiac mass is diagnosed, immediate surgical intervention is the best course of action. In the period between diagnosis and surgical intervention, mortality may occur due massive embolization or obstruction, according to the literature (6). In our series, the mean duration between diagnosis and surgical intervention was 1.7 days, ranging from 1 to 3 days.

Cardiac myxomas are most common in women in the fourth and sixth decades of life (7), a finding consistent with our patient series.

In patients with cardiac mass, the typical triad of obstructive cardiac sign, embolic event, and constitutional manifestation determines the symptoms and presentation. Cardiac mass symptoms are generally non-specific, with dyspnea being the most commonly reported sign in patients (5). The presenting symptoms of patients with cardiac masses in our study were dyspnea in six (60%), angina in two (20%), palpitation in one (10%), and stroke in two (20%), similar to the literature. Due to their intracardiac location and fragile nature, cardiac masses might be an embolism source. Myxomas, particularly those found in the left cavities of the heart, have the potential to embolize into the circulation and cause infarction and ischemic symptoms by moving to the cerebral, renal, femoral, coronary, and other visceral arteries (8). Systemic embolism has been documented in the literature in 30 to 45 percent of left atrial myxoma patients. Stroke was the first finding in 2 (20%) of our patients, leading to the diagnosis of cardiac mass.

As a first step in the diagnostic process, cardiac mass should be suspected in patients with persistent dyspnea, a history of systemic embolization, an acute neurological disorder, and a family history of cardiac mass, even though approximately 3.2 to 46.4 percent of patients with cardiac mass are asymptomatic (9). In this patient population, TTE should be considered as a primary diagnostic method. With a sensitivity of 95%, TTE is the most effective initial modality for the diagnosis of myxoma, whereas TEE has a sensitivity of 100% (10). However, to differentiate between a primary cardiac tumor and other cardiac masses, echocardiography is not very accurate. Especially, cardiac myxoma is frequently misdiagnosed with atrial thrombus. For a thorough assessment of cardiac masses, MRI and CT have proven to be an effective technique. These imaging techniques are non-invasive, provide a wide field of view, and enable direct multiplanar imaging. Although echocardiography was used to diagnose all of the patients in our study, MRI was also used in three (30%) of them. Although we thoroughly evaluated the patients prior to surgery by integrating clinical findings and imaging modalities such as TTE, TEE, and MR, the pathological diagnosis in 3 patients was thrombus and in 2 patients it was

chronic inflammation. Especially, the pathological diagnosis of thrombus was unexpected to us, even though the excised cardiac mass in one of our patients was preoperatively thought to be a myxoma due to contrast enhancement on MRI. In addition to imaging techniques, the patient's age, gender, symptoms, and underlying comorbid disorders must be taken into account when evaluating intracardiac masses.

Depending on where the tumor is located, various approaches have been presented in the literature. Left and/or right atriotomy, biatrial and transseptal approaches are commonly the most favoured techniques (11). Key things to consider while selecting the surgical approach to the mass include minimal manipulation of the mass, adequate exploration for resection, and the possibility to entirely remove the mass. We decided our surgical strategy based on the location of the cardiac mass: we performed right atriotomy for right atrial masses and left atriotomy for left atrial masses. With this method, none of our patients experienced complications such as postoperative systemic embolism, arrhythmia, or evidence of inadequate excision in the follow-up period. The advantages of the biatrial method were stated by Jones et al. (11) as direct visibility, little manipulation, appropriate excision, and evaluation of all heart chambers. However, there have been concerns that the high rate of arrhythmias and conduction disturbances following tumor removal may outweigh the benefits of this strategy.

Bahnson and Newman (12) completed the first successful removal of an intracardiac mass with inflow occlusion technique around 400 years after Realdo Colombo described the first cardiac tumor (13). In the years that followed, in 1954, Professor Crafoord successfully excised the left atrial myxoma for the first time by the use of extracorporeal circulation (13). In recent years, minimally invasive procedures have been adopted widely in the field of heart surgery. According to a comparative study by Kadiroğulları et al. (14) robotic-assisted endoscopic surgery for cardiac myxoma excision has been shown to be safe and effective, with benefits including a shorter hospital stay, reduced pain, and less blood product consumption, along with better cosmetic outcomes than conventional median sternotomy. However, concerns have been raised regarding the application of this technique to cardiac mass resection due to the increased manipulation of the mass, which increases the risk of local and systemic embolization, and the limited use for concomitant procedures.

The retrospective design with a small sample size is the major limitation of our study. However, the lack of a control group and the inability to make comparisons are other limitations of the study. It is important to point out that this is because cardiac masses are relatively uncommon.

Conclusion

The cardiac masses can present with a wide variety of clinical manifestations, and when indicated, successful surgical resection can be performed with minimal morbidity and mortality with the assistance of preoperative sophisticated imaging modalities and detailed clinical evaluation.

Ethics

Ethics Committee Approval: The study protocol was approved by the Local Ethics Committee of the Selçuk University Medical Faculty (approval number: 2022/267 and approval date: 24.05.2022).

Informed Consent: Each patient signed a written informed consent form.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Ö.F.Ç., M.B., A.O., H.A., Concept: Ö.F.Ç., M.B., Design: Ö.F.Ç., A.O., Data Collection or Processing: Ö.F.Ç., İ.E.Ö., A.T., Analysis or Interpretation: Ö.F.Ç., H.A., Literature Search: Ö.F.Ç., İ.E.Ö., Writing: Ö.F.Ç., A.T.

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Original Article / Özgün Araştırma

Long-term Results of Hook Plate Technique in Acromioclavicular Joint Dislocations

Akromiyoklavikuler Eklem Çıkıklarında Hook Plate Tedavisinin Uzun Dönem Sonuçları

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Abstract

Objective: The aim of this study is evaluating the long-term results and complications of hook plate (HP) used in acromioclavicular joint (ACJ) dislocations.
Materials and Methods: Sixty-two cases who were operated for ACJ separation with HP technique between 2010 and 2015 were evaluated retrospectively. Clinical and radiological outcomes and long term complications evaluated.
Results: The mean disabilities of the arm, shoulder and hand questionnaire score was 5±1, the mean Constant-Murley score was 92±6. Coracoclavicular distance measurement on the first day after surgery showed an overcorrection compared to the intact extremity. Some reduction loss was detected in the fifth year measurements.
Conclusion: Decrease in reduction loss at the end of the fifth year did not have a negative effect on the clinical outcomes of the shoulder.

Keywords
Acromioclavicular joint separation, hook plate, long-term outcomes

Öz

Amaç: Bu çalışmanın amacı; akromiyoklavikuler eklem (ACJ) çıkıklarına uygulanan hook plate'in (HP) uzun dönem sonuçlarını ve komplikasyonlarını değerlendirmektir.
Gereç ve Yöntemler: ACJ separation nedeni ile 2010 ila 2015 yılları arasında HP kullanılarak opere edilen 62 olgu retrospektif olarak değerlendirildi. Klinik, radyolojik sonuçlar ve uzun dönem komplikasyonlar incelendi.
Bulgular: Klinik değerlendirmeler sonucunda ortalama kol, omuz ve el sorunları anketi skoru 5±1, Constan-Murley skoru ortalama 92±6 idi. Korakoklaviküler mesafe ameliyat sonrası ilk gün ölçümünde sağlam ekstremite ile kıyaslandığında fazladan düzeltme (overcorrection) görüldü. Beşinci yıl ölçümlerinde ise bir miktar redüksiyon kaybı tespit edildi.
Sonuç: Beşinci yılın sonunda redüksiyon kaybındaki bu düşüşün omuz klinik sonuçlarına negatif etkisi görülmemiştir.

Anahtar Kelimeler
Akromiyoklaviküler eklem çıkığı, hook plate, uzun dönem sonuçlar

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Introduction

Acromioclavicular joint (ACJ) separation accounts for 9% to 12% of all shoulder girdle injuries (1). Rockwood type I and II injuries in which the coracoclavicular ligaments are still intact are usually treated conservatively (2). In Type III-V injuries, surgical treatment is generally recommended (3).

Up to know several surgical procedures including; open reduction and fixation methods with many different methods such as screw, plate, Kirschner wire, sutures and hook plate (HP) have been described in the surgical treatment of ACJ separation (4). Despite the fact that the HP technique being used frequently, it is associated with a high rate of complications needing implant removal (3). However, current literature lacks of long term follow-up studies to show exact results with HP technique. Although there are some short-term studies after HP technique, studies showing long-term results are needed.

The aim of the current study is to evaluate the long-term clinical and radiological results of ACJ separation cases operated with HP.

Materials and Methods

The study protocol was approved by the Ethics Committee from Aydın Adnan Menderes University Faculty of Medicine (protocol number: 2022/103, date: 09.06.2022). Seventy-four patients who operated with HP between 2010 and 2015 for ACJ separation were evaluated retrospectively. Informed consent has been provided from all patients before surgery. Patients aged 18 years and over and treated surgically by HP method were included in the study. Total sixty-two patients who met the inclusion criteria were included in the study. The shortest follow-up period was determined to be five years. No additional pathology, no previous surgical history from the same extremity were required in the inclusion criteria. Those who did not come for follow-up after 5 years (n=9), those who had additional injuries (n=2), and those who had a previous upper extremity surgery (n=1) were excluded from the study.

All patients were operated with same procedure and same trauma surgeon: open reduction and internal fixation with HP. In this procedure patients were operated in beach chair position. After incision and dissection, ACJ reducted and held with a reduction clamp. HP with 18 mm offset were used. Final location of hook was just near to acromial joint posterior border. No repair were performed for ligament recontruction.

The ACJ separation classification of all cases were carried out according to the Rockwood classification with preoperative X-rays (5). Implant removal times were evaluated as well. The coracoclavicular distance (CCD) was measured to evaluate the reduction quality. CCD measurements on radiographs were carried-out without using weights, as suggested by Bossart et al. (6). CCD was measured preoperatively for injured and intact extremities. In addition, CCD measurements were carried out on the first day after surgery and at the last follow-ups after five years.

In the rehabilitation program, passive movements were applied in the first postoperative week, active training of shoulder muscle groups after 3-6 weeks, active sports and strength exercises were started after 6 months, for a total of 1 year.

The disabilities of the arm, shoulder and hand questionnaire (DASH) and Constant-Murley score (CMS) were used to evaluate clinical outcomes (7). DASH scores were evaluated at the first follow-up and fifth year. CMS was measured preoperatively for intact and injured extremities. Then, CMS evaluation of the injured extremity was performed on the first postoperative day and at the last follow-up by the same orthopedic surgeon. CMS of postoperative first day and last measurement were compared. Range of movement (ROM) of injured side and additional potential complications were evaluated and noted at the last follow-ups as well.

Statistical Analysis

SPSS (IBM Statistics, Version 26) was used for statistical analysis of the data. Categorical variables were summarized as number (n) and percent (%), continuous variables as mean ± standard deviation, median (min-max). Since continuous variables were not normally distributed (Kolmogorov-Smirnov and Shapiro-Wilk $p<0.05$), test were used to evaluate continuous variables. The changes between preoperative and postoperative values were evaluated with Wilcoxon signed ranks analysis. $P<0.05$ was considered to be statistically significant.

Results

The mean age was 45.35±13.65. Twenty-nine of the patients were female and 33 were male. The

operated shoulder was right in 33 and left in 29 patients. The cause of injury was sports injury in 12 (19.3%) patients, traffic accident in 20 patients, and simple traumas in 30 patients. While trauma exposure was direct in 46 patients, it was indirect in 16 (25.8) patients.

According to the Rockwood classification, 13 patients were type III, 3 patients were type IV, and 46 patients were type V injuries. The mean follow-up period was 65.82±4.65 months. Implant removal was performed in all patients after one year. The mean time of removal of the implant was determined as 409.14±32.5 (377-432) days (Table 1).

Radiological results revealed that, the mean CCD was 8.92±2.8 in the non-injured extremity. While the mean preoperative measurements were 20.26±6.87 on the injured side, it decreased to 8.59±2.69 on the first postoperative day ($p<0.001$). Compared to the intact extremity, the CCD was lower in the operated extremity, and overcorrection was achieved with surgery ($p<0.001$). In CCD final control measurements, the mean was 11.49±3.99. Compared to the first day after surgery, a significant increase in the amount of CCD was observed at the last follow-up ($p<0.001$) (Table 2,3).

Considering the clinical results, at the last follow up: the mean DASH score of the patients was 5.06±0.82, the CMS score was 92.29±6.5.

At the last follow up a palpable pain on ACJ was evident in 10 patients and during active ROM in 4 patients. ACJ separation reoccurred in one case on the 10th postoperative day. This case operated using a longer HP and did not encounter a following complication during five year follow ups.

Superficial infection was observed in two patients and the infection regressed in both patients after oral antibiotic treatment. Heterotopic ossification was observed in two patients. He was followed up without any intervention. (Table 2).

Discussion

In our study, the long-term radiological and clinical results of the patients in which HP technique was used for ACJ separation were evaluated. The applied method was evaluated with clinical scoring systems and successful results were obtained. As a result of radiological evaluations, disadvantages such as increased CCD and loss of reduction were detected. However, these results had no effect on the clinical evaluation of the patient. Data on long-term results

Table 1. Demographic and clinical data of patients

Age (year) Mean ± SD, Median (min-max)		**45.35±13.65**	**45 (22-74)**
Gender	Female	29	46.8
	Male	33	53.2
Injured side	Right	33	53.2
	Left	29	46.8
Cause for ACJ separation	Sport	12	19.4
	Traffic accident	20	32.3
	Everyday routine	30	48.4
Trauma mechanism	Direct impact	46	74.2
	Indirect impact	16	25.8
Rockwood clasification	Type 3	13	21.0
	Type 4	3	4.8
	Type 5	46	74.2
Follow-up time (month) Mean ± SD, median (min-max)		65.82±4.65	64 (60-80)
Time of implant removal (day) Mean ± SD, median (min-max)		409.14±32.59	406.5 (365-502)

min-max: Minimum-maximum, SD: Standard deviation, ACJ: Acromioclavicular joint

of HP surgery for ACJ seperation are not available in literature. In this sense, this study, which evaluates long-term results with a follow-up period of at least five years, may be a pioneer in the literature.

Many surgical options have been described in the treatment of ACJ dislocations, and the advantages and disadvantages of these treatments have been reported in the literature (8). HP technique, which is one of the most commonly used methods in surgical treatment, is widely used because it provides stable fixation against deforming forces in the rotational, horizontal and vertical axis and allows early movements (9). CMS scores are generally used in studies to evaluate the clinical success of the HP technique. For example Koukakis et al. (10) was found CMS score 6 months after plaque removal 96.4 and it was found to be 97 in Salem and Schmelz's (9) 30-week follow-up study (9-10). Di Francesco et al. (11) had reported a mean 91.79 CMS after mean 18 months follow-up. Also Jafary et al. (12), had reported a mean 94.5 point CMS result after 9 months of follow up. In our study, the mean CMS value was 92.29±6.5. Although implant removal was performed later in this study compared to other studies, similar results were obtained in CMS values when compared with short and medium-term studies. These results showed us that there is no harm in performing implant removal in the late period.

Complications such as implant failure, implant loosening, instability, penetration into the humeral head, subacromial impingement, rotator cuff muscle degeneration, infection, subacromial erosion, and neurovascular injury have been reported after HP technique (13,14). Huang et al. (15) had reported the complication rate after HP to be 37.5%. In our study, the complication rate was found to be 11.3% in the follow-ups of the cases who were operated using HP for fixation. However, in our study, subacromial erosion could not be measured because of insufficient measurement with direct radiography. Current literature reports that the rate of subacromial erosion evaluated with computed tomography (CT) to be 30% (16). There is a need for a study measuring subacromial erosion in patients with late implant removal and in cases with long follow-up. Our treatment choice was in the direction of HP due to our clinical experience and surgeons preference.

There is no definite consensus regarding the time of removal of the implant in patients who underwent HP. Generally, removal of the implant depends on the patient's symptoms, and if there is persistent pain in the postoperative period, it is recommended to be performed within the first 3 months (9-11,13,17-20). If the patient does not have shoulder pain, it is recommended to remove the implant after the first 3 months (12,17-20).

Table 2. Clinical and radiological outcome of patients

DASH score last control		5.06±0.82	5 (4-9)
CMS uninjured side-preoperation		93.63±6.58	94 (52-100)
CMS last control		92.29±6.5	92 (54-100)
VAS last control		0.11±0.48	0 (0-3)
CCD uninjured side-preoperation Mean ± SD, median (min-max)		8.92±2.8	7.9 (3-15.2)
CCD injured side-preoperation Mean ± SD, median (min-max)		20.26±6.87	18.35 (6.9-34.2)
CCD injured side-postoperation 1. day Mean ± SD, median (min-max)		8.59±2.69	7.55 (2.5-16)
CCD last control Mean ± SD, median (min-max)		11.49±3.99	10.4 (3.1-23.8)
Pain around the ACJ with palpation	Yes	10	16.1%
	No	52	83.9%
Pain during motion around the ACJ	Yes	4	6.5%
	No	58	93.5%

DASH: The disabilities of the arm, shoulder and hand questionnaire, CMS: Constant-Murley score, VAS: Visual analogue scale, CCD: Coracoclavikuler distance, ACJ: Acromioclavicular joint, min-max: Minimum-maximum, SD: Standard deviation

Table 3. Comparative results of coracoclavicular distance measurement

	p-value
CCD injured side-preoperation - CCD injured side postoperation 1. day	<0.001
CCD injured side-postoperation 1. day - CCD last control	<0.001
CCD uninjured side - CCD injured side postoperation 1. day	<0.001

CCD: Coracoclavicular distance

Contrary to the general opinion, there are articles recommending that implant removal should not be performed in the first three months, even if there is pain and limitation of movement. however, there are also studies suggesting removal of the implant in early period (21-23).

Pain scores have been reported to better when implant removal performed in 3 months after surgery (9-11,13,17-20). Koukakis et al. (10) have reported a mean 0.87 VAS, Sarrafan et al. (19) reported a mean 4 VAS, and Steinbacher et al. (20) had reported a mean 1.8 VAS. In these studies, the implant removal time is earlier than in our study. In our study, as per our clinical practice, if there was no severe limitation of movement or intolerable pain, implant removal was performed one year later at the earliest. Despite the altered implant removal time in our study, a lower pain score is detected compared to the medium and short-term results.

Loss of reduction after implant removal is one of the controversial issues. Reduction loss has been reported for 3-67% of cases after removal of the HP (9,10,17,21). In the study of Hemmann et al. (24), an increase in CDD distance of 3.8±0.6 was observed when the implant was removed after a mean 64±2 days of surgery. Although radiological findings of reduction loss are obtained, there are articles showing that this loss is not reflected in clinical results. In the article published by Smith et al. (25) in 2011, conservative treatment and surgical treatment were compared. In this study, it was stated that anatomical reduction is not essential for good functional outcomes. In our study, implant removal was performed after a mean 409.14±32.59 days, and 2.9 mm increase in CCD was detected. In our study, the implants were removed later than in the literature and it was observed that the reduction loss was less.

The study has several limitations. Retrospective design and the inaccessibility of data for some of the patients can be seen as the most important limitations when evaluating long-term results. In addition, due to our clinical practice in radiological evaluation, not having stress radiographs and not using CT before implant removal prevent drawing exact results. It would be illuminating to conduct prospectively designed, multicenter studies on this subject with a specific patient group.

Conclusion

Fixation of ACJ separation with HP appears to have satisfactory long-term clinical outcomes. The most important disadvantage is the need for an exact implant removal and reduction loss.

Ethics

Ethics Committee Approval: The study protocol was approved by the Ethics Committee from Aydın Adnan Menderes University Faculty of Medicine (protocol number: 2022/103, date: 09.06.2022).

Informed Consent: Informed consent has been provided from all patients before surgery.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: A.Ş., Ş.Ö.Ş., Concept: A.Ş., C.P., Ş.Ö.Ş., Design: A.Ş., Ş.Ö.Ş., Data Collection or Processing: A.C.Ç., C.P., Analysis or Interpretation: A.Ş., A.C.Ç., Literature Search: A.C.Ç., C.P., Writing: A.Ş.

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Original Article / Özgün Araştırma

The Association Between Body Mass Index, Intraocular Pressure and Central Corneal Thickness in Children

Çocuklarda Beden Kitle İndeksi, Göz İçi Basıncı ve Santral Kornea Kalınlığı Arasındaki İlişki

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Keywords
Body mass index (BMI), central corneal thickness (CCT), children, intraocular pressure (IOP), non-contact air puff tonometer (NCT), obese, overweight

Anahtar Kelimeler
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Abstract

Objective: To compare intraocular pressure (IOP) and central corneal thickness (CCT) of normal, overweight, and obese children and evaluate the effects of body mass index (BMI) on IOP and CCT.

Materials and Methods: In this prospective, cross-sectional study, children aged 6 to 18 years without any ocular disease were included. IOPs and CCTs were measured with a non-contact air-puff tonometer (NCT) and optical coherence tomography (OCT), respectively. According to their BMI, children were divided into three groups as follows: group1 (normal), BMI ≤85; group 2 (over-weight), BMI 86-94; group 3 (obese), BMI ≥95. The IOP and CCT measurements of these groups were compared.

Results: Of all 73 patients (43 males, 30 females), 146 eyes were investigated in this study. The mean of IOP was 15±2.89, 16.50±3.10 and 19.50±4.15 mmHg in group 1 (n=62), group 2 (n=24) and group 3 (n=60), respectively (p<0.001). IOP was significantly higher in obese girls than in normal weight girls (20±3.82 mmHg, 15±2.50 mmHg, p<0.01). BMI and age had a significant effect on IOP (p=0.048 and p=0.025). A 1 standard deviation increase in BMI and age increased IOP of 0.175 and 0.187 mmHg, respectively.

Conclusion: In our study, IOPs measured with NCT were significantly higher in obese children, especially in girls, compared to normal and overweight children. Since the increase in intraorbital adipose tissue may lead to increased episcleral venous pressure resulting in increased IOP and impaired ocular perfusion, IOP measurements should be carefully evaluated in obese children.

Öz

Amaç: Normal, fazla kilolu ve obez çocukların göz içi basıncı (GİB) ve santral kornea kalınlığını (SKK) karşılaştırmak ve beden kitle indeksinin (BKİ) GİB ve SKK üzerindeki etkilerini değerlendirmek.

Gereç ve Yöntemler: Bu ileriye yönelik, kesitsel çalışmaya, 6-18 yaş arası, herhangi bir oküler hastalığı olmayan çocuklar dahil edildi. GİB'ler ve SKK'ler sırasıyla non-kontakt hava üflemeli tonometre (NKT) ve optik koherens tomografi (OKT) ile ölçüldü. Çocuklar BKİ'lerine göre üç gruba ayrıldı: grup 1 (normal), BKİ ≤85; grup 2 (fazla kilolu), BKİ 86-94; grup 3 (obez), BKİ ≥95. Bu grupların GİB ve SKK ölçümleri karşılaştırıldı.

Bulgular: Bu çalışmada 73 hastanın (43 erkek, 30 kadın) 146 gözü incelendi. GİB ortalamaları grup 1'de (n=62), grup 2'de (n=24) ve grup 3'te (n=60) sırasıyla 15±2,89, 16,50±3,10 ve 19,50±4,15 mmHg idi (p<0,001). GİB, obez kızlarda normal kilolu kızlara göre anlamlı olarak daha yüksekti (20±3,82 mmHg, 15±2,50 mmHg. p<0,01). BKİ ve yaşın GİB üzerinde anlamlı etkisi vardı (p=0,048 ve p=0,025). BKİ ve yaştaki 1 standart sapmalık artış, GİB'de sırasıyla 0.175 ve 0.187 mmHg'lik bir artışla sonuçlandı.
Sonuç: Çalışmamızda, obez çocuklarda, özellikle kızlarda NKT ile ölçülen GİB'ler normal ve fazla kilolu çocuklara göre anlamlı olarak daha yüksekti. Göz içi yağ dokusundaki olası artış, episkleral venöz basıncın artmasına neden olarak GİB'in artmasına ve oküler perfüzyonun bozulmasına neden olabileceğinden, obez çocuklarda GİB ölçümleri dikkatle değerlendirilmelidir.

Introduction

Obesity is becoming an important health problem due to reduced daily physical activity and changes in eating habits, especially in children, with effects on their adult lives. The coronavirus disease-2019 (COVID-19) pandemic in which people are stuck at home has also contributed to this problem recently. Considering that childhood obesity leads to a predisposition to many chronic diseases, the importance of studying the ophthalmologic effects of it is clear (1).

Recent studies have shown that obesity could be associated with increased intraocular pressure (IOP) (2). Increased IOP is the most important risk factor for primary open-angle glaucoma both for development and progression (3). It has been shown in adults that one of the independent risk factor for increased IOP is obesity. However, in children, the association between IOP and body mass index (BMI) is still controversial (4,5). So, we tried to investigate the association between BMI, IOP, and central corneal thickness (CCT) in children in this study.

Materials and Methods

This cross-sectional, prospective study was conducted after receiving the approval from the Aydın Adnan Menderes University Ethical Committee and Review Board in consistence with the Declaration of Helsinki, (decision no: 18 date: 05.03.2020).

All patients aged 6 to 18 years who were admitted to the Aydın Adnan Menderes University Ophthalmology Clinic between March and July 2020, without any ophthalmic pathology or systemic diseases like hypertension and diabetes mellitus were enrolled in this study. Visual acuity, slit-lamp anterior segment biomicroscopy, and fundoscopy examinations were performed. IOP and CCT measurements were made using a non-contact air-puff tonometer (NCT) (TONOREF II, Nidek, Tokyo, Japan) and optical coherence tomography (OCT) (Cirrus HD-OCT 500, Carl Zeiss, Dublin, LA), respectively. The patients weight is divided by the square of the height of the patients (kg/m^2) in order to calculate their BMI. Children were categorized as group1 (normal-weight; BMI ≤85), group 2 (overweight; BMI 86-94), and group 3 (obese; BMI ≥95) according to the Turkish percentile reference values (Olcay Neyzi percentile calculation system) (6).

Statistical Analysis

Before statistical analysis, to assess the assumption of normality of numeric variables the Kolmogorov-Smirnov test was performed. Continuous variables of the groups were shown as mean ± standard deviation and compared with one-way analysis of variance (ANOVA) and the Kruskal-Wallis H test. A chi-square test was used to compare categorical variables and expressed as numbers and percentages (%). A p-value of less than 0.05 was accepted as statistically significant.

Results

A total of 146 eyes of 73 children aged 6 to 18 years (43 male and 30 female) were evaluated in this study. Table 1 demonstrates the general characteristics, IOP, and CCT values of groups. No statistically significant correlation was detected between age and IOP or age and CCT (p=0.022, p=0.967). However, BMI and CCT were in a negative correlation with each other (r=0.146, p=-0.07) (Figure 1). CCT and IOP were found to have a positive correlation (r=0.246, p=0.03) (Figure 2). Furthermore, categorical regression analysis showed that age and BMI had a significant effect on IOP (p=0.048 and p=0.025, respectively). A 1 standard deviation increase in BMI and age resulted in 0.175 and 0.187 standard deviation increases in IOP, respectively. Female and male patients did not differ

Table 1. Demographic features, IOP, and CCT values of groups				
	Group 1 (n=62)	Group 2 (n=24)	Group 3 (n=60)	p-value
Mean age	12.5±3	13.2±3.1	13.5±2.6	0.158
Gender Male/female	30 (48.4)/ 32(51.6)	18 (75)/6 (25)	38 (63.3)/22 (36.7)	0.053
IOP (mmHg)	15.0±2.89	16.5±3.10	19.5±4.15	<0.001
CCT (µm)	550±14.9	566.5±15.7	551±17.3	0.053
IOP: Intraocular pressure, CCT: Central corneal thickness, n: Number				

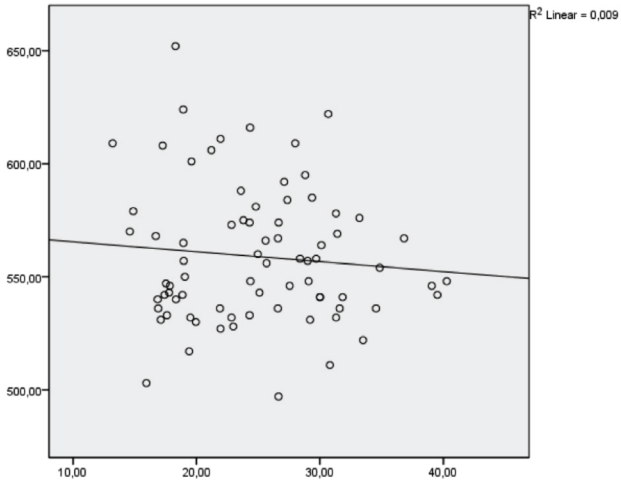

Figure 1. Negative correlation between BMI and CCT
BMI: Body mass index, CCT: Central corneal thickness

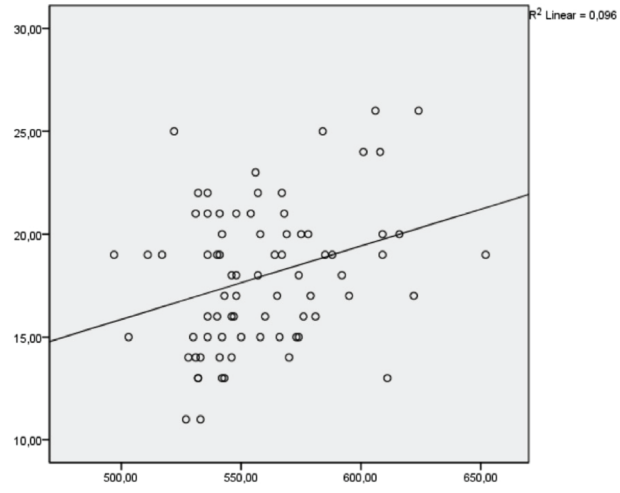

Figure 2. Positive correlation between CCT and IOP
CCT: Central corneal thickness, IOP: Intraocular pressure

in terms of CCT with each other statistically, but IOP and BMI were significantly higher in females than in male patients (p<0.001).

Discussion

Obesity is one of the most common chronic diseases in childhood (7). Understanding and investigating the correlation between childhood obesity and IOP may be important for early diagnosis and, more importantly, for the prevention of glaucoma in their later ages. The correlation between obesity and IOP has been reported in adults, but it is still controversial in children.

It has shown in adults that higher IOP is associated with obesity, BMI is an independent risk factor for increase in IOP, and IOP also decreases with weight loss (8). Moroever, studies investigating the effect of bariatric surgery on IOP have shown a significant reduction in IOP after surgery and subsequent rapid weight loss (9). There are some suggestions, in the literature, about the aetiology of increased IOP in obesity. Increased intra-abdominal pressure causes increased episcleral venous pressure or intraorbital adipose tissue contributes to the elevation of IOP by increasing episcleral venous pressure and decreasing aqueous outflow facility (10). Also, obesity related to hyperlipidemia might increase blood viscosity and excessive blood viscosity decreases the outflow capacity of episcleral veins, which can result in high IOP (11). Furthermore, analysis of serum leptin levels and leptin gene mutations have shown that there has been monogenic leptin deficiency in early childhood obesity, and recombinant leptin therapy might be a great choice for them (12). Leptin has a neuroprotective effect on a number of neurodegenerative diseases in the central nervous system (13). Glaucomatous neurodegeneration, similar to this neurodegenerative diseases. Therefore, leptin has proposed as a potential neuroprotective agent in the clinical management of glaucoma (14). Imparement of this leptin responce in obese children

may also contribute to development of glaucoma. In this prospective, cross-sectional study, the potential effect of BMI on IOP and the relation between CCT values of obese, overweight, and normal-weight children were investigated. Although CCT showed no significant difference among the groups, the mean IOPs of the obese children were significantly higher than the overweight and normal ones in our study. Our results, showing no significant differences in CCT, age, or sex among the groups, revealed that obesity alone might be a risk factor for increased IOP in children. A 1 standard deviation increase in BMI of the children resulted in an increase in IOP of 0.175 mmHg in our study. Similar to our result, some studies showed a positive correlation between BMI and IOP (15). Moreover, in the first study in the literature, Akinci et al. (5) reported a diurnal variation of IOP in obese children. However, in contrast to these reports, a few studies indicated no statistically significant association between IOP and BMI (16). These different results may be due to methodological variations in the studies, such as the instruments used for the measurement of the IOP, the determination of study groups, or a relatively small number of patients.

Another finding of our study was that the relationship between BMI and IOP was stronger in females compared to male patients. A positive correlation between BMI and IOP is more pronounced in female patients. Saguş Aydın et al. (4) reported a positive correlation between BMI and IOP in adults, finding that every 10 kg/m^2 increase would cause an IOP increase of 1.4 mmHg. Moreover, they also reported that this correlation was found to be more significant in female patients than in male patients, which is similar to our results in children. Estrogen could play a role on this result as it promotes collagen growth in cornea and sclera and this could alter the IOP levels in female (17). Anthropometric parameters differ among female and male children. It was shown that body composition especially fat mass/muscle mass ratio in females was significantly higher than those in males (18). A meta-analysis study suggested that greater fat mass has a higher risk of elevated IOP, notedly in females, and abdominal adiposity has a positive association with glaucoma (19). Adipose tissue also could play a role as an endocrine organ and synthesize sex hormones such as estrogen (20). In addition, estrogen may effect IOP; not only by influencing fat distribution but also by decreasing aqueous production and facilitating outflow systems in female patients (21).

There was a positive correlation between age and IOP. In our study, every 1 year increase in age caused an increase in IOP of 0.187 mmHg. While most studies in Western countries reported a positive association between age and IOP, Japanese researchers observed a negative association (22). In obese children, elevated IOP may not be diagnosed until optic nerve becomes evident. Therefore, IOP measurements in obese children should be repeated annually.

CCT is one of the most important factors affecting IOP measurements and the correlation between CCT and IOP has been well described; the thicker CCTs can cause false higher IOP readings (23). It was reported that CCT is also associated with higher BMI and metabolic syndrome. They suggested adults with a higher BMI have a thicker CCT and this increases IOP measurements. On the contrary, Sahinoglu-Keskek et al. (24) have showed a relationship between metabolic syndrome and higher IOP but not CCT in adults. There was no statistically significant difference among the groups in terms of CCT, in our study.

One of the limitations of our study is that IOPs were measured using a NCT instead of a Goldmann applanation tonometer, which is accepted as the gold standard for IOP measurement. During the procedure, compression of the chest or holding the breath may increase venous pressure, and applanation tonometry may cause false results in overweight patients (25). Second, IOPs were not measured at the same time interval of the day. Therefore, diurnal variation of IOP was not evaluated in our study.

Conclusion

Our study revealed that IOPs were significantly higher in obese children, especially females. BMI and age had a significant effect on IOP. Further studies with a larger sample size are needed.

Ethics

Ethics Committee Approval: This cross-sectional, prospective study was conducted after receiving the approval from the Aydın Adnan Menderes University Ethical Committee and Review Board in consistence with the Declaration of Helsinki, (decision no: 18 date: 05.03.2020).

Informed Consent: This cross-sectional, prospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: F.V., A.İ.A.Ü., T.Ü., A.A., Concept: A.İ.A.Ü., S.D., T.Ü., A.A., Design: A.İ.A.Ü., S.D., Data Collection or Processing: F.V., T.Ü., A.A., Analysis or Interpretation: A.İ.A.Ü., S.A.E., S.D., T.Ü., A.A., İ.K.Ö., Literature Search: F.V., S.A.E., Writing: F.V., A.İ.A.Ü., S.A.E., İ.K.Ö.

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Original Article / Özgün Araştırma

Comparison of Three Different Rotavirus Antigen Tests for Rotavirus Detection in Fecal Samples: A Retrospective Analysis

Dışkı Örneklerinde Rotavirüs Tespitinde Üç Farklı Antijen Testinin Karşılaştırılması: Retrospektif Bir Analiz

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Keywords
Rotavirus, antigen, stool, test, agreement

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Abstract

Objective: Direct antigen tests are the most commonly used methods in most laboratories to detect rotavirus rapidly in stool samples. This study aimed to evaluate the performance of three commercially available test methods for detecting rotaviruses in fecal specimens and compare the results with those of the reverse transcription-polymerase chain reaction (RT-PCR), which is considered a gold standard test.

Materials and Methods: The presence of rotavirus antigens in stool samples was investigated by an enzyme-linked immunosorbent assay (ELISA), an immunochromatographic test (ICT), and a latex agglutination test (LAT), which were commercially available. The results of these tests were compared with those of a multiplex RT-PCR as a reference test. Sensitivity, specificity, and positive and negative predictive values were calculated, and agreement with RT-PCR was evaluated by Cohen's kappa test.

Results: A total of 85 patients (51.8% male and 48.2% female, aged 0-32 years) were included in this study. The sensitivities of the ICT, LAT, and ELISA tests were 78.6%, 78.6%, and 96.4%, respectively; the specificities of the tests were 69.0%, 72.4%, and 69.0%, respectively. According to kappa tests, moderate agreement was found between RT-PCR and ICT (κ=0.464, p<0.001); moderate agreement was found between RT-PCR and LAT (κ=0.493, p<0.001); substantial agreement was found between RT-PCR and ELISA (κ=0.694, p<0.001). The ELISA test showed the highest sensitivity and a high level of agreement with RT-PCR.

Conclusion: ICT and LAT are quick and practical tests for rotavirus detection. However, in this study, it was seen that they were not superior to the ELISA test in terms of accuracy of diagnosis.

Öz

Amaç: Rotavirüsün hızlı tespitinde direkt antijen testleri çoğu laboratuvarda en yaygın olarak kullanılan yöntemlerden biridir. Bu çalışmanın amacı, dışkı örneklerinde rotavirüs antijenlerinin tespiti için ticari olarak piyasada bulunan üç farklı tanı yönteminin performanslarını değerlendirmek ve sonuçlarını altın standart test olarak kabul edilen ters transkripsiyon-polimeraz zincir reaksiyonu (RT-PCR) testinin sonuçlarıyla karşılaştırmaktır.

Gereç ve Yöntemler: Dışkı örneklerinde rotavirus antijenlerinin varlığı ticari enzyme-linked immunosorbent assay (ELISA), immünokromatografik test (ICT), ve lateks aglütinasyon testi (LAT) ile araştırıldı. Testlerin sonuçları referans test olarak bir multipleks RT-PCR'ınkilerle karşılaştırıldı. Duyarlılık, özgüllük, pozitif ve negatif prediktif değer hesaplandı ve RT-PCR ile uyumluluk Cohen'in kappa testi ile değerlendirildi.

Bulgular: Çalışmaya toplam 85 hasta (%51,8 erkek ve %48,2 kadın, 0-32 yaş) dahil edildi. ICT, LAT ve ELISA testlerinin duyarlılıkları sırasıyla %78,6, %78,6 ve %96,4; testlerin özgüllükleri sırasıyla %69,0, %72,4 ve %69,0 idi. Kappa testlerine göre, RT-PCR ve ICT arasında orta düzeyde (κ=0,464, p<0,001), RT-PCR ve LAT arasında orta düzeyde (κ=0,493, p<0,001), RT-PCR ve ELISA arasında ise iyi derecede bir uyum belirlendi (κ=0,694, p<0,001). ELISA testi, RT-PCR ile en yüksek duyarlılık ve yüksek düzeyde uyum gösterdi.

Sonuç: ICT ve LAT, rotavirüs tespiti için hızlı ve pratik testlerdir. Ancak bu çalışmada tanı doğruluğu açısından ELISA testinden daha üstün olmadıkları görülmüştür.

Introduction

Rotavirus is a leading cause of acute viral gastroenteritis throughout the world, and most children are infected by 5 years of age (1). Many studies conducted in both developing and developed countries report that group A rotaviruses are responsible for 13-50% of all cases of viral gastroenteritis in children under 5 years of age (2). Despite the availability of a rotavirus vaccine, more than 200,000 deaths occur per year under the age of five worldwide (1). In studies conducted in Turkey, rates of rotavirus positivity in children with gastroenteritis ranged from 18.7% to 53% (3-10).

The rapid and accurate diagnostic tests for the detection of the virus in patients with acute gastroenteritis are important not only for the diagnosis of viral gastroenteritis but also to prevent the spread of the disease (11). The specific methods available for detecting the rotavirus in stool specimens include electron microscopy (EM), immuno-EM, cultivation techniques, rapid antigen tests, polyacrylamide gel electrophoresis (PAGE), and reverse transcriptase-polymerase chain reaction (RT-PCR). EM, cultivation, and PAGE are not recommended because they are expensive, time-consuming, and technically difficult (4). Several rapid antigen tests, such as the latex agglutination test (LAT), the immunochromatographic test (ICT), and the enzyme-linked immunosorbent assay (ELISA), are inexpensive, easy-to-perform, and commercially available.

In routine diagnostics, it is important to know the sensitivity and specificity of these rapid tests. Rapid and accurate diagnosis could prevent unnecessary and potentially harmful antibiotic treatment and improve knowledge of the epidemiology of rotavirus infections (12). These rapid tests have good performance for determining rotavirus, and they are frequently used by physicians as an aid to diagnosis. The RT-PCR is the most sensitive molecular method to detect and confirm rotavirus (13,14).

The objective of the presented study is to evaluate the performance, sensitivity, and specificity of three commercially available rotavirus antigen tests: ELISA, ICT, and LAT, compared with a reference test, a multiplex reverse transcription-PCR (mRT-PCR), for detecting rotavirus in fecal specimens from patients with acute gastroenteritis over medical records retrospectively.

Materials and Methods

In this study, the results of three different commercial rotavirus antigen tests, ELISA, ICT, and LAT, which were studied to detect rotavirus in stocked stool samples sent to our laboratory from patients with acute gastroenteritis between January and December 2014, were compared with the results of the RT-PCR, which was used as a reference test. The study was approved by the Aydın Adnan Menderes University Ethics Committee (protocol no: 2022/176, date: 10/11/2022).

Detection of rotavirus by ELISA: The ProSpecTTM® Rotavirus Microplate Assay (Oxoid, Ltd., Basingstoke, Hampshire, UK) is a qualitative sandwich ELISA and was used to detect rotavirus group A antigen in stool samples.

Detection of rotavirus by ICT: The CerTest® Rota-Adeno Card Test (CerTest, Biotec, Spain) was used to detect rotavirus antigen in stool samples. This test is a one-step lateral flow ICT that simultaneously detects group A rotavirus and adenovirus in stool samples.

Detection of rotavirus by LAT: The Virotect Rota (Omega Diagnostics, Scotland, UK) wasbasically a rapid

LAT for the detection of rotavirus in fecal samples.

Multiplex RT-PCR: The commercially available Seeplex® Diarrhea ACE Detection multiplex PCR (Seegene, Seoul, Korea) was used to simultaneously detect group A rotaviruses, AdV 40 and 41 (species F), noroviruses GI and GII, and astroviruses. Nucleic acids were extracted from fecal suspensions by using the QIAamp DNA Mini kit (Qiagen, Hilden, Germany) and the QIAcube platform (Qiagen). The nucleic acid was amplified using a PTC-200 thermal cycler (Bio-Rad, Hercules, CA, USA), and the PCR products were visualized after electrophoresis on a 1.5% agarose gel. Under ultraviolet light, the DNA products with 650 base pairs (bp) for Astrovirus, 411 bp for enteric adenovirus, 541 bp for group A rotavirus, 304 bp for norovirus group I, and 214 bp for norovirus group II showed positive results. All test kits were performed according to the manufacturer's instructions.

Statistical Analysis

Descriptive statistics were presented as percentages, the mean with standard deviation, or the median with minimum and maximum values. Based on the RT-PCR results (15), sensitivity, specificity, and positive and negative predictive values were calculated for the ICT, LAT, and ELISA. Test agreement with the RT-PCR results accepted as the gold standard was assessed using Cohen's kappa with 95% confidence intervals (CI). The strength of agreement was based on Cohen's kappa value: <0 poor, 0.0-0.20 slight, 0.21-0.40 fair, 0.41-0.60 moderate, 0.61-0.80 substantial, and 0.81-1.00 almost perfect (16). All data entries were made in SPSS 26.0, and the tables were prepared with it. Calculations were performed and evaluated according to the references mentioned before.

Results

In the study, which included 85 people in total, 25 (29.4%) of them were under 1-year-old and 43 (50.6%) were under 5-years-old. The median age was two years (0-32), and 41 were female and 44 were male.

Out of the 85 samples, 56 (65.9 %) tested positive for rotavirus by RT-PCR. Out of all the samples, 63 (74.1%), 53 (62.4%), and 52 (61.2%) were found to be positive in the ELISA, ICT, and LAT kits, respectively.

In Table 1, it was shown that the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated using the RT-PCR as a gold standard. These values for the ICT test were found to be 78.6%, 69.0%, 83.0%, and 62.5%, respectively. These values were 78.6%, 72.4%, 84.6%, and 63.6% for the LAT and 96.4%, 69.0%, 85.7%, and 90.9% for the ELISA, respectively.

Considering Cohen's kappa, moderate agreement was found between the RT-PCR and the ICT (κ=0.464 (95% CI, 0.268-0.660), p<0.001); moderate agreement was found between the RT-PCR and the LAT (κ=0.493 (95% CI, 0.303-0.683), p<0.001); and substantial agreement was found between the RT-PCR and the ELISA (κ=0.694 (95% CI, 0.529-0.859), p<0.001) (Table 1).

Discussion

Rapid and accurate rotavirus detection is required to ensure the administration of appropriate treatment plans and infection control. Several rapid test kits, including latex agglutination, ICTs, and enzyme immunoassays for detection of rotavirus infection, are used in routine diagnosis. In our study, we evaluated the performance, sensitivity, and specificity of three commercial rotavirus antigen tests compared with a mRT-PCR assay for detecting rotavirus in fecal specimens from patients with acute diarrhea.

In the presented study, RT-PCR was accepted as the reference method, the ELISA was the antigen test with the highest sensitivity (96.4%), and the LAT was the test with the highest specificity (72.4%) among the three tests compared. When PPV and NPV were examined, it was observed that the ELISA had the highest values (85.7% and 90.9%, respectively). The test with the highest agreement with the RT-PCR was found to be ELISA (κ=0.694). Considering our findings, it can be thought that the performances of all three tests are not at the desired level; nevertheless, ELISA is the best among these tests.

The ICT is rapid and easy to perform (17). We found that out of 85 samples, 53 (62.4%) were positive in the ICT. The sensitivity and specificity of ICT were 78% and 69%, respectively. Studies comparing the ICT test with the reference method, the PCR test, showed that the sensitivity of the ICT test was 80-100% and the specificity was 89-100%. In the presented study, the sensitivity and specificity of the ICT test were lower than in previous studies. The latex agglutination assay is faster and simpler but less sensitive than the ELISA

Table 1. The sensitivity, specificity, positive and negative predictive value, and agreement for rotavirus group A detection in fecal samples by three tests compared with the RT-PCR

	ICT	LAT	ELISA
Sensitivity (%)	78.6	78.6	96.4
Specificity (%)	69.0	72.4	69.0
Positive predictive value (%)	83.0	84.6	85.7
Negative predictive value (%)	62.5	63.6	90.9
Cohen's kappa with 95% CI	0.464 (0.268-0.660)	0.493 (0.303-0.683)	0.694 (0.529-0.859)

LAT: Latex agglutination test, ICT: Immunochromatographic test, ELISA: Enzyme-linked immunosorbent assay, RT-PCR: Reverse transcription-polymerase chain reaction, CI: Confidence interval

(17). In our study, while the LAT test was similar to the ICT, it was less sensitive and specific than the ELISA test. In a previous study, it was reported that the latex agglutination assay had a sensitivity of 63.6% and a specificity of 86.8% when compared with the RT-PCR (18). Xiang et al. (19) found 81.03% and 97.44% for the LAT sensitivity and specificity, respectively. Unlike previous studies, we found 78.6% sensitivity and 72.4% specificity for the LAT.

Although the ELISA is the standard test for detecting rotaviruses, it is time-consuming and not cost-effective. In this study, 63 (74.1%) samples were positive for rotavirus by the ELISA and the sensitivity and specificity of the ELISA were 96.4% and 69%, respectively, when compared with the RT-PCR. Gautam et al. (20) conducted a comparative analysis study of three commercial EIA kits. Using the RT-PCR as the gold standard, the sensitivities were between 75 and 82.1% and all the specificities were found to be 100%. Ibrahim et al. (13) observed that the ELISA had a good performance with 88% sensitivity and 100% specificity when compared with the RT-PCR results. Considering our study, the ELISA test was more sensitive than the the other ELISA tests mentioned but less specific.

In a study in which 95 stool samples were studied for LAT, three ICTs, and ELISA tests and compared with the RT-PCR, it was reported that the sensitivity and specificity were 85.7% and 100% for LAT, 100% and 95% for two ICTs, 86.7% and 87.5% for another ICT, and 98.1% and 97.3% for the ELISA, respectively (21). We found the sensitivity of the ELISA to be higher than LAT and ICT, and the specificity to be lower. In another study comparing rapid tests, three different commercial immunologic tests for rapid detection of group A rotavirus (the ICT method, LAT, and ELISA) were used to evaluate 228 stool specimens obtained from children with acute gastroenteritis. The sensitivity and specificity values of the ELISA, ICT, and LAT methods were 96% and 68%, 99% and 99%, 99%, and 96%, respectively. In the study conducted by Wilhelmi et al. (22), the sensitivity and specificity for the ELISA and ICT were found to be higher than LAT.

Agreement with the RT-PCR for the ELISA was the highest compared with the ICT and LAT. In a previous study (23), the analytical and clinical performance of ICT was investigated, and the test results were compared to the ELISA and RT-PCR, found a high level of agreement (κ=0.857). Although there was lower agreement, we also found the highest agreement with the RT-PCR in our study with the ELISA.

The LAT and ICT were rapid and easy to perform but showed lower sensitivity than the ELISA. The ELISA was the best test in terms of sensitivity and specificity but had limitations, such as generating results that were difficult to interpret and being time-consuming. Because of the nature of convenience sampling, the results of this study are not generalizable. However, similar results have been obtained in many studies. In this study, the samples were not fresh; frozen samples were thawed and studied.

Conclusion

In summary, the ELISA test for rotavirus detection showed the highest sensitivity and a high level of agreement with the RT-PCR. Even though it is a less sensitive test, the ICT and LAT may be used alternatively for the rapid screening of group A rotavirus in stool samples, especially during the acute gastroenteritis outbreak seasons. In clinical practice, the possibility of false positive and false negative results with rotavirus should be kept in mind. A false positive antigen test could be caused by cross reactivity with other microorganisms or interference.

When testing with rapid kits yields a negative result in suspected patients, testing with RT-PCR should not be delayed.

Ethics

Ethics Committee Approval: The study was approved by the Aydın Adnan Menderes University Ethics Committee (protocol no: 2022/176, date: 10/11/2022).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: S.K., N.E., Design: S.K., N.E., Data Collection or Processing: S.K., N.E., F.K., V.Y., M.A., Analysis or Interpretation: S.K., H.Ö., Literature Search: S.K., N.E., H.Ö., M.A., Writing: S.K., N.E., H.Ö.

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

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