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Original Article / Özgün Araştırma

The Hemostatic Effect of Ankaferd Blood Stopper in Rat Bleeding Models with Antithrombotic Drug Therapy: An Experimental *In Vivo* Study

Antitrombotik İlaç Tedavisi Uygulanan Sıçan Kanama Modellerinde Ankaferd Kanama Durdurucusunun Hemostatik Etkisi: In Vivo Deneysel Bir Çalışma

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Ankaferd blood stopper, bleeding, bleeding control, antithrombotic therapy

Anahtar Kelimeler
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Abstract

Objective: Ankaferd blood stopper is a mixture of five medicinal plant extracts used as a hemostatic agent for management of external hemorrhage. The aim of this study was to evaluate the hemostatic effects of Ankaferd blood stopper (ABS) on bleeding after tooth extraction *in vivo* models with taking antithrombotic drug.

Materials and Methods: Forty-eight male Albino Wistar rats were divided into six groups of 8 animals each. Maxillary right first molar tooth of the rats were extracted under general anesthesia. 2 mL saline solutions were applied topically to Control group (group 1), Warfarin group (group 3) and Heparin group (group 5) on the sockets immediately after extraction. Two ml ABS's were applied topically to Ankaferd group (group 2), Warfarin-Ankaferd group (group 4) and Heparin-Ankaferd group (group 6) likewise. The bleeding time and the amount of bleeding were compared among 6 groups just following the tooth extraction. The collected data results were analysed statistically by the ANOVA followed by Tukey test for pair-wise comparisons.

Results: The bleeding time was longer in Warfarin and Heparin group than the Control group ($p<0.05$), Ankaferd, Warfarin-Ankaferd and Heparin-Ankaferd groups ($p>0.05$). Similiarly, the amount of bleeding of Warfarin group was significantly higher than those of the Control and Warfarin-Ankaferd group ($p<0.05$). The amount of bleeding were lower in Control, Heparin and Heparin-Ankaferd groups but the differences were not statistically significant ($p>0.05$).

Conclusion: Topically administered ABS is less effective on bleeding control of Warfarin-induced bleeding model than Heparin-induced bleeding model in wistar rats. Resulting small difference in between warfarin and heparin should be investigated in future studies.

Öz

Amaç: Ankaferd kanama durdurucu, eksternal hemorajinin tedavisinde hemostatik bir ajan olarak kullanılan beş tıbbi bitki özütünün bir karışımıdır. Bu çalışmanın amacı, Ankaferd kanama durdurucusunun (ABS) antitrombotik ilaç almış *in vivo* modellerde diş çekimi sonrası kanama üzerine hemostatik etkilerini değerlendirmektir.

Gereç ve Yöntemler: Kırk sekiz erkek Albino Wistar sıçan, her biri 8 hayvandan oluşan altı gruba ayrıldı. Sıçanların sağ üst birinci molar dişleri genel anestezi altında çekildi. Ekstraksiyon işleminden hemen sonra soketler üzerinde kontrol (grup 1), Varfarin (grup 3) ve Heparin (grup 5) gruplarına 2 mL salin solüsyonları topikal olarak uygulandı. Ankaferd (grup 2), Warfarin-Ankaferd (grup 4) ve Heparin-Ankaferd (grup 6) gruplarına ise benzer şekilde 2 mL ABS uygulandı. Diş ekstraksiyonunu takiben 6 grupta kanama zamanı ve kanama miktarı karşılaştırıldı. Toplanan veri sonuçları, önce ANOVA ile ardından ikili karşılaştırmalar için Tukey testi ile istatistiksel olarak analiz edildi.

Bulgular: Kanama zamanı, Varfarin ve Heparin grubunda Kontrol grubu ($p<0,05$) ile Ankaferd, Varfarin-Ankaferd ve Heparin-Ankaferd gruplarına göre ($p>0,05$) daha uzun bulundu. Benzer şekilde, Varfarin grubunda kanama miktarı Kontrol ve Varfarin-Ankaferd grubuna göre anlamlı derecede yüksekti ($p<0,05$). Kanama miktarı Kontrol, Heparin ve Heparin-Ankaferd gruplarında daha düşüktü, ancak fark istatistiksel olarak anlamlı değildi ($p>0,05$).

Sonuç: Topikal olarak uygulanan ABS, wistar sıçanlarda, varfarin kaynaklı kanama modelinin kanama kontrolü üzerinde heparin kaynaklı kanama modeline göre daha az etkilidir. Varfarin ve heparin arasında oluşan küçük fark ilerideki çalışmalarda araştırılmalıdır.

Introduction

Bleeding is a common challenging problem especially in patients who take an anticoagulant or antiaggregant treatment (i.e., patients with clotting disorders). Severe bleeding can be a life-threatening condition and should be managed effectively in a variety of ways including mechanic sponge pressing on the bleeding area; vessel ligation; applying chemical or electro-cauterization and cryotherapy; using topical haemostatic or vasoconstrictor agents and etc (1-4). Various haemostatic agents have been investigated for their role in haemostasis for decades (2,4).

As a commonly used blood stopper agent in our country, Ankaferd blood stopper [ABS (Ankaferd Health Products Ltd., İstanbul, Turkey)] is a traditional folk medicinal plant extract that has been approved in the management of cutaneous, dental and postoperative external bleeding. In addition, it has been reported that ABS had bacteriostatic effects on gram positive and gram negative bacterial flora and induced wound healing (3,5-13). Safety, efficacy, sterility and nontoxicity of the product have been shown (http://www.ankaferd.com) (4).

ABS is a unique mixture of five plant extracts (*Thymus vulgaris, Glycyrrhiza glabra, Vitis vinifera, Alpinia officinarum and Urtica dioica*) (2,3,5,6,10,14). One of the noteworthy studies on this topic has revealed that use of ABS caused the rapid formation of protein network within the serum and plasma (5).

The mechanism of the haemostatic action for ABS is the formation of an encapsulated protein network for vital physiological erythrocyte aggregation. This protein network formation with blood cells, particularly erythrocytes, covers the primary and secondary haemostatic system without independently acting on coagulation factors and platelets (5,6). Other than haemostatic effect, Mihmanli et al. (13) has reported a proliferative effect of ABS on human leukocytes.

In the light of the above-mentioned data, this study aimed to investigate the *in-vivo* haemostatic effect of ABS on warfarin-induced and heparin-induced bleeding model in rats.

Materials and Methods

The study protocol and experimental design were in accordance with the European Community Council Directive of November 24, 1986 (86/609/EEC) and were approved by the institutional review board and Animal Ethics Committee of Gaziosmanpaşa University Faculty of Medicine (approval number: 51879863-42).

Ankaferd Blood Stopper®

ABS (Ankaferd Health Products Ltd., İstanbul, Turkey) products are available as a liquid (solution), spray or in a dressing (tampon) forms that contain a standardized mixture of 5 medicinal plant extracts. The liquid form of ABS was used in the study and it includes active ingredients as follows:

0.16 mg of dried leaf of Vitis vinifera, 0.12 mg of dried root of Urtica dioica, 0.14 mg of dried leaf of Alpinia officinarum, 0.18 mg of dried leaf of Glycyrrhiza glabra, and 0.10 mg of dried leaf of *Thymus vulgaris* (1).

Animals

Forty-eight male Wistar rats, with an average weight of 270-320 g, were used in this study. They were housed in specially designed wire cages and maintained on a 12 h-12 h light-dark cycle with a constant room temperature of 23 °C. Rats were

allowed access to water and standard rodent diet ad libitum. All procedure was performed by the same specialist who authorized to animal experiments.

Experimental Design

Because of the limitations of ethics committee on the number of rats, they were not determined by using power analysis. So, 48 male Wistar rats were selected by the directions of Animal Ethics Committee of University and randomly divided into six groups of 8 animals each. The six groups were as follows:

1. Control group (group 1) rats had no pretreatment with any drug.
2. Ankaferd group (group 2) rats had no pretreatment with any drug.
3. Warfarin group (group 3) rats had pretreated with warfarin dissolved in saline (2 mg/kg) orally by a feeding catheter custom-made of silver for 3 consecutive days before experiment.
4. Warfarin-Ankaferd group (group 4) rats had pretreated with warfarin dissolved in saline (2 mg/kg) orally by a feeding catheter custom-made of silver for 3 consecutive days before the experiment.
5. Heparin group (group 5) rats were given an equal volume (0.25 mL) of standard heparin sodium (640 IU/kg) intraperitoneally 3 times a day for 3 consecutive days before the experiment.
6. Heparin-Ankaferd group (group 6) rats were given an equal volume (0.25 mL) of standard heparin sodium (640 IU/kg) intraperitoneally 3 times a day for 3 consecutive days before the experiment.

The maxillary right first molars were extracted under general anaesthesia (Alfamine 10%, Ege-Vet and Rompún®, Bayer HealthCare AG cocktail) using dental instruments (Figure 1). The extraction comprised fiberotomy, luxation, and tooth removal. The surgical wounds were left for secondary healing. Two ml of saline solution was applied to the each empty tooth sockets in group 1, 3 and 5 immediately after the extraction. Two mL of ABS was applied to the extraction sockets in group 2, 4 and 6 (Figure 1) immediately after the extraction.

The animal number in each group was determined according to literature (11,14) knowledge and local ethic committee advice.

Bleeding Time Assay

The bleeding time was measured with a chronometer and the amount of bleeding was measured with a milligram sensitive scale in all 6 groups just started following the tooth extraction. Bleeding time was detected as the time passed after the start of bleeding just following the tooth extraction to cessation of bleeding. Bleeding times were recorded by a single specialist with using a chronometer.

Amount of Bleeding Assay

The amount of bleeding was measured by means of a cotton wool roll. Each cotton roll was weighed before the procedure on a 0.1-g accurate scale by an investigator blinded to the treatment. Immediately after the extraction, cotton roll was inserted to the socket area and when the roll was filled with blood it is removed and weighed again. This process was carried on until the bleeding stopped. The difference in weight was considered as the amount of bleeding.

Statistical Analysis

The collected data results were analysed and also compared among 6 groups statistically by the ANOVA followed by Tukey test for pair-wise comparisons. P values less than 0.05 were considered as statistically significant.

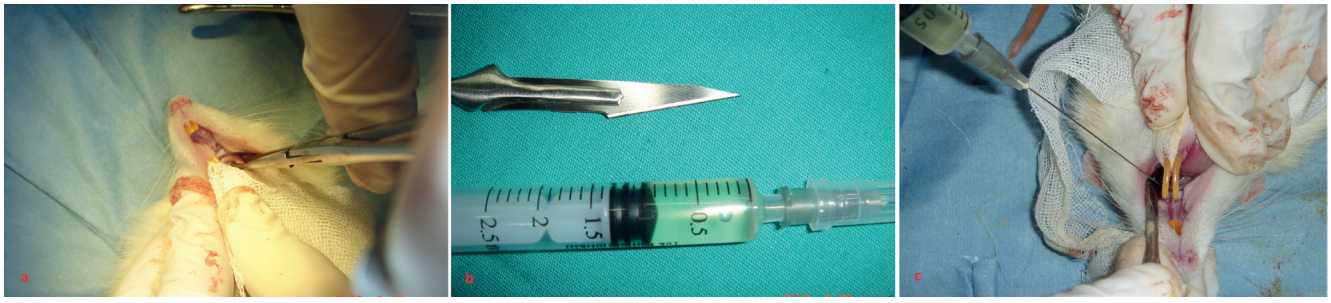

Figure 1. Figure shows the bleeding of rat models which ABS was applied topically; (a) extraction of upper first molar under general anaesthesia using dental instruments, (b) ready-to-use abs solution in dental injector, (c) 2 mL of ABS and saline solutions were applied to the extraction sockets

ABS: Ankaferd blood stopper

Results

There were not any complications observed during the study period and surgical procedure.

Bleeding Time

The mean bleeding time values of all groups were shown in the Table 1. The most prolonged bleeding time was observed in the warfarin group as 47.50 seconds and the shortest bleeding time was observed in Ankaferd Group as 2.87 seconds. Besides, there were not any statistical differences between Ankaferd and Control groups. Regarding the bleeding time, there was no statistically significant difference between warfarin and heparin groups ($p>0.05$). In the Warfarin and Heparin groups, the bleeding time was higher than those of the control and Ankaferd groups and the difference was statistically significant ($p<0.05$). The results showed that ABS administration decreased the bleeding time in both Warfarin-Ankaferd and Heparin-Ankaferd groups, but Ankaferd alone achieved no statistically significant difference when compared with control group (Table 1). The bleeding time of Heparin group was higher than those of the Heparin-Ankaferd, and Warfarin-Ankaferd groups ($p<0.05$).

Although the bleeding time was shortest in the Ankaferd group, there were no statistically significant differences among control, Ankaferd and Heparin-Ankaferd groups ($p>0.05$).

Amount of Bleeding

The amount of bleeding was highest in the Warfarin group as 0.041 grs, and this result was statistically significant when compared with Control and Ankaferd groups ($p<0.05$) (Table 1). There were no statistically significant differences among the Control, Ankaferd, Warfarin-Ankaferd, Heparin and Heparin-Ankaferd groups ($p>0.05$). However, when the results of Warfarin-Ankaferd, Heparin and Heparin-Ankaferd groups were compared with Warfarin group, there were statistically significant differences between them ($p<0.05$).

Warfarin group came first when both bleeding time and amount of bleeding results of all groups were taken into account.

Discussion

Purpose of the present study was to evaluate the haemostatic effect of ABS on warfarin and heparin-induced rat bleeding models. Rat bleeding model is a well-known study model for bleeding control studies. Besides, tooth extraction model was used in order to induce bleeding. Tooth extraction causes bleeding, and bleeding normally stops in a few seconds without intervention in rats. In fact, ABS use in not-drugged rats is not essential for bleeding control but it was necessary to compare the amount and time of the bleeding in this study. Tooth extraction was selected for ABS bleeding models due to frequency of its practice in dental surgery area.

Two distinct anticoagulant drugs were utilized to investigate the haemostatic effects of ABS, warfarin and heparin. It is well-known that these drugs, especially warfarin, cause an enormous bleeding in tooth extraction sockets (15). Warfarin is the most commonly prescribed oral anticoagulant in humans especially who require dental extractions (15,16). Considering dental extractions, patients who take warfarin medication for prevention of cardiovascular thrombosis are at an increased risk of perioperative thromboembolism if the medication is interrupted but there is also a possible increased risk of bleeding

Table 1. The comparison of the bleeding time and the amount of bleeding in between each group

Groups	Control	Ankaferd	Warfarin	Warfarin-Ankaferd	Heparin	Heparin-Ankaferd
Bleeding time (second; sc)	14.87±5.13	2.87±2.10	47.50±13.37[a,b]	18.62±12.59[b,c]	40.50±10.14[a,b,d,e]	9.00±4.40[c]
Amount of bleeding (gram; gr)	0.007±0.005	0.001±0.003	0.041±0.036[a,b]	0.009±0.018[c]	0.015±0.007[c]	00±00[c]

[a]$p<0.05$ differences compared to Control, [b]$p<0.05$ differences compared to Ankaferd, [c]$p<0.05$ differences compared to Warfarin, [d]$p<0.05$ differences compared to Warfarin-Ankaferd, [e]$p<0.05$ differences compared to Heparin

if the medication is continued (15). Heparin has also similar status of its use. It is usually used by parenteral route (16).

The main indication for using anticoagulant therapy such as warfarin, heparin, low molecular weight heparins and etc. is to prevent or manage for arterial and venous thrombosis (7,16). The use of such anticoagulant agents alters the coagulation cascade in the body (1,16). One of the most important factors in coagulation is the vitamin K. Warfarin is one of the most common used vitamin K antagonist agent and it is quickly adsorbed and well tolerated by oral route. The anticoagulant effect of warfarin is slowly established but has a relatively prolonged effect, through a half-life of at least 48 hours (14,16). Heparin has some different effects on the coagulation but also activates anti-thrombin and other proteases involved in the blood clotting system that causes coagulation problems (1). The results of the present study showed that warfarin and heparin, as anticoagulant drugs, caused an important increase on bleeding time. ABS administration normalized the increase and stopped the bleeding as well. According to results of study, these anticoagulant drugs also increased the amount of the bleeding.

According to previous studies, oral surgical and clinical approach to the patients who take anticoagulant medication made it necessary to stop the anticoagulant treatment for several days or reduce the dose of anticoagulant drug for preventing severe life-threatening hemorrhage (7). But, currently, this radical approach for the treatment of patients undergoing anticoagulant therapy were replaced to an approach that advises to perform the oral surgical procedures without any intervention of anticoagulant treatment but with attention on the local bleeding control methods (7). Therefore, ABS was used as a local haemostatic agent to control warfarin and heparin-induced bleeding in present study. As a result, bleeding control was achieved by using ABS in rats which have been receiving either of these anticoagulant drugs or not.

Ankaferd blood stopper is a unique standardized herbal mixture being used as a haemostatic agent in Turkey for many years. The exact mechanism of ABS is yet unknown but there is a growing body of evidence on the efficacy of ABS on homeostasis (1,3-5,7,8,10-12,14). Additionally, it was found that ABS shortened the bleeding time in the rats receiving and not receiving anti-coagulant therapy both. Meric Teker et al. (17) reported that ABS-induced formation of the protein network affected the physiological haemostatic process without affecting any individual clotting factor. The present study also supports this literature knowledge.

Similar to the present results, Cipil et al. (14) reported that the bleeding time was reduced to 44% with ABS use in animals pretreated with warfarin. Sacco et al. (16) also demonstrated that ABS had a haemostatic effect on the cut tails of rats alone or in the presence of heparin or aspirin medication. Iynen et al. (1) showed that ABS irrigation effectively prevented nasal bleeding in rats pretreated with heparin sodium. Meric Teker et al. (17) reported the ABS as an effective, safe, quick, and easy-to-use alternative to the phenylephrine in patients with anterior epistaxis. But, they also revealed secondary haemorrhage in a few patients with using ABS. Iynen et al. (1) reported that ABS successfully shortened the haemostasis time and decreased the bleeding volume.

Warfarin and heparin have different biochemical anti-haemostatic actions on coagulation cascade mentioned in the literature (1,16). In contrast, the previous results have some contradictions. ABS does not affect the entire blood clotting system including coagulations factors (7,14,18-21). But, heparin and warfarin affect entire clotting system with various different ways (14). ABS has a different level of action on both anti-haemostatic agents.

The present study revealed that ABS more effective on heparin experiment models than warfarin without statistically significance. In addition the previous result of the present study, ABS should be used on warfarin models as a local haemostatic agent due to statistically significance between Warfarin group, Control group, and Warfarin-Ankaferd group in terms of bleeding time and amounts ($p<0.05$). Similarly, these previous results valid on heparin including groups ($p<0.05$).

Study Limitations

The present study had a limitation as international normalization ratio (INR) test was not used for standardization of anti-coagulation level of blood. According to the literature, Clinicians should attempt to identify contributing factors for prolonged non-

therapeutic INR. So, the risk of coagulation can be minimized, as well as costs of hospital stay and laboratory exams can be reduced (22). It is important to achieve the therapeutic levels of INR in the patients treated by anticoagulants (23).

Conclusion

The results of the present study concluded that topically administered ABS to empty tooth sockets immediately after the extraction is effective for homeostasis in warfarin and heparin-induced bleeding in rats. But, efficiency of ABS over warfarin-induced and heparin-induced bleeding patterns and whether if it affects in the same way or not, should be investigated in further studies. Also, the exact mechanism of ABS over haemostasis and the necessity of this agent in clinic use should be investigated in the future studies.

Ethics

Ethics Committee Approval: Animal Ethics Committee of Gaziosmanpaşa University Faculty of Medicine (approval number: 51879863-42).

Informed Consent: It was not taken.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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Original Article / Özgün Araştırma

Evaluation of Changes in Quality of Life After Dental Treatment in Children

Çocuklarda Diş Çürüğü Tedavisi Sonrası Yaşam Kalitesindeki Değişimlerin Değerlendirilmesi

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Abstract

Objective: Various instruments can be used in children to determine the impact of Oral Health Related Quality of Life (OHRQoL). Child Perceptions Questionnaire (CPQ_{8-10}) can be used to evaluate the oral symptoms, functional limitations, emotional well-being and social well-being of subjects. Our study aimed at evaluating the impact of dental caries on OHRQoL and the changes in quality of life of subjects after treatment of dental caries compared to subjects without dental caries using CPQ_{8-10}.

Materials and Methods: Study included a total of 200 systemically healthy subjects aged 8-10 years (110 girls, 90 boys; 9.10±0.8) who referred to Adnan Menderes University Pediatric Dentistry clinic between June 2016 and May 2017. Based on the results of clinical and radiological examination, two groups were created consisting of subjects with (n=100, 9.14±0.81) and without (n=100, 9.05±0.82) dental caries and treatment needs, and CPQ_{8-10} questionnaire was used to evaluate the quality of life. Questionnaire was repeated 4 weeks after the completion of treatment of subjects with dental caries. Results were subjected to statistical evaluation.

Results: Pre-treatment CPQ_{8-10} scores of subjects with dental caries were higher than those of subjects without dental caries (17.46±11.66 and 6.86±4.76, respectively; $p<0.001$) OHQoL scores were decreased after dental treatment (4.06±5.40) and the difference was statistically significant ($p<0.001$). CPQ_{8-10} scores after dental treatment were lower than those of subjects without dental caries who did not need treatment ($p<0.001$).

Conclusion: We have determined that the untreated dental caries negatively affect the quality of life in children aged 8-10 years, and the quality of life substantially improves after treatment of dental caries.

Keywords
Quality of life, dental caries, CPQ_{8-10}

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Yaşam kalitesi, diş çürüğü, CPQ_{8-10}

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Öz

Amaç: Ağız sağlığının yaşam kalitesi üzerine etkisinin Oral Health Related Quality of Life (OHRQoL) belirlenmesi için çocuklarda farklı ölçekler uygulanabilmektedir. "Çocuk Algıları Ölçeği" [Child Perceptions Questionnaire" (CPQ_{8-10})] ile gönüllülerin ağız içi semptomları, fonksiyonel kısıtlılıkları, duygusal problemleri ve sosyal etkiler değerlendirilebilmektedir. Çalışmamızda diş çürüğünün OHRQoL üzerine etkisinin değerlendirilmesi, diş çürüklerinin tedavisi sonrasında yaşam kalitesindeki değişimlerinin CPQ_{8-10} ile diş çürüğü olmayan gönüllülerle karşılaştırılması amaçlanmıştır.

Gereç ve Yöntemler: Adnan Menderes Üniversitesi Diş Hekimliği Pedodonti Kliniği'ne, Haziran 2016-Mayıs 2017 tarihleri arasında başvuran 8-10 yaş arasındaki

sistemik olarak sağlıklı 200 gönüllü (110 kız, 90 erkek, 9,10±0,8) çalışmaya dahil edilmiştir. Klinik ve radyolojik muayene sonucunda, diş çürüğü ve tedavi ihtiyacı olan (n=100, 9,14±0,81) ve diş çürüğü ve tedavi ihtiyacı olmayanlardan (n=100, 9,05±0,82) oluşan iki grup oluşturulmuş ve yaşam kalitesinin değerlendirilmesinde CPQ_{8-10} anketi uygulanmıştır. Diş çürüğü olan gönüllülerin, tedavilerinin tamamlanmasından 4 hafta sonra anket tekrarlanmıştır. Sonuçlar istatistiksel olarak değerlendirilmiştir.

Bulgular: Diş çürüğü bulunan gönüllülerin tedavi öncesi CPQ_{8-10} skorları (17,46±11,66) diş çürüğü olmayanlara (6,86±4,76) göre daha yüksek bulunmuştur (p<0,001). Diş tedavileri sonrasında, yaşam kalitesi skorları (4,06±5,40) düşüş göstermiştir ve bu fark istatistiksel olarak anlamlıdır. (p<0,001). Diş tedavisi sonrasındaki $CPQ8_{8-10}$ skorları, tedavi ihtiyacı olmayan çürüksüz gönüllülerden daha düşük olarak tespit edilmiştir (p<0,001).

Sonuç: Sekiz-on yaş aralığındaki çocuklarda tedavi edilmemiş diş çürüğünün yaşam kalitesini olumsuz yönde etkilediği ve diş çürüklerinin tedavi edilmesi sonrasında yaşam kalitesinin önemli derecede arttığı belirlenmiştir.

Introduction

Oral and dental health problems may cause negative effects on functional, social and psychological status of children. Evaluation of impact of oral and dental health on quality of life of children can improve the communication between patients, parents and dentists. In addition, evaluation of Oral Health-Related Quality of Life (OHRQoL) may be helpful in assessing the treatment needs, setting the priorities and assessing the outcomes of treatment strategies and initiatives (1).

Assessments have been conducted in recent years to investigate the impacts of dental caries, traumatic dental injuries, gingival diseases and malocclusions on quality of life in children (2-4). Various questionnaires and scales have been used to conduct such assessments. Early Childhood Oral Health Impact Scale (ECOHIS) is a method that is applied to children aged 2-5 years and responded by the parents (5). Oral Impact on Daily Performance scale evaluates the impacts of oral and dental health on daily activities (6). Child Perceptions Questionnaire (CPQ), which was also used in our study, was developed by Jokovic et al. (7) in 2004. This questionnaire can be applied to two different age groups of 8-10 years and 11-14 years. CPQ questionnaires, which contain questions that evaluate the functional, emotional, social and oral symptoms, have been adapted to many languages and found to be successful in determining OHRQoL (8-11).

Studies performed using CPQ_{8-10} questionnaire have shown that the dental caries negatively affect the quality of life in children (12,13). The aim of our study was to determine if treatment of dental caries eliminated such negative effects, using CPQ_{8-10} questionnaire.

Materials and Methods

Our study was conducted at Adnan Menderes University Faculty of Dentistry. Ethics committee's approval was obtained (Adnan Menderes University, Faculty of Medicine, Non-Interventional Ethics Committee/2016-876) before initiation of the study. The CPQ_{8-10} was used to evaluate OHRQoL in both groups. Questionnaire consisted of 25 questions including 5 questions for oral symptoms, 5 questions for functional, 5 questions for emotional and 10 questions for social status. Questions were directly asked to children and they were asked to respond the questions as "never", "a few times", "sometimes", "often" and "almost every day". Responses were scored between 0 and 4 according to 5-point Likert scale. Besides these 25 questions, the following 2 questions were also asked to children to obtain a general evaluation about their oral health: "What do you think about your mouth and teeth?" and "How often do you feel uncomfortable with your mouth and teeth?" (Questions 26 and 27). Study included 200 children aged 8-10 years who referred to pediatric dentistry clinic for examination between June 2016 and May 2017. Three calibrated dentists carried out the dental examinations. Based on the results of clinical and radiological examination, two groups were created consisting of subjects with dental caries who have no pain last 4 week, have no need radicular pulpal treatments and only need restorative dental treatment (n=100) and without (n=100) dental caries, and CPQ_{8-10} was used to evaluate the quality of life. Age, gender, health insurance status and income levels were also recorded in addition to questions evaluating the quality of life. Status of dental caries was reported as decayed, missing and filled teeth (DMFT)/dft, decayed-missing-filled surfaces (DMFS)/dfs in consequence of clinical examination.

Following the completion of clinical examination and questionnaire, treatment of patients with dental caries was initiated by a single specialist (MD). Oral hygiene training were given to patients after completion of preventive treatments and asked to return to clinic for control visit 4 weeks for repeating the questionnaire by two different specialist (KUGG and IS). All patients included were informed aboult the study.

Statistical Analysis

Data of group of healthy subjects and pre and post-treatment data of patients with dental caries were recorded and statistically evaluated by IBM SPSS Statistics 21.0 program (Armonk, NY, USA). Results were evaluated using chi-square test, Mann-Whitney U and Kruskal-Wallis tests.

Results

Records of 110 girls and 90 boys included in two groups consisting of subjects with (n=100, age= 9.14±0.81 year) and without (n=100, age= 9.05±0.82 year) dental caries and treatment needs were evaluated. No statistically significant relationship was found between the age, gender, social insurance status and the OHRQoL; however, OHRQoL was observed to increase with the increase in income level and a statistically significant relationship was found (p=0.017) (Table 1). In group of patients with dental caries, mean DMFT/dft and mean DMFS/dfs values were calculated to be 2.51±1.99 ve 3.62±2.53 and 3.62±3.39 ve 7.39±5.97, respectively.

CPQ_{8-10} values of groups caries free and dental caries pretreatment and post-treatment were found to be 6.86±4.76, 17.46±11.66 and 4.06±5.4, respectively (Table 2). When the responses to Questions 26 and 27 were evaluated, the mean values were determined to be 1.64±0.905, 1.08±0.849 and 1.82±0.657 and 1.31±0.961, 1.71±0.891 and 43±0.537, respectively in caries free, dental caries pretreatment and post-treatment subjects (Table 3).

Discussion

Most of the current OHRQoL instruments could manage to measure the impact of oral health on physical, functional, social and emotional well-being of a person. As in adults, oral and dental health disorders can affect diet, health and quality of life in children (14). Results of our study also showed that the oral and dental health affect quality of life. A significant difference was observed between the OHRQoL values of caries free children and children with dental caries, and dental caries were determined to negatively affect the quality of life. According to results of our study, quality of life is not affected by age, gender and health insurance status.

Table 1. Distribution of age, gender, income level and health insurance in groups

	Caries free subjects (n)	Subjects with dental caries (n)	Total	Rate (%)
Age				
8	31	27	58	29.0
9	33	32	65	32.5
10	36	41	77	38.5
Gender				
Girls	55	55	110	55.0
Boys	45	45	90	45.0
Income level				
TRY <1.000	13	22	35	17.5
TRY 1.000-2.999	48	60	108	54.0
TRY 3.000-5.999	25	14	39	19.5
TRY >6.000	14	4	18	9.0
Health insurance				
Yes	95	94	189	94.5
No	5	6	11	5.5

Table 2. Study groups and oral health related quality of life values (p<0.001)			
	Caries free	Dental caries pretreatment	Dental caries Post-treatment
Oral symptoms (Questions 1-5)	2.50±2.24b	5.75±2.88c	0.91±1.34a
Functional limitations (Questions 6-10)	1.31±1.60b	4.08±3.50c	0.89±1.33a
Emotional well-being (Questions 11-15)	1.89±2.31b	4.59±4.64b	1.13±2.25a
Social well-being (Questions 16-25)	1.15±1.88a	3.17±4.56b	1.17±2.32a
Total (Questions 1-25)	6.86±4.76b	17.46±11.66c	4.06±5.40a
a,b,c: A statistically significant differences is shown with the letter a, b, c in the same column			

Table 3. Responses to questions 26 and 27 and mean values by groups			
	Caries free	Dental caries pre-treatment	Dental caries post-treatment
Question 26	1.64±0.90b	1.08±0.84a	1.82±0.65c
Question 27	1.31±0.96b	1.71±0.89c	0.43±0.53a
a,b,c: A statistically significant differences is shown with the letter a, b, c in the same column			

Children of parents with higher education and family income levels are likely to have a better quality of life. It has been shown that lower education level causes decreased income (15) and the low income level is associated with poverty (16). Children from poor families have limited access to healthcare services and preventive interventions that avoid poor quality of life (17). Results of our study indicated a significant relationship between family income level and quality of life. Children from families with higher income level were observed to have a superior quality of life.

Untreated dental caries in children can cause difficulties in chewing, sleeping and socializing, and affect self-confidence, growth and weight gain, thereby decreasing quality of life (18). Such effect of dental caries has given rise to investigations into whether dental treatments could improve the quality of life again. In recent years, investigators evaluated pre- and post-treatment quality of life of children given dental treatment under general anaesthesia using ECOHIS scale, and reported that dental treatment positively affected the quality of life (19-21). These studies were usually conducted in patients aged 6 years and below. In a study conducted in an older age group by administering treatment under clinical conditions, CPQ_{8-10} questionnaire was applied to a total of 186 children divided into 2 groups as healthy children and children with dental caries. Questionnaire was repeated 4 weeks after the completion of treatment in patients who needed treatment. Based on the results of study, it was concluded that the treatment of dental caries positively affected the quality of life (22). These results are consistent with the results of our study.

Study Limitations

Our study showed that the post-treatment OHRQoL values were significantly higher compared to group of healthy subjects. This difference might be associated with the resolution of pre-treatment symptoms such as pain and dental fear and the reduction of anxiety.

Orthodontic problems are also known to affect quality of life. Healey et al. (23) evaluated the relationship between malocclusion and the quality of life using CPQ_{11-14} questionnaire and reported a significant relationship between them. Orthodontic evaluations were not conducted in subjects with and without dental caries in our study, and the patients presented to our clinic with orthodontic problems were asked to complete the questionnaire without receiving orthodontic treatment. This might have affected the OHRQoL values and can be considered as a limitation of our study.

As another limitation, the relationship between treatment type and the quality of life could not be evaluated in our study. More traumatic procedures like tooth extraction and the number of such procedures are believed to increase the possibility of problems such as post-treatment eating difficulty in children (24). On the other hand we did not evaluate the validity of CPQ_{8-10} in Turkish children. During the development and evaluation of the CPQ for 8- to 10-year-olds, questions were selected from the CPQ_{11-14} (7). The translation and validation of CPQ_{11-14} version into Turkish was carried out by Aydogan (25). The CPQ_{8-10} was used to evaluate OHRQoL in both groups. We approved the validation of CPQ_{11-14} version into Turkish children for assesing the 8-10 years old. Further studies are needed to development of the CPQ_{8-10} in Turkish children for more accurate estimation.

Conclusion

Our study has shown that the quality of life of 8 to 10-years-old children with dental caries that need to be treated is negatively affected compared to children without dental caries and treatment needs, and the quality of life is significantly improved after treatment. Thus, it appears that the dental treatments are likely to improve the quality of life in children.

Ethics

Ethics Committee Approval: Adnan Menderes University Faculty of Medicine, Non-Interventional Ethics Committee, (approval number: 2016-876).

Informed Consent: All patients included were informed about the study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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Original Article / Özgün Araştırma

Antibacterial Efficiency of Different Irrigation Solutions, Lasers and Photodynamic Therapy with Indocyanine Green in Root Canals Infected By *Enterococcus Faecalis*

Enterococcus Faecalis ile Enfekte Edilmiş Kök Kanallarında Farklı İrrigasyon Solüsyonlarının, Lazerlerin ve İndosiyanin Yeşili ile Fotodinamik Terapinin Antibakteriyel Etkinliği

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Abstract

Objective: The aim of this study was to evaluate bacterial reduction after using different irrigants, lasers and photodynamic therapy (PDT) in root canals infected by *Enterococcus faecalis*.

Materials and Methods: Seventy human maxillary incisors were used in this study. After endodontic preparation, sixty teeth were infected by 20 µl *E. faecalis* (ATCC 29212) and specimens were incubated for 72 hours. Groups were formed according to the irrigation protocol used: group 1: negative control, group 2: positive control, group 3: 10 mL 2% chlorhexidine (CHX), group 4: 10 mL 5% NaOCl, group 5: diode laser, group 6: Er:YAG laser, and group 7: indocyanine green (ICG)-diode laser (PDT). After disinfection procedures the samples isolated from root canals were placed into blood agar, incubated at 37 °C for 24 h and then were counted for colony-forming units. One-Way ANOVA was used for the statistical evaluation of data. For multiple comparisons, Tamhane's T2 test was used.

Results: All investigated disinfection procedures had a significant reduction in the bacterial population ($p<0.001$). CHX, NaOCl and ICG-diode laser groups were equally effective in reducing *E. faecalis* populations ($p>0.05$) and statistically more effective than both laser groups ($p<0.05$).

Conclusion: Promising results were obtained by using PDT with ICG. This alternative disinfection method found effective on *E. faecalis*.

Öz

Amaç: Bu çalışmanın amacı, *Enterococcus faecalis* ile enfekte edilmiş kök kanallarında farklı irrigasyon solüsyonları, lazerler ve fotodinamik tedavi (FDT) kullandıktan sonra bakteri sayısındaki azalmayı değerlendirmektir.

Gereç ve Yöntemler: Bu çalışmada yetmiş adet insan üst çene kesici dişi kullanıldı. Kök kanal preparasyonundan sonra altmış diş 20 µL *E. faecalis* (ATCC 29212) ile

enfekte edildi ve numuneler 72 saat inkübasyona bırakıldı. Gruplar, kullanılan dezenfeksiyon protokolüne göre oluşturuldu: grup 1: Negatif kontrol, grup 2: Pozitif kontrol, grup 3: 10 mL %2 klorheksidin (KH), grup 4: 10 mL %5 NaOCl, grup 5: Diyot lazer, grup 6: Er:YAG lazer ve grup 7: İndosiyanin yeşili (İSY)-diyot lazer (FDT). Dezenfeksiyon prosedürlerinden sonra kök kanallarından izole edilen numuneler kanlı agara yerleştirildi, 37 °C'de 24 saat inkübe edildi ve daha sonra koloni oluşturan birimler sayıldı. Verilerin istatistiksel değerlendirilmesi için tek yönlü varyans analizi, çoklu karşılaştırmalar için Tamhane'nin T2 testi kullanıldı.

Bulgular: İncelenen tüm dezenfeksiyon prosedürleri bakteri popülasyonunda belirgin bir azalmaya neden olmuştur ($p<0,001$). KH, NaOCl ve İSY-diyot lazer grupları, *E. faecalis* popülasyonlarının azaltılmasında eşit derecede ($p>0,05$), her iki lazer grubundan ise istatistiksel olarak anlamlı seviyede daha etkindi ($p<0,05$).

Sonuç: İSY'nin kullanıldığı FDT'de umut verici sonuçlar elde edildi. Bu alternatif dezenfeksiyon yöntemi *E. faecalis* üzerinde etkili bulundu.

Introduction

The main objective of endodontic therapy is to eliminate pathogenic microorganisms which are the major confounding factor in the treatment of infected root canals and periapical healing (1).

Disinfection of dentinal tubules, accessory canals, isthmuses and ramifications that can be found in root canal system is not possible by mechanical preparation alone (2). Therefore, irrigation is one of the most important steps of endodontic treatment, which is recommended both before and during root canal shaping because of the antibacterial effects and necrotic tissue dissolving properties (3). The most commonly used irrigant in root canal therapy is sodium hypochlorite (NaOCl). Concentrations between 0.5% and 6% are preferred (4). Two-percent chlorhexidine (CHX) gluconate is an alternative endodontic solution (5).

The prevalence of endodontic infections depends on microorganisms' ability to adapt to environmental changes. Many different mechanisms are used by bacteria including biofilm formation, physiological modifications, exchange of genetic materials and formation of cell subpopulations (6). Despite the technological and scientific advances in endodontics, there are many unsuccessful cases due to microbial factors. *Enterococcus faecalis* is the most commonly isolated bacteria in teeth with apical periodontitis and is thought to be responsible for the majority of failed root canal treatments (7). *E. faecalis* may penetrate up to depths of 400-1000 µm in the dentin tubules, can live in conditions with poor nutrition and can develop resistance to root canal disinfectants (8).

Endodontic disinfectants have toxic and inadequate inorganic tissue dissolving properties, can not completely eliminate bacteria and cannot be tolerated by periapical tissues when extruded from apex (9). These factors have prompted researchers to develop novel methods and materials for endodontic procedures (10-13).

Photodynamic therapy (PDT) shows promise as an antimicrobial therapy that can eliminate microorganisms observed in root canal infections. The PDT utilizes a non-toxic photosentizing agent that has the capacity to generate highly reactive species, which are harmful for microorganisms and then irradiates through the application of the laser (14,15).

Recently used methylene blue and toluidine blue as photoactive agents were shown to cause discoloration of anterior teeth (12,16). Indocyanine green (ICG) is a photosensitizing agent that recently developed and used in many areas of medicine (17,18). Previous studies have reported that ICG did not lead to discoloration of teeth and did not produce any toxic or allergic effects. ICG displayed a strong adhesion to bacterial membranes (*Porphyromonas gingivalis, Prevotella intermedia, Fusobacterium nucleatum, Tannerella forsythia, Camphylobacter recta, Eikenella corrodens and Treponema denticola*) and demonstrated bactericidal effect when activated by a diode laser (19-21).

The aim of present study was to evaluate antibacterial effect of different irrigation solutions, two types of laser and PDT with ICG in infected root canals by *E. faecalis.* The null hypothesis tested was that these disinfection protocols had no influence on the eradication of *E. faecalis.*

Materials and Methods

The study was reviewed and approved by the Ethics Committee of Gaziosmanpaşa University Faculty of Medicine (approval number: 14-KAEK-246). Seventy human maxillary incisors were used in this study. Mesio-distal and bucco-lingual direction radiographs

were taken from the teeth to confirm the presence of a single canal. Teeth were decoronated and 15 mm long roots were obtained, approximately 15 mm in length. Roots were prepared up to protaper F3 file (Dentsply-Maillefer, Ballaigues, Switzerland). After each file, 2 mL of 5% NaOCl (Whitedentmed, Erhan Kimya, İzmir, Turkey) was used. Five mL of 5% NaOCl, 5 mL of 15% ethylenediaminetetraacetic acid (Imicryl, Konya, Turkey) and 5 mL of 5% NaOCl solutions were used for the final irrigation respectively. Root canals were dried with paper points. Root apexes were sealed using composite resin. Specimens were placed into Eppendorf tubes with silicone impression material. Teeth were packaged one by one and sterilized in an autoclave at 121 °C for 30 minutes. After sterilization, 10 teeth were not infected to confirm the accuracy of the sterilization and were evaluated as a negative control group. *E. faecalis* cultures (ATCC 29212) were incubated for 72 hours and were placed in Brain Heart Infusion broth medium. Solution was prepared to be 0.5 Mc Farland. Each sample was inoculated with 20 µL of the prepared solution and specimens were incubated at 37 °C for 72 hours in the incubator. No disinfection procedure was applied to 10 samples, which were evaluated as the positive control group. In all experimental steps after sterilization samples were handled with sterile gloves, gauze and instruments in a laminar flow chamber to avoid contamination. Groups were formed as follows:

Group 1: Negative control (n=10). Specimens were sterilized and were not infected by *E. faecalis.*

Group 2: Positive control (n=10). Specimens were infected by *E. faecalis* but did not have a disinfection technique applied.

Group 3: CHX (n=10). Root canals were irrigated with 10 mL 2% CHX and 10 mL sterilized distilled water, respectively.

Group 4: NaOCl (n=10). Root canals were irrigated with 10 mL 5% NaOCl and 10 mL sterilized distilled water, respectively.

Group 5: Diode laser (n=10). Root canals were irradiated for 1 minute with a 940 nm wavelength diode laser (Epic, Biolase Tech., CA, USA) at 1W power utilizing the continuous mode with endodontic tips.

Group 6: Er:YAG (Erbium Yttrium Aluminium Garnet laser) (n=10). Root canals were irradiated for 1 minute with a 2940 nm wavelength Er:YAG laser (Kavo Key 3+, KaVo, Biberach, Germany) with endodontic tips (diameter ISO 30). Laser Parameters were 1W, 10 Hz, and 100 mJ.

Group 7: ICG-diode laser (n=10). 25 mg sterile ICG mixed with 5 mL sterile distilled water in a sterile bottle. Each root canal was irrigated with 0.5 mL ICG solution then allowed to sit for 30 seconds. Then root canals were then irradiated for 1 minute (30 seconds wet, 30 seconds dry) with a 940 nm wavelength diode laser (Epic, Biolase Tech., CA, USA) at 1W power using the continuous mode setting with endodontic tips (diameter 200 µm).

Three sterile paper points for each tooth were moistened with 0.5 mL of sterile distilled water and were placed into the root canal one by one for 1 minute. Paper points were placed into sterile Eppendorf tubes with brain heart infusion medium and specimens were sent to the microbiology laboratory. Vortexing was performed on samples and 20 µL of liquid medium was inoculated on blood agar plates. Specimens were incubated at 37 °C for 24 hours and the colony-forming units (CFU) were counted at the end of incubation. Written consent wasn't obtained because operation was made on extracted teeth.

Statistical Analysis

One-Way ANOVA was used to evaluate the data. For multiple comparisons, Tamhane's T2 test was used. The significance was defined as $p<0.05$. Analyses were performed using SPSS 19.0 (IBM SPSS Statistics 19, SPSS inc., an IBM Co., Somers, NY, USA).

Results

Results were summarized in Tables 1 and 2 and Figure 1. The highest bacterial colonization was observed in the positive control group. While there was no difference between negative control, NaOCl and the ICG-diode laser groups ($p>0.05$), there were statistical differences between the negative control and all other groups ($p<0.05$). There was no statistical difference between Er:YAG laser and diode laser groups ($p>0.05$). There were no statistically significant difference between CHX, NaOCl and PDT (ICG-diode laser) groups ($p>0.05$); bacterial colonization has been shown to be decreased when compared with laser groups used alone. Positive and negative bacterial growth three days after disinfection procedures were completed and are shown in Table 2. Laser groups did not completely eliminate *E. faecalis*, despite being quite effective in reducing bacterial colonization.

Table 1. Multiple comparisons between disinfection methods

Groups	CFU/mL Mean ± SD	p
Negative control	0.00±0.00^{A}	<0.001
Positive control	8200±3794.73^{D}	
Chlorhexidine	9.40±7.07^{B}	
Sodium hypochlorite	6.90±15.37^{A,B}	
Diode laser	86.20±29.55^{C}	
Er:YAG laser	67.20±37.83^{C}	
ICG-diode laser (PDT)	7.20±10.16^{A,B}	

The results were considered statistically significant for p<0.05. For groups different uppercase letters (A,B,C,D) (One-Way ANOVA) indicate a statistically significant difference, SD: Standard deviation, CFU: Colony-forming units, ICG: Indocyanine green, PDT: Photodynamic therapy

Table 2. Bacterial growth after three days from disinfection of root canal system

Groups	Negative growth-count (n)	Positive growth-count (n)
Negative control	10	0
Positive control	0	10
Chlorhexidine	2	8
Sodium hypochlorite	7	3
Diode laser	0	10
Er:YAG laser	0	10
ICG-diode laser (PDT)	6	4

ICG: Indocyanine green, PDT: Photodynamic therapy

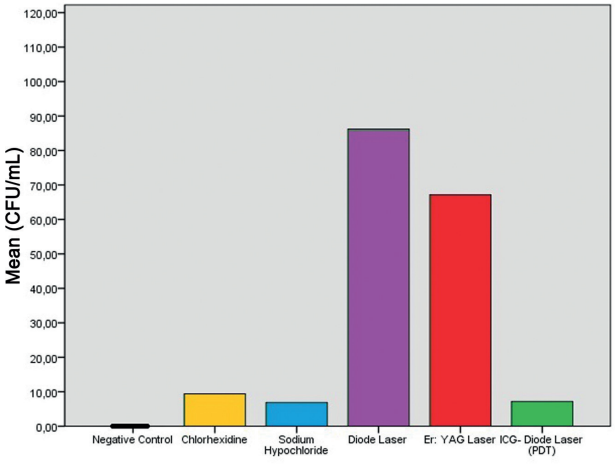

Figure 1. Graphic view of the results. The number of viable *E. faecalis* after each disinfection protocole was counted

CFU: Colony-forming units, ICG: Indocyanine green, PDT: Photodynamic therapy

Positive bacterial growth was observed in 10 samples from both the Er:YAG and diode laser groups. There were no bacterial growth in 7 samples in the NaOCl group, 6 samples in the ICG-diode laser group, and 2 samples from CHX group.

Discussion

Irrigation in addition to mechanical preparation is required in order to increase the efficiency of preparation and effectively eliminate bacteria from the root canal system (22). These irrigation solutions should eliminate endodontic pathogens, persistent infection and resistant bacteria. For this purpose, many solutions and their various concentrations and different disinfection methods are used in clinics and research (11,23-26).

However, the cytotoxic properties of the solutions beside antibacterial activity have prompted researchers to seek other options (27). Recently, new systems and materials have been proposed to replace the traditional chemomechanical process or supporting their impact to improve root canal disinfection (28). We aimed to demonstrate the effects of two types of lasers used alone and PDT with ICG on *E. faecalis* by comparison with conventional endodontic disinfectants. According to our results, NaOCl, ICG-diode laser group and CHX were more effective in eliminating *E. faecalis* than Er:YAG laser and diode laser disinfection methods. Therefore, the null hypothesis that the eradication of *E. faecalis* was not affected by the disinfection protocols was rejected.

Various studies have reported the antibacterial efficacy of PDT and have stated that PDT can be used safely without harming the host tissue (29-36).

Bonsor et al. (29) compared antimicrobial effect of PDT and 2.5% NaOCl on facultative anaerobic microorganisms on patients who were diagnosed irreversible pulpitis or periradicular periodontitis. Tolonium chloride as a photosensitizer and a diode laser as a light source were used. Researchers reported that 2.5% NaOCl provided an 80.9% bacterial reduction whereas PDT caused a bacterial elimination rate of 91.3% (29).

Fimple et al. (30) evaluated the effects of PDT application with methylene blue and a diode laser at the 665 nm wavelength in experimentally infected root canals by *Actinomyces israeli, F. nucleatum,*

P. gingivalis and P. intermedia and found an 80% bacterial decrease of CFUs. The findings of the study demonstrated that bacterial elimination could be achieved by more than 1 log^{10} with an increasing concentration of methylene blue and light energy. Authors have concluded that PDT effectively helps the standard disinfection process with optimized parameters (30).

In another study that used conjugates of polyethyleneimine and chlorine as a photosensitizer with a 660 nm wavelength diode laser with 40 mW energy as a light source, Garcez et al. (31) investigated the effects of PDT on antibiotic resistant microflora and retreatment of necrotic pulp teeth. Authors reported that using PDT after traditional chemomechanical preparation provided a microorganism-free root canal system and suggested the use of PDT in the presence of multi-drug-resistant organisms (31).

Pagonis et al. (32) evaluated the effects of PDT with nanoparticles loaded with methylene blue and a 665 nm wavelength diode laser on *E. faecalis* in the planktonic phase and reported that application of PDT led to a 1-2 log^{10} bacterial reduction in CFUs (32).

Rios et al. (33) evaluated the antimicrobial effect of PDT with toluidine blue and a 638 nm wavelength light source on root canals infected by *E. faecalis*. They found that PDT resulted in a reduction of the bacterial count and suggested that PDT be used as a supportive antimicrobial process (33). The aforementioned study results are in agreement with this study results.

Nagayoshi et al. (34) studied the antimicrobial effects of PDT with ICG and an 805 nm wavelength diode laser at 5 W power in root canals infected by *E. faecalis* by using *in vitro* apical periodontitis models. The authors concluded that the diode laser irradiation combined with photosensitizer provided closer antimicrobial effect as obtained with 2.5% NaOCl and 60 seconds irradiation was more effective than 30 seconds (34). Similarly, Silva et al. (12) used a 660 nm wavelength diode laser at 40 mW power with methylene blue and malachite green as photosensitizing agents to evaluate disinfection of root canals by PDT. Authors suggested performing PDT with a diode laser and concluded that both photosensitizers for 60 and 120 seconds created more effective antibacterial effect against the *E. faecalis* than 30 seconds. (12). Therefore, in the present study, ICG solution was irradiated with a diode laser for 60 seconds in order to more closely simulate an actual clinical application period.

Souza et al. (35) examined the antimicrobial effects of PDT on *E. faecalis* by using methylene blue and toluidine blue as photosensitizers combined with NaOCl or sodium chloride and a 660 nm wavelength diode laser at 40 mW power as the light source. Authors concluded that PDT application in addition to traditional chemomechanical preparation and irrigation reduced populations of *E. faecalis* in root canals although the decrease was not statistically significant (35). These results are in contrast to the results of the present study. The difference in results may be attributed to the performed disinfection procedures and the used photosensitizer.

Juric et al. (36) investigated the antimicrobial efficacy of PDT in addition to retreatment. Researchers completed the endodontic therapy in a single session after using phenothiazine chloride photosensitizer and a 660 nm wavelength diode laser at 100 mW power in addition to chemomechanic preparation. Authors reported that PDT's use in combination with traditional chemomechanical root canal preparation led to significantly greater reductions of CFUs to indicate a decrease of the remaining bacteria or, in some cases, completely eliminated bacteria (36).

PDT was applied in dental practice in different wavelengths (between 488-906 nm) (14,37). A new 940 nm wavelength diode laser and ICG were used in the present study. As can be understood from our literature review, the role of PDT in the endodontic disinfection process had been tested with the use of the different photosensitizers, light sources and different laser parameters resulting in promising data indicating a reduction of *E. faecalis* in root canals. The results of the present study are consistent with what the researchers mentioned above found. PDT led to significantly higher bacterial reduction when compared with the diode laser group used alone and increased efficiency of the diode laser. However there was not a statistically significant difference in bacterial elimination when PDT was compared with traditional irrigants (5% NaOCl and 2% CHX).

Bago et al. (38) examined the antimicrobial activities of PDT and the traditional root canal disinfection techniques in *E. faecalis* infected root canals. For PDT applications, toluidine blue was used as a photosensitizer with a 660 nm wavelength diode

laser with 100 mW power was used in one group. Phenothiazine chloride as a photosensitizer and a 660 nm wavelength diode laser with 100 mW power was used in the other group. According to the findings of the study, PDT had the capacity to eradicate *E. faecalis* and is superior to traditional disinfection of 2.5% NaOCl (38). Although Bago et al. (38) found outstanding antibacterial activity following use of PDT when compared with NaOCl, their results partially coincide with present study results. In the present study, PDT was found to have the capacity to eliminate *E. faecalis* in serious rate but that PDT showed equivalent antibacterial activity as in NaOCl and CHX. We conclude that the reasons of this difference are time of application, different laser parameters and type of photosensitizing agent.

Tooth staining and discoloration are the main concerns of PDT, prompting the evaluation of the effectiveness of chemical components in an attempt to overcome this disadvantage. Carvalho Edos et al. (39) found an effective methodology to prevent tooth discoloration caused by methylene blue during PDT by using endo-PTC cream (10% urea peroxide, 15% tween 80 and 75% carbowax). The results of the present study showed that ICG did not lead to any visible tooth discoloration during the current application.

The *in vitro* model and using planktonic bacteria are the major limitations of this study. Unlike as in our model system, endodontic infections are typically polymicrobial by nature and have interactions between various microorganisms. Because of this, an *in vitro* culture may not reflect the *in vivo* condition and biofilm formation may affect results.

Conclusion

NaOCl is an irrigant that most commonly used in endodontics, easily available and inexpensive. Besides, NaOCl cannot be tolerated by periapical tissues and cytotoxic properties of NaOCl is disadvantage. Promising results were obtained by using PDT with ICG. ICG increased bacterial effectiveness of diode laser. There is need for further *in vivo* studies to support the *in vitro* promising results.

Ethics

Ethics Committee Approval: The study was reviewed and approved by the Ethics Committee of Gaziosmanpaşa University Faculty of Medicine (project number: 14-KAEK-246).

Informed Consent: Written consent wasn't obtained because operation was made on extracted teeth.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Concept: İ.Ö., Material: İ.Ö., H.G., U.S.Ş.C., F.A., Design: İ.Ö., H.G., Data Collection or Processing: İ.Ö., H.G., U.S.Ş.C., F.A., Analysis or Interpretation: İ.Ö., H.G., U.S.Ş.C., F.A, Literature Search: İ.Ö., H.G., F.A., Writing: İ.Ö., H.G.

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Original Article / Özgün Araştırma

Color Stability of NeoMTA Plus and MTA Plus when Mixed with Anti-washout Gel or Distilled Water

Çözünmeye Dirençli Jel veya Distile Suyla Hazırlanan NeoMTA Plus ve MTA Plus Materyallerinin Renk Stabilitelerinin Değerlendirilmesi

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Abstract

Objective: This study aims to evaluate the effect of the special anti-washout gel mixing agent on the color stability of mineral trioxide aggregate (MTA) Plus and NeoMTA Plus in contact with distilled water, ethylenediaminetetraacetic acid (EDTA) and sodium hypochlorite (NaOCl).

Materials and Methods: One set of three specimens was mixed using the anti-washout gel and the other set of three specimens was mixed using distilled water. Then, one specimen from each group was immersed in distilled water, 5% NaOCl or EDTA solutions for 24 hours. The color change of each specimen was assessed using spectrophotometer. The data were evaluated statistically with two-way ANOVA and post-hoc Tukey tests.

Results: It was found that there was no statistically significant differences among the color changes of the MTA Plus and NeoMTA Plus mixed with either distilled water or gel when immersed in distilled water and EDTA ($p>0.05$). Immersion to NaOCl resulted in the highest discoloration in all materials compared to their immersion to distilled water and EDTA. MTA Plus mixed with distilled water showed significantly greatest discoloration after contact with NaOCl ($p<0.05$). MTA Plus mixed with gel showed the highest color stability regardless of the solution type.

Conclusion: Mixing with MTA Plus and NeoMTA Plus powder with the special anti-washout gel within their kits contributes to the color stability of the materials.

Keywords
Discoloration, MTA Plus, NeoMTA Plus, spectrophotometer

Öz

Amaç: Bu çalışma, özel çözünme direnci sağlayan jel karıştırma ajanlarının mineral trioksit agregat (MTA) Plus ve NeoMTA Plusın renk stabilitelerine etkisini materyallerin distile su, etilendiamintetraasetik asit (EDTA) ve sodium hipoklorit (NaOCl) temasları sonrası değerlendirmeyi amaçlamaktadır.

Gereç ve Yöntemler: Her iki materyalden üç numune içeren bir set çözünmeye dirençli jel kullanılarak, diğer set ise distile su kullanılarak hazırlandı. Daha sonra, her setten birer numune 24 saat boyunca distile su, %5'lik NaOCl veya EDTA solüsyonları içerisinde tutuldu. Her materyalde meydana gelen renk değişikliği

Anahtar Kelimeler
Renklenme, MTA Plus, NeoMTA Plus, spektrofotometre

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spektrofotometre kullanılarak değerlendirildi. Veriler iki-yönlü ANOVA ve post-hoc Tukey testleri kullanılarak istatistiksel olarak değerlendirildi.
Bulgular: MTA Plus ve NeoMTA Plus materyalleri jelle veya distile suyla karıştırılıp hazırlandıklarında distile su ve EDTA ile temasları sonucu meydana gelen renk değişiklikleri arasında istatistiksel olarak anlamlı fark olmadığı bulundu (p>0,05). NaOCl da bekletilen örneklerin tamamında distile su ve EDTA içinde bekletilen örneklere kıyasla yüksek renklenme görüldü. Distile su ile hazırlanan MTA Plus, NaOCl ile temas sonrası en yüksek renk değişimini gösterdi (p<0,05). Jel ile karıştırılan MTA Plus bekletildiği solüsyon tipinden bağımsız olarak en yüksek renk stabilitesini gösterdi.
Sonuç: MTA Plus ve NeoMTA Plus tozlarının ürün kitlerindeki özel çözünme engelleyici jelle karıştırılmaları materyallerin renk stabilitelerine katkı sağlamaktadır.

Introduction

Discoloration of dental structures following application of endodontic materials is an unpleasant matter that harms treatment quality and patient satisfaction. The exact mechanism behind the discoloration following endodontic procedures is not known, yet. Material penetrations into the dentinal tubules and reflection of discoloration through hard tissues have been indicated as the principal mechanisms of tooth discoloration over time (1,2). Therefore, material selection to be used in the aesthetic zone is crucial to prevent further discoloration.

Mineral trioxide aggregate (MTA) includes bismuth oxide and modified Portland cement (3). MTA has been utilized for many endodontic procedure including pulpotomy, pulp capping, perforation repair, apexification, (4) and revascularization procedures (5). Since previous case reports associated the use of grey MTA with coronal tooth discoloration (6,7), white MTA was developed (8). Nevertheless, discoloration within the mass of wProRoot MTA (9) and tooth discoloration has also been reported (10,11).

Bismuth oxide within the MTA and MTA-like materials' composition has been associated with tooth discoloration, which has led to the invention of different materials with similar compositions that will not induce discoloration. MTA Plus (Avalon Biomed Inc, Bradenton, FL, USA) is a cost-effective, tricalcium silicate-based MTA-like material developed for similar indications to MTA. MTA Plus has been reported to have smaller particle sizes when compared to MTA (12). The MTA Plus kit consists of MTA Plus powder and a gel, which provides washout resistance to the setting material. Recently, the manufacturer of MTA Plus marketed NeoMTA Plus for the aesthetically important areas by changing the bismuth oxide with tantalum oxide within its composition. NeoMTA is also mixed with MTA Plus gel within the kit.

Powders of MTA and MTA-like materials can also be mixed with distilled water and local anaesthetic agents due to the depletion of liquids/gels before powder. Manufacturer provides these materials with either water or gel for mixing (12). The aim of this study was to evaluate the effect of anti-washout gel on the color stability of MTA Plus and NeoMTA Plus in contact with different irrigation solutions.

Materials and Methods

Specimen Preparation

The study did not include any human or animal information, tissue or material. Thus, no ethical approval was required. Two groups of materials were used in this study as MTA Plus and NeoMTA Plus with each group containing 3 specimens. All materials were mixed homogeneously according to the manufacturers' instructions. One group was mixed using the anti-washout gel and the other group was mixed using distilled water. Cylindrical specimens, 5 mm in diameter and 2 mm high, were obtained by curing them in molds according to each material's setting time at 100% humidity and 37 °C. The specimens mixed with gel were incubated for 60 minutes, whereas specimens mixed with distilled water were incubated for 5 hours, as suggested by the manufacturer. Then, the setting of each specimen was controlled and the specimens were separated from the molds. One specimen from each group was immersed in the following three irrigation solutions for 24 hours: distilled water, 5% sodium hypochlorite (NaOCl) solution (Wizard, Ankara, Turkey) or 17%

ethylenediaminetetraacetic acid (EDTA) (Werax, Ankara, Turkey). After 24 hours of immersion, the specimens were dried and the colors of each were assessed before and after immersion. The study did not include any human. Thus, informed consent was not required.

Spectrophotometric Analysis

Spectrophotometer (VITA Easyshade® compact; VITA Zahnfabrik, Bad Sackingen, Germany) was utilized to calculate total color change under constant conditions (same laboratory light and the same operator). Prior to each measurement the device was calibrated. Each measurement was repeated three times and the mean value was calculated. The Commission Internationale de l'eclairage (CIE) system was utilized to calculate the differences in color.

The color differences of the samples were calculated by using the following formula:

$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]$ ½

ΔL represents the change in luminosity, Δa represents the change in red-green parameter and Δb represents the change in yellow-blue parameter. ΔE values, which were equal to or greater than 3.3 were accepted as clinically perceptible discoloration (13).

Statistical Analysis

The data were evaluated with two-way ANOVA by using SPSS software (PASW Statistics 20.0; SPSS Inc, Chicago, IL, USA). The significant effects and interactions were further investigated with post-hoc Tukey test. The level of statistical significance was set at $p<0.05$.

Results

The mean values for changes in color of materials are presented in Table 1. Distilled water and EDTA caused clinically perceptible discoloration of the immersed materials apart from the MTA Plus mixed with distilled water. No statistically significant difference was detected regarding the type of mixing agent in the specimens immersed in either distilled water or EDTA ($p>0.05$).

All of the tested materials showed clinically perceptible discoloration when they were immersed in NaOCl solution. MTA Plus mixed with distilled water showed significantly greatest discoloration after contact with NaOCl ($p<0.05$). Mixing with distilled water significantly increased the ΔE value of MTA Plus and NeoMTA Plus compared to mixing with gel ($p<0.05$). MTA Plus mixed with gel showed the highest color stability regardless of the solution type.

Discussion

The present study evaluated the effect of the mixing agents on the color stability of 2 novel calcium silicate-based materials, as MTA Plus and NeoMTA Plus in contact with different irrigation solutions. Irrigation is an indispensible phase of root canal disinfection and NaOCl has been a standard irrigant for disinfection procedures and cannot be eliminated from the

Table 1. Mean and standard deviation values of materials' color changes (ΔE) after immersion of different irrigation solutions

	Distilled water	EDTA (17%)	Sodium hypochlorite (5%)
MTA Plus + distilled water	4.02 ± 0.24^{Aa}	5.76 ± 0.69^{Aa}	35.64 ± 5.42^{Ba}
MTA Plus + gel	3.10 ± 1.84^{Aa}	5.07 ± 3.59^{Aa}	18.22 ± 0.35^{Bb}
NeoMTA Plus + distilled water	3.20 ± 0.05^{Aa}	4.92 ± 1.52^{Aa}	12.92 ± 2.85^{Bc}
NeoMTA Plus + gel	1.87 ± 0.69^{Aa}	1.93 ± 0.78^{Aa}	6.91 ± 2.89^{Bd}

Different superscript lower letters in the same column show statistically significant difference ($p<0.05$). Different superscript capital letters in the same line show statistically significant difference ($p<0.05$)

EDTA: Ethylenediaminetetraacetic acid, MTA: Mineral trioxide aggregate

treatment protocol. Avoidance of the contact of NaOCl and the applied material or the use of a material that will provide color stability in contact with NaOCl are precautions for possible discoloration. However, avoidance of contact with NaOCl does not seem realistic. In our study, set specimens were immersed in irrigation solutions for 24 hours. Since, NaOCl has been reported to crystallize and occlude dentinal tubules; it could not be completely eliminated from root canal system (14). Residual NaOCl within tubules might come into contact with chlorhexidine gluconate as well as with calcium silicate-based materials during regenerative endodontic applications or during the repair of resorption cavities and the management of perforations. Prolonged immersion into irrigation solutions are preferred to simulate this long contact duration of the solution and calcium silicate-based materials in the root canal system.

Visual inspection and the use of specific devices have been utilized for the measurement of color changes of dental materials. CIE system is an ISO acknowledged system for international standardization of color change measurement (15). Instrumental measurements of color change using the CIE system include the use of colorimeters and spectrophotometers. Visual spectrophotometry has been regarded as a gold standard technique (16). In the present study, spectrophotometric analysis was preferred due to the technique's sensitivity, instrument's objectivity and repeatability (13).

MTA Plus consists of bismuth oxide as radiopacifier, whereas NeoMTA Plus consists tantalum oxide. Contact of bismuth oxide with NaOCl has been reported to result in discoloration of the material (17,18). Bismuth oxide discolors via two mechanisms depending on the environmental conditions. In the absence of oxygen, bismuth oxide has been reported to dissociate into metallic bismuth and oxygen when exposed to light (19,20). However, previous studies have reported that bismuth oxide also discolors due to overoxidation as a result of their contact with NaOCl (16). Due to the oxidation process, the oxygen from bismuth oxide becomes unstable and reacts with the carbon dioxide in the air. This reaction produces bismuth carbonate, which is sensitive to light and lead discoloration (21). A previous study evaluated the color stability of MTA Plus and NeoMTA Plus in contact with NaOCl and distilled water. Authors reported that MTA Plus discolored following contact with NaOCl and NeoMTA Plus exhibited color stability (22). In the present study contact of NaOCl and MTA Plus mixed either with distilled water and gel resulted in significantly greater discoloration than the contact of NaOCl and NeoMTA Plus. The findings of the present study were in accordance with the results of that study, since MTA Plus mixed with gel induced significant discoloration compared to NeoMTA Plus mixed with the same agent (17). The presence of bismuth oxide within MTA Plus powder could contribute to the higher ΔE values of the material compared to NeoMTA Plus, which replaced bismuth oxide with zirconium oxide.

In the present study, contact with distilled water or EDTA did not significantly increase ΔE values of the materials irrespective of the mixing agents. Only the contact of EDTA with NeoMTA Plus mixed with gel did not resulted in clinically perceptible discoloration of the material, whereas contact of EDTA with the other materials exceeded 3.3 ΔE threshold values. However, the differences were not statistically significant.

In the present study, contact with NaOCl and MTA Plus mixed with distilled water led to significantly greater discoloration than MTA Plus mixed with the gel. MTA Plus has been reported to have a finer particle size than the ProRoot MTA, which is further associated with higher ion release, porosity, solubility, and water sorption when compared to the ProRoot MTA (12,23). These properties enhance the ability of the MTA Plus to release calcium and prolong the bioactivity of the material (23). However, certain physical properties, such as the increased porosity, fluid uptake, and sorption values, were also correlated with blood-induced discoloration in a recent study (24). In this study, the staining agents, such as blood, which could be absorbed by the material's surface, were not used. Direct comparison could not be made because no other study has evaluated the effect of mixing agent on the color stability of MTA Plus or NeoMTA Plus. Distilled water, which was used as a mixing agent for materials might contribute increased discoloration via structural surface changes. The fluid uptake ability

and surface porosity of the MTA Plus and NeoMTA Plus might increase when mixed with distilled water instead of the gel and lead to discoloration after contact with NaOCl.

Previous literature suggests the application of a double layer dentin bonding material on the access cavity or treatment with internal bleaching to prevent discoloration caused by calcium silicate-based materials (25,26).

Conclusions

Within the limitations of this study, mixing with distilled water negatively affects the color stability of MTA Plus and NeoMTA Plus materials in contact with NaOCl. Anti-washout gel also contributed to the color stability of the materials.

Ethics

Ethical Committee Approval: The study did not include any human or animal information, tissue or material. Thus, no ethical approval was required.

Informed Consent: The study did not include any human. Thus, informed consent was not required.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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Original Article / Özgün Araştırma

Oxidative Alteration in Gingival Fibroblast Cells Induced By Bulk-Fill and Conventional Flowable Composites

Bulk-Fill ve Geleneksel Akıcı Kompozitlerle Uyarılan Dişeti Fibroblast Hücrelerindeki Oksidatif Değişim

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Abstract

Objective: The release of components from dental materials may cause oxidative stress which is crucial factor for tissue damage and cell apoptosis or death. The aim of this study was to evaluate the cytotoxicity of different flowable composites and this materials effect on total antioxidant capacity (TAC) and total oxidant status (TOS) level in human gingival fibroblast cell culture.

Materials and Methods: Gingival fibroblast cells obtained from healthy persons were used for evaluation the cytotoxicity and oxidant status. Six flowable composites used were: two bulk-fill flowable composites (SureFil SDR, X-tra base), a self-adhering flowable composite (Vertise Flow), a highly filled flowable composite (GrandioSO Flow), two conventional flowable composites (Filtek Ultimate, Clearfil Majesty). Specimens in 3 mm diameter, 2 mm height were prepared from each composite (n=6) and were transferred to 24 well plates. Wells without composite material were used as the control group. After 24 h incubation period, cytotoxicity was determined by using the 3-(4,5 dimetylthiazol-2-yl)-2,5 diphenlytetrazolium bromide (MTT) assay. Oxidative alterations were assessed using TAC and TOS assay kits. Data were analyzed using the ANOVA and least significant differences post-hoc test.

Results: Cytotoxicity of six materials was significantly different from the control group ($p<0.05$). Vertise flow was the most cytotoxic material. TAC levels of Vertise flow were significantly different from X-tra base and GrandioSO. TOS levels increased in SureFil SDR and Vertise flow groups but it was not statistically significant difference.

Conclusion: All of the materials used in this study showed cytotoxic effect in human gingival fibroblast cell culture. These materials did not have a significant effect on TOS level. However, TAC level could not prevent the rise of TOS level in Vertise and sureFil SDR.

Öz

Amaç: Dental materyallerden salınan komponentler doku hasarı ve hücre apoptosisi veya ölümünde önemli bir faktör olan oksidatif stres oluşumuna sebep olabilir. Bu çalışmanın amacı; farklı akıcı kompozit rezinlerin sitotoksisitesini ve bu materyallerin insan dişeti fibroblast hücre kültüründe total antioksidan kapasite (TAK) ve total oksidan durum (TOD) seviyesine etkisini değerlendirmektir.

Gereç ve Yöntemler: Sağlıklı bireylerden elde edilen diş eti fibroblast hücreleri sitotoksisite ve oksidan durum değerlendirmesi için kullanıldı. Bulk-fill akıcı

kompozit (SureFil SDR, X-tra base), kendiliğinden bağlanan akıcı kompozit (Vertise Flow), yüksek dolduruculu akıcı kompozit (GrandioSO Flow), geleneksel akıcı kompozit (Filtek Ultimate, Clearfil Majesty) olmak üzere altı farklı materyal kullanılarak 3 mm çapında 2 mm yüksekliğinde örnekler hazırlandı (n=6) ve 24'lük doku kültür kabına yerleştirildi. Örnek yerleştirilmeyen kuyucuklar kontrol grubu olarak belirlendi. Yirmi dört saat boyunca inkübe edildikten sonra 3-(4,5 dimetylthiazol-2-yl)-2,5 diphenlytetrazolium bromide (MTT) analizi ile materyallerin sitotoksisitesi değerlendirildi. Oksidatif değişim TAK ve TOD analiz kitleri ile değerlendirildi. Elde edilen verilerin analizinde ANOVA ve en önemsiz farkla post-hoc testi kullanıldı.

Bulgular: Uygulanan altı materyalin sitotoksisitesi kontrol grubu ile anlamlı farklılık gösterdi ($p<0,05$). Vertise flow en sitotoksik materyal olarak tespit edildi. Vertise flow uygulanan grupta TAK seviyesi X-tra base ve GrandioSO'ya göre anlamlı farklılık gösterdi. SureFil SDR ve Vertise flow grubunda TOD seviyesi artış gösterdi fakat bu artış anlamlı değildi.

Sonuç: Çalışmada kullanılan tüm materyaller sitotoksik etki göstermiştir. Bu materyaller TOD seviyesi üzerinde önemli etki oluşturmamıştır. Ancak TAK seviyesi, Vertise ve SureFill SDR grubunda TOD seviyesindeki yükselmeyi engelleyememiştir.

Introduction

Flowable composites were introduced in 1990s and they have been designed to provide improved adaptation and polymerization stress relief. The low viscosity of these materials allows them to shape itself to fit in the difficult access cavity areas (1,2). Flowable composites are suitable for small class III or class V restorations, enamel defects, margin repairs or as cavity liners (3,4). However, there are some problems associated with their poor mechanical properties, lower filler content, polymerization shrinkage and weak adhesion (1,3). Manufacturers have recently introduced new generation flowable composites, so called higher filler loading flowable resin composites, bulk-fill flowable composites and self-adhering flowable composites for elimination of negative effects and improving clinical requirements (3,5). Higher filler loading flowable resin composites for posterior restorations have better wear resistance compared with some resin composites (6). Bulk-fill composite resins specifically designed for placement in single layers of 4 to 5 mm and have lower polymerization shrinkage and stress values when compared with conventional flowable composite resins (7,8). Self-adhesive flowable composites includes acidic monomer like glycerol phosphate dimethacrylate (GPDM) and can be bonded to tooth structures without using adhesive systems (9).

The biocompatibility has gained considerable interest during recent decades and is the important factor for evaluation of materials clinical success, as well as the physical properties (10). Chemical composition of composite material, degradation of material, degree of monomer conversion, surface treatment and conditions within the oral cavity may cause to release substances into the oral environment (11,12). Inadequate polymerization can cause to release methyl methacrylate (MMA), hydroxyethyl methacrylate (HEMA), bisphenol A diglycidyl dimethacrylate (Bis-GMA), triethyleneglycol dimethacrylate (TEGDMA), and urethane dimethacrylate (UDMA) from the resin matrix (13,14). These monomers are associated with cytotoxicity and oxidative stress in tissue or cell and influence the signal transduction pathways and complex regulatory cellular networks. Moreover, this monomer deplete the amount of glutathione and cause to increase the formation of reactive oxygen species (ROS) (14,15). As a result of aerobic metabolism, ROS are occurred in cells. Low concentrations of ROS are compatible with normal physiological functions, whereas high concentrations of ROS are considered to be harmful to cells, leading to oxidative stress (16). Antioxidant systems neutralize these reactive molecules and protect cells from potential cytotoxic effects. But when the balance between oxidants and antioxidants is disrupted, oxidative stress occurs (15,17).

The objective of this study was to evaluate *in vitro* cytotoxicity of different flowable composites materials and their effects on total antioxidant capacity (TAC) and total oxidant status (TOS) levels in human gingival fibroblast (HGF) cell culture.

Materials and Methods

This study was granted ethical approval by the Ethical Committee of the Atatürk University Faculty of Dentistry (22.04.2016/23) and conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all patients. The cytotoxic effects of flowable composites on HGF cell culture, were evaluated using the direct contact test method and 3-(4,5-dimethylthiazol-2-yl)-2,5-

diphenyltetrazolium bromide (MTT) assay. Oxidative alterations were determined by measuring of TAC and TOS level.

Preparation of Samples

Six different flowable composite materials were used in the study. The materials used and their contents are demonstrated in Table 1. Samples in a disc shape, 3 mm diameter and 2 mm height, were prepared from each restorative material. Six samples in disc shape using a teflon mold were prepared from each restorative material. The materials placed in the mold were covered with transparent tape (mylar strip) and left to harden between two glasses. Resin containing materials was polymerized using a visible blue LED light device (Elipar Freelight II, 3M-ESPE, St. Paul, MN, USA) at a wavelength of 450 nm according to manufacturer instructions. The edges and surfaces of samples were straightened using polishing discs (Sof-Lex; 3M ESPE, St. Paul, MN, USA). Samples were transferred to well plates, Wells without flowable composite material were determined as the control group.

Preparation of the Cell Culture

Cultured HGF cells were used in this study. Gingival tissue samples were obtained from young healthy donors tissue overlying impacted third molars by the informed written consent of the patients (aged 18-25). The gingival tissue pieces immediately were placed in 2 cc Dulbeccos Modified Eagles Medium/F12 (DMEM: Gibco BRL, New York, USA). The samples were washed by normal saline and then by DMEM medium for 2-3 min. Gingival tissue was cut into approximately 1x1 mm pieces in size and placed in 50 cc falkon for washing small parts and blood cells residues.

The samples were seeded in the well plate (Corning, New York, USA) then 2 cc DMEM/F12 medium with 10% heat-inactivated fetal calf serum (FCS), 100 U/mL penicillin, 100 µg/mL streptomycin and 1% amphotericin B (Gibco BRL, New York, USA) added and incubated at 37 °C in a humidified atmosphere of 95% air and 5% CO_2 (Incubator ESCO, SINGAPORE) and the old medium was replaced with fresh medium twice a week. The morphology of primary HGF resulted in spindle shaped cells (Figure 1). When the plate obtain 70-80% confluency (the cell number was $1x10^6$ cells/

Table 1. Flowable composites used in this study

Material name	Material type	Content	Manufacturer/lot no
SureFill SDR Flow	Bulk- fill flowable composites	UDMA with hybrid glass filler. Barium and strontium, alumino-fluoro-silicate glasses	DENTSPLY, milford, USA 1312000155
X-tra base	Bulk-fill flowable composites	Bis-EMA, Aliphatic dimethacrylate	VOCO, GmbH, Cuxhaven, GERMANY 1422161
Vertise™ flow	Self-adhering flowable composites	GPDM, HEMA, methacrylate co-monomers	KERR,USA 2894473
Clearfil majesty flow	Highly filled, microhybrid flowable composites	TEGDMA, hydrophobic aromatic dimethacrylate, Silanated barium glass filler Silanated colloidal silica dl-Camphorquinone Accelerators Pigments	KURARAY, Medical Inc, Okayama, JAPAN BM0004
Grandio SO flow	Highly filled flowable composites	HEDMA, Bis-GMA, TEGDMA, Bis-EMA	VOCO, GmbH, Cuxhaven, GERMANY 1331164
Filtek™ Ultimate	Nanohybrid flowable composites	Bis-GMA, TEGDMA, Silane treated ceramic, Ytterbium fluoride	3M ESPE, St. Paul, MN, USA N629174

UDMA: Urethane dimethacrylate, Bis-EMA: Ethoxylated Bisphenol A dimetacrylate, Bis-GMA: Bisphenol A diglycidyl dimethacrylate, GPDM: Glycerol phosphate dimethacrylate, HEMA: Hydroxyethyl methacrylate, TEGDMA: Triethyleneglycol dimethacrylate

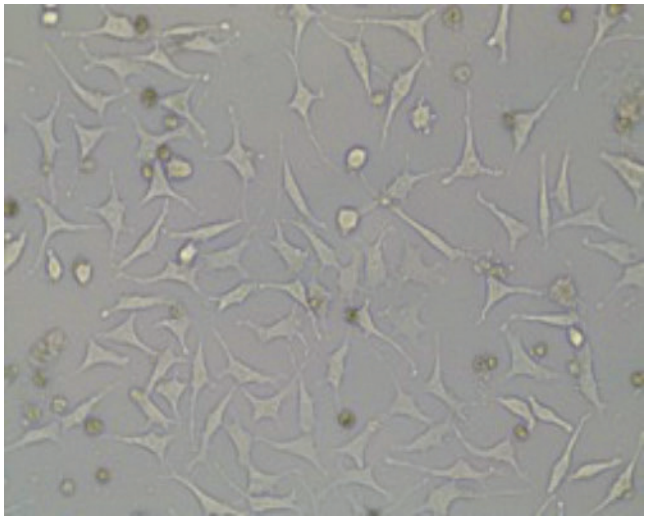

Figure 1. The morphology of cultured cells, growing from the tissue origin (100 µ diameter).

mL), the primary HGFs were trypsinate. HGFs were used in the third passages.

MTT Assay

After 24 h material-cells interaction periods, MTT assay was carried out with a commercially available kit (Sigma, USA). MTT reagent (10 µL) was added into the cell culture. The plate was incubated in CO_2 incubator at 37 °C for 4 h, in this periods NAD(P)H oxidoreductases reduced a purple formazan intracellular and then 100 µl of crystalline solvent solution was added to each well. The intensity of the formazan was measured at 570 nm with a biotek Spectrophotometer (µ Quant, Biotek, Winooski, USA).

Total Antioxidant Capacity and Total Oxidant Status Assay

TAC assay kit was used to determine antioxidant levels of samples by inhibiting formation of a free radical, 2,20-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) compound (Rel Assay Diagnostics, Gaziantep, Turkey) on HGF cell cultures for 24 h. The assay calibrated with Trolox equivalent as stable antioxidant. After cells incubated for 24 h, the medium was removed from the well plate and new medium placed. Standard solutions were added to wells, according to kit protocol, reagent 1 solution was added to each well and first spectrophotometric reading was carried out at 660 nm. Reagent 2 solution was added to each well and the well plate was incubated at room temperature for 10 min. Second spectrophotometric reading was obtained at 660 nm. In TAC assays kits, ascorbic acid used as organic antioxidant compounds for determined positive control.

The TOS assay was carried out with a commercially available kit (Rel Assay Diagnostics, Gaziantep, Turkey) on HGF cell cultures for 24 h. According to assay protocol, complexes formed with the ferric ion are oxidized to ferrous ion by oxidants presented in the sample. In the acidic environment ferrous ions form chromogen colored. The pigment density measured spectrophotometrically is related to the total amount of oxidant molecules in the sample. Hydrogen peroxide (H_2O_2) was used for assay calibration. After 24 h cells incubation periods, medium removed from the plate and new medium placed. Standard solutions in kit were added to each well. According to assay protocol reagent 1 solution was added to each well. First spectrophotometric reading was carried out at 530 nm. After the first reading, reagent 2 solution was added to each well and then incubated for 10 min at room temperature. Second spectrophotometric reading was done at 530 nm. In TOS assays, H_2O_2, ROS was used as a positive control.

Statistical Analysis

Analysis of variance (ANOVA) was used to analyse the effects of the six flowable composite resins to MTT, TAC and TOS level. Significant main effects were analysed post-hoc using LSD multi comparison test. All statistical analyses were performed via SPSS 20 (SPSS Inc., Chicago, IL, USA) using a confidence interval of 95%.

Results

The cytotoxicity of flowable composites was measured by using MTT assay and expressed as a percentage of the control groups (Figure 2). Cell viability in all experimental groups was significantly decreased compared with the control group ($p<0.05$). Vertise flow exhibited significant cytotoxicity on cultured HGF and caused cytotoxicity at levels of 41%. Filtek ultimate and X-tra base showed lower cytotoxicity at levels of 18.5 and 21.7%. Vertise flow had statistically significant difference compared with X-tra base and Filtek ultimate ($p=0.034$, $p=0.015$).

Table 2 presents the level of TAC and TOS in HGF cell culture after application of dental flowable composites. TAC level of Vertise was significantly different from X-tra base and GrandioSO ($p=0.034$,

p=0.049). TAC level of Filtek Ultimate was significantly different from X-tra base and GrandioSO (p=0.025, p=0.036). TOS level increased in SureFil SDR and Vertise groups but it was not statistically significant difference. The lowest TOS level was seen in Filtek Ultimate. Table 3 demonstrates the differences (p value) between groups in terms of MTT, TAC and TOS.

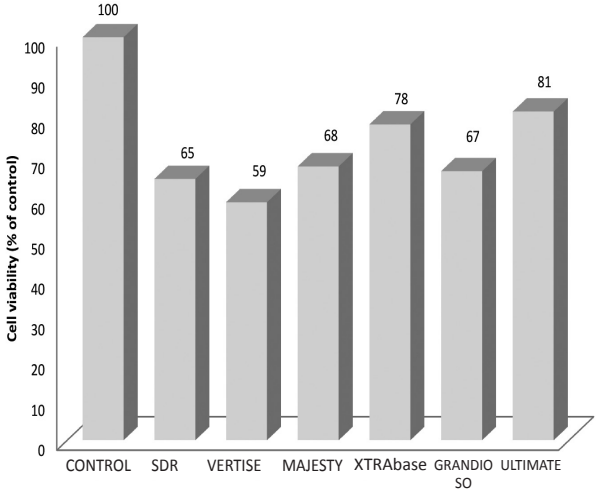

Figure 2. Cytotoxicity of flowable composites were measured by using MTT assay and expressed as a percentage of the control groups. Low cell viability means high cytotoxicity
MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

Discussion

Cell culture assay has gained interest in recent years and are assumed to be appropriate methods to evaluate the biocompatibility of the restorative dental materials, since they are standardized, easy to apply, repeatable, cost-effective and take less time (18,19). In the present study biocompatibility of six different flowable composites on HGF were investigated with the MTT assay and TAC and TOS biological parameters assay in cell culture.

Pulp and gingival fibroblasts are highly exposed to resin monomers after releasing from composite fillings to the oral cavity and this materials cause morphological changes and inflammatory reaction in cell (11). Therefore, primary cultures of human pulp and gingival fibroblasts were selected as optimal for biocompatibility testing of dental materials. Several approaches are possible to determine the cytotoxicity of material like LDH release, MTT formation, XTT formation, neutral red uptake, kenacid blue binding, acid phosphatase activity, sulforhodamine B binding and resazurin binding (20). But, MTT and NR tests are more sensitive and more reliable in the evaluation of a material's toxic properties (21,22). According to the ISO 10993-5 specification, direct tests, indirect tests

Table 2. Mean and standart deviation of MTT, total antioxidant capacity and total oxidant status in human gingival fibroblast cell culture after 24 h application of dental flowable composites

Groups	Sample size (n)	MTT Mean ± SD (count of cell/μm^2)	TAC Mean ± SD (mmol trolox Equiv./L)	TOS Mean ± SD (mmol H_2O_2 Equiv./L)
Control	6	0.251±0.03^{a}	0.89±0.01^{ab}	3.64±0.96^{a}
Surefil SDR flow	6	0.162±0.04b^{c}	0.89±0.01^{ab}	4.03±0.64^{a}
Vertise flow	6	0.148±0.04^{c}	0.90±0.01^{a}	3.91±1.28^{a}
Clearfil majesty flow	6	0.170±0.03b^{c}	0.89±0.01^{ab}	3.37±1.06^{a}
X-tra base	6	0.196±0.04^{b}	0.88±0.01^{b}	3.60±1.44^{a}
Grandio SO flow	6	0.167±0.01b^{c}	0.89±0.01^{b}	3.64±0.67^{a}
Filtek ultimate	6	0.204±0.03^{b}	0.90±0.00^{a}	2.90±0.82^{a}

a,b,c: In the same column, the groups identified by different superscript lowercase are statistically different (p<0.05)
MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, TAC: Total antioxidant capacity, TOS: Total oxidant status, SD: Standard deviation

Table 3. P-value between groups in terms of MTT, total antioxidant capacity and total oxidant status

	Control			Surefil SDR flow			Vertise flow			Clearfil majesty flow			X-tra base			Grandio SO flow			Filtek ultimate		
	MTT	TAC	TOS	MTT	TAC	TOS	MTT	TAC	TOS	MTT	TAC	TOS	MTT	TAC	TOS	MTT	TAC	TOS	MTT	TAC	TOS
Control	x	x	x																		
Surefil SDR flow	0.000	0.736	0.519	x	x	x															
Vertise flow	0.000	0.300	0.659	0.514	0.481	0.838	x	x	x												
Clearfil majesty flow	0.001	0.680	0.644	0.724	0.454	0.271	0.317	0.151	0.368	x	x	x									
X-tra base	0.018	0.257	0.941	0.131	0.145	0.472	0.034	0.034	0.606	0.242	0.468	0.698	x	x	x						
Grandio SO flow	0.01	0.331	0.989	0.827	0.193	0.510	0.385	0.049	0.649	0.892	0.572	0.654	0.193	0.870	0.952	x	x	x			
Filtek ultimate	0.041	0.240	0.218	0.064	0.398	0.65	0.015	0.887	0.098	0.129	0.116	0.437	0.718	0.025	0.246	0.100	0.036	0.223	x	x	x

MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, TAC: Total antioxidant capacity, TOS: Total oxidant status

and extract tests are used for material and cellular contact methods in tests for *in vitro* cytotoxicity. In previous studies demonstrated a good association between direct and indirect contact tests, while the least sensitivity was seen in the extract tests (23,24). Based on previously performed studies direct material cellular contact method and MTT assay were used in this present study. The *in vitro* antioxidant/oxidant capacity of materials was determined by measuring TAC and TOS levels with commercially available kits. The major advantage of this test is to measure the antioxidant capacity of all antioxidants and all oxidant in a biological sample.

The main finding obtained from this study is that the restorative materials showed different degrees of cytotoxicity. The rank order of cytotoxicity for materials; Filtek Ultimate<X-tra base<Clearfil Majesty Flow<Grandio SO flow<SureFill SDR Flow<Vertise. This is not surprising; substances released from dental materials cause toxicity in cell culture (13). Self-adhering flowable composite material Vertise flow showed higher cytotoxicity. This might be due to the presence of an acidic monomer in compound. Two main components of this material are an acidic functional monomer (GPDM) and a functional monomer (HEMA). GPDM etches dentine and enamel, and improves wettability (25). Tadin et al. (11) reported serious toxic effects of Vertise flowable composites on pulpal fibroblast and to cause increasing apopthotic cells. X-tra base resin formulated with ethoxylated bisphenol A dimetacrylate (Bis-EMA) showed cell viability at level 78%. In a toxicity study by Barbosa et al., (26) UDMA and Bis-EMA showed lesser cell death than HEMA. Bis-GMA and TEGDMA are proven cytotoxic agent. However cytotoxicity caused by Bis-GMA is higher than that caused by

the other monomers. Lower concentrations of Bis-GMA cause necrosis of HGF while higher ones cause cell apoptosis (27,28).

Imbalance between antioxidant defence system and free radicals cause oxidative stress. In the current study, alterations in the oxidant and anti-oxidant level of HGF cells were determined after exposure to flowable composite materials. Experimental studies support that, resin monomer effect redox balance and enhance ROS production and there are evidences about materials toxicity related with ROS production (16,29). Vertise and Filtek Ultimate had significant effect on TAC. SDR was slightly but not significantly induced level of TAC. Although increased TAC level, TOS level increasing was not prevented in Vertise and SDR. The decrease in cell survival was also observed by application of these two flowable materials. Increased TOS level and cytotoxicity were closely associated for SDR and Vertise flowable composites. Because oxidative stress is a common final mechanism of cell death (30). Schweikl et al. (31) reported dental monomers exposed cells indicated oxidative stress as a result of increased ROS and this oxidative stress acts as a signal for pathways activation which control cell death and viability through the redox sensitive activation of antioxidant proteins. Gallorini et al. (32) observed in their study that the resin monomer HEMA differentially caused oxidative stress in RAW264.7 mouse macrophages. In a study performed by Chang et al. (33) showed that Bis-GMA induced the expression of hemeoxygenase-1 which is an oxidative responsive gene and stimulate ROS production, apoptosis and cell death in pulp cell culture.

Conclusion

The result of this study provides a better understanding toward the protective effect of antioxidant capacity against formation of oxidative stress induced by flowable resin composite. The limitation of this study included testing only total levels of antioxidant and oxidant status. Evaluation of the different antioxidant enzyme such as superoxide dismutase, catalase, glutathione is recommended for further studies.

Ethics

Ethics Committee Approval: This study was granted ethical approval by the the Ethical Committee of the Atatürk University Faculty of Dentistry Atatürk (date/approval number: 22.04.2016/23).

Informed Consent: Informed consent was obtained from all patients.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Concept: N.Ç., M.İ.Y., Design: A.T., M.İ.Y., Data Collection or Processing: A.T., Analysis or Interpretation: A.T., Literature Search: M.İ.Y., N.Ç., Writing: N.Ç., M.İ.Y.

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

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Original Article / Özgün Araştırma

Cone Beam Computed Tomographic Analysis of Paranasal Variations, Osteomeatal Complex Disease, Odontogenic Lesion and Their Effect on Maxillary Sinus

Paranazal Varyasyonların, Osteomeatal Kompleks Hastalığının ve Odontojenik Lezyonların Maksiller Sinüse Olan Etkisinin Konik Işınlı Bilgisayarlı Tomografi ile İncelemesi

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Keywords
Concha bullosa, nasal septal deviation, maxillary sinus mucosal thickening, odontogenic lesions, cone beam computed tomography

Anahtar Kelimeler
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Abstract

Objective: The objective of this study was to establish the prevalence of nasal septal deviation, concha bullosa, and osteomeatal complex disease (obstruction), and odontogenic lesions, along with their potential relationships with maxillary sinus mucosal thickening.

Materials and Methods: (CBCT) images of 200 patients (101 males, 99 females) with 396 exposed maxillary sinuses, were inspected for presence of nasal septal deviation, concha bullosa, osteomeatal complex disease (obstruction), and odontogenic lesions related to maxillary sinus and maxillary sinus mucosal thickening.

Result: One hundred nineteen patients (59%) had nasal septal deviation, 100 (50%) had at least one concha bullosa, 26 (13%) had osteomeatal complex disease on at least one side, 39 (19.5%) had odontogenic lesions related to maxillary sinuses, and 112 (56%) had evidence of mucosal thickening. There was a statistically significant relationship among osteomeatal complex disease, odontogenic lesion and maxillary sinus mucosal thickening (p=0.00). No statistically significant relationship could be established among the presence of concha bullosa, nasal septal deviation and maxillary sinus mucosal thickening (p>0.05).

Conclusion: Odontogenic lesions and osteomeatal complex diseases have association with maxillary sinus mucosal thickening. CBCT imaging could be a diagnostic tool for assessment potential reasons of maxillary sinus mucosal thickening.

Öz

Amaç: Bu çalışmanın amacı nazal septum deviasyonu, konka bulloza, osteomeatal kompleks tıkanıklığı ve odontojenik lezyon varlığının, maksiller sinüs mukozal kalınlaşmasıyla olan potansiyel ilişkisinin ortaya konmasıdır.

Gereç ve Yöntemler: İki yüz hastaya (101 erkek, 99 kadın) ait 396 maksiller sinüsün konik ışınlı bilgisayarlı tomografi görüntülerinde; maksiller sinüs mukozal kalınlaşmasıyla ilişkili nazal septum deviasyonu, konka bulloza, osteomeatal kompleks tıkanıklığı ve odontojenik lezyon varlığına bakılmıştır.

Bulgular: Yüz on dokuz hastada (%59) nazal septal deviasyon, 100 hastada (%50) en az bir konka bulloza, 26 hastada (%13) en az bir tarafta osteomeatal kompleks

hastalığı, 39 hastada (%19,5) maksiller sinüsle ilişkili odontojenik lezyon ve 112 hastada (%56) mukozal kalınlaşma tespit edilmiştir. Osteomeatal kompleks hastalığı ve odontojenik lezyon ile maksiller sinüs mukozal kalınlaşması arasında istatistiksel olarak anlamlı ilişki bulunmuştur (p=0,00). Konka bulloza ve nazal septum deviasyonu ile maksiller sinüs mukozal kalınlaşması arasında istatistiksel olarak anlamlı ilişki bulunmamıştır (p>0,05).

Sonuç: Odontojenik lezyon ve osteomeatal kompleks hastalığı ile maksiller sinüs mukozal kalınlaşması arasında ilişki bulunmuştur. Konik ışınlı bilgisayarlı tomografi görüntüleme maksiller sinüste mukozal kalınlaşmaya neden olan potansiyel etmenleri belirlemede tanı aracı olabilir.

Introduction

Maxillary sinuses are air-filled spaces located at the lateral of the nasal cavity and are connected with it through an ostium. They are extending to the apexes of posterior teeth inferiorly. Odontogenic lesions (OL) with close proximity to sinuses such as granulomas, cysts and periodontitis can cause odontogenic maxillary sinusitis (OMS). MS can occur when mucosal membrane of sinuses is irritated by extention of pulp infecton or chronic infection and destruction of tooth socket (1). Maxilary sinusitis is caused by temporary and/or reversible mucociliary dyskinesia (2), which could be caused by some osteomeatal obstructions, allergy, and among other conditions, as well as abnormal growths, such as nasal septal deviations (NSD), concha bullosa (CB) (3-7). Maxillary sinus mucosal thickening (MT) is the radiographical appearance of MS, defined as inflammation of maxillary sinuses (2).

Osteomeatal complex is a term referring to the maxillary sinus ostium, ethmoidal infundibulum, hiatus semilunaris and frontal recess. It is a common channel that links the frontal sinus, anterior and middle ethmoid sinuses and the maxillary sinus to the middle meatus that allows air flow and mucociliary drainage (8). Osteomeatal complex disease (OMD) is an inflammatory mucosal disease which affects the anterior ethmoid cells, the ethmoid infundibulum, and the drainage pathways leading to the middle meatus. Anatomic variations can narrow or block pathways which resulting OMD. Opening the obstruction could result in an improvement of the MS. It is generally believed that OMD, which have strong relationship with septal deviation, may impede ventilation and mucociliary clearance from the sinuses, thus predisposing the affected individual to sinusitis (9,10).

CB is a pneumatized cavity of the concha, which is one of the most frequent variations of nasal cavity also known as a middle turbinate pneumatization. Bolger et al. (11) have classified pneumatization of the concha according to the site as bulbous CB (pneumatization of the bulbous part), lamellar CB (pneumatization of the vertical part) and extensive CB (pneumatization of both lamellar and bulbous parts) (12). It may leads to MS by the obstruction of the middle meatus (13-15).

Computed tomography (CT) is a standard radiographic modality for accurately assessment mucosal changes and variations of nasal cavity and paranasal sinuses. With the growing use of cone beam CT (CBCT) which has lower radiation dose compared with CT, dentists/maxillofacial radiologists and otolaryngologists are more able to define pathological conditions and anatomical aberrancies within the tissues of the sinonasal structures (11,16).

The aim of this study is to determine the prevalence of CB, NSD, OMD, and OL and examine their potential relationship with MT.

Materials and Methods

The Ethical Committee of the University of Erciyes approved the study protocol (approval no: 2017/440, date: 15.09.2017) that has, therefore, performed in accordance with ethical standards laid down in the Declaration of Helsinki in 1964.

This retrospective study utilized the CBCT (Newtom 5G, QR, Verona, Italy, 110 kVp and 0.57 mA 12-18s, 0.25 mm isotropic voxel size) images of subjects who were referred to our clinic, because of some dental problems (implant planning, cysts, impacted teeth). All CBCT images were evaluated by the same radiologist who has experience in assessing CBCT volumetric data.

The inclusion criteria:

1. Good quality of the CBCT images,

2. Good visibility of both maxillary sinuses with selected field of view (fov),

3. Bilateral existence of at least one of maxillary posterior tooth.

The exclusion criteria:

1. Evidence of trauma or developmental anomalies on CBCT images,

2. Subjects with mucosa thickening of all sinus walls,

3. Subjects with a history of prior nasal or sinus surgery.

CBCT images of 200 subjects (99 females and 101 males) were utilized. The images were examined in a darkroom, using the inbuilt software (NNT) in a Dell Precision T5400 workstation (Dell, Round Rock, TX, USA), with a 32-inch Dell LCD screen of 1.280 × 1.024 pixels resolution. The contrast and brightness of the images were adjusted using the image processing tool provided as a part of the software, to ensure optimal visualization.

Scans were reviewed for sinonasal region abnormalities and pathologies, with specific evaluation on the presence of CB and its types, NSD, OL, OMD and MT. CB was defined and classified as the presence of pneumatization of the superior, middle or inferior turbinates (13,16). Deviation of the nasal septum was defined as a deviation of more than 4 mm from the midline (16). Presence of pneumatization of the nasal septum was also investigated. Each of the maxillary sinuses and osteomeatal obstructions were assessed separately. Presence of MTs exceeding 2 mm was defined as MT (16,17). Teeth with apical lesions (granulomas, cysts), which were in contact with or exposed the sinus floor, were identified and their respective sides recorded (Figure 1a-f).

Statistical Analysis

Statistical analysis was performed by SPSS 16.0 for Windows (SPSS, Chicago, IL) to determine the prevalence of NSD, type of CB, MS, OMD, and OL. Categorical variables were shown, along with their respective percentage values, and were compared using the χ^2 tests. For this purpose, $p<0.05$ was considered as statistically significant.

Results

CBCT images of 396 sides of 200 patients were examined. Ninety-nine (49.5%) of them were female and 101 (50.5%) were male.

Osteomeatal complex disease: OMD was present in 26 subjects. Seven were on the left side, 9 on the right side, and 10 were bilaterally. While 22 were male, the number of female was 4. There was statistically significant difference in accordance to gender (p=0.00) (Tables 1, 2)

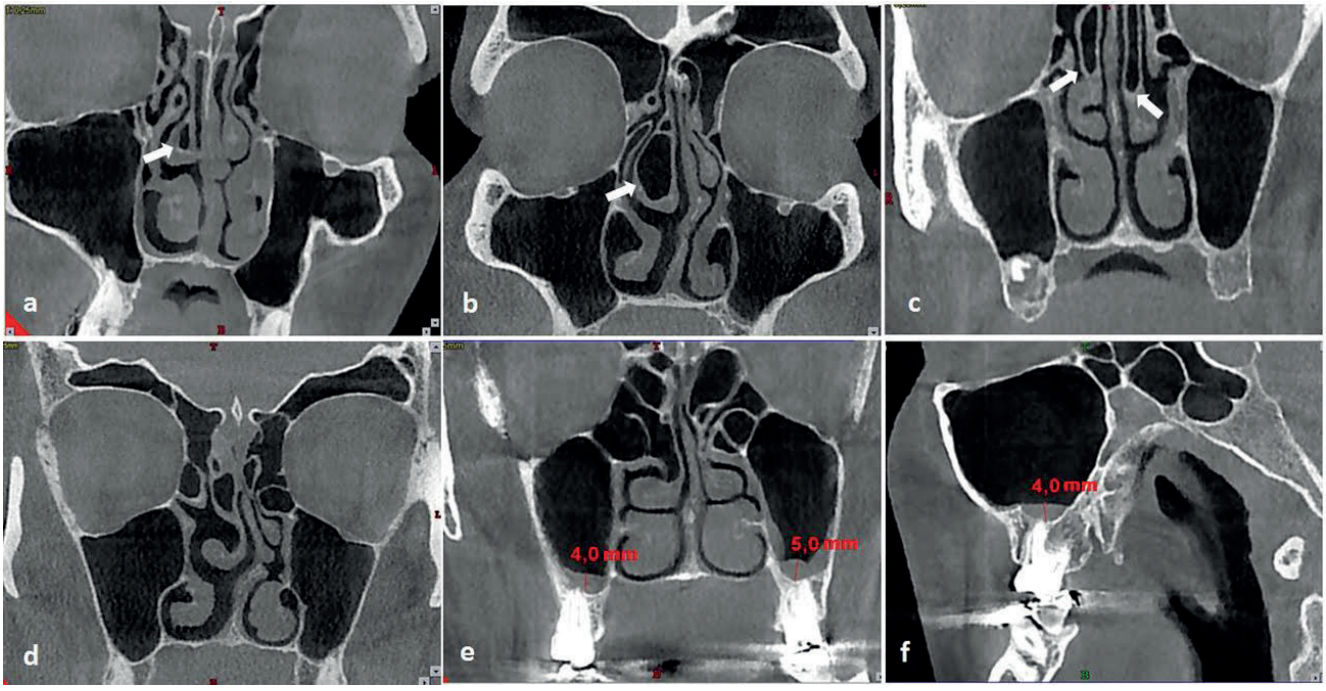

Figure 1. a) Bulbous type of concha bullosa, b) Extensive type of concha bullosa, c) Lamellar type of concha bullosa, d) Left sided septal deviation, e, f) mucosal thickening related with odontogenic lesion

Table 1. Distribution of the pathological conditions according to gender

Condition / Gender	Osteomeatal complex disease		Concha bullosa		Nasal septal deviation		Odontogenic lesion		Mucosal thickening	
	Present	Absent	Present	Absent	Present	Absent	Present	Absent	Present	Absent
Female	22 (84.6%)	79 (45.4%)	45 (45%)	56 (56%)	69 (57.9%)	31 (38.2%)	23 (58.9%)	78 (48.4%)	64 (57.1%)	37 (42%)
Male	4 (15.4%)	95 (54.6%)	55 (55%)	44 (44%)	50 (42%)	50 (61.7%)	16 (41%)	83 (51.6%)	48 (42.9%)	51 (58%)
Total	26 (13%)	174 (87%)	100 (50%)	100 (50%)	119 (59%)	81 (41%)	39 (19.5%)	161 (80.5%)	112 (56%)	88 (44%)

Table 2. Distribution of the pathological conditions according to the effected side

Condition / Effected side	Osteomeatal complex disease		Concha bullosa		Nasal septal deviation		Odontogenic lesion		Mucosal thickening	
	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
Left side	7	26.9	10	10	63	52.9	8	20.5	18	16
Right side	9	34.6	35	35	56	47.1	16	41	33	29.5
Bilateral	10	38.5	55	55	-	-	15	38.5	61	54.5
Total	26	100	100	100	119	100	39	100	112	100

Concha bullosa: CB was observed in 100 (50%) of the subjects (55 female and 45 male). All were noted in the middle concha. Of the 155 CBs, 10 were seen only in the left, 35 were located only in the right, and in 55 were detected bilaterally. Reverse curvature and absence of concha were not detected. Bulbous CB was found in 48 cases, extensive type CB in 8 cases, and lamellar CB in 99 cases. (Figure 1a-c) There was no significant statistical difference in accordance to gender (p>0.05) (Tables 1, 2).

Nasal septal deviation: NSD was found in 119 (59.5%) cases (50 female and 69 male). Pneumatisation of nasal septum was not detected. Left-sided deviation was found in 63 (15.9%) cases, and right-sided deviation of the nasal septum was found in 56 (14.5%) cases (Figure 1d). In accordance to gender, no significant statistical difference was found (p>0.05) (Tables 1, 2).

Odontogenic lesion: Fifty- four OLs were detected in 39 (19.5%) of all cases (16 female and 23 male). Eight (20.5%) were at the left, 16 (41%) were at the right and 15 (38.5%) were present bilaterally (p>0.05) (Tables 1, 2).

Maxillary sinus mucosal thickening: In 112 (56%) of cases MT was detected (48 female, 64 male). While 18 (16%) was left-sided, 33 (29.5%) was right-sided MT, and bilateral MT was found in 61 (54.5%) of the cases. No significant statistical difference was found in accordance to gender (p>0.05) (Tables 1, 2).

The concomitant presence of CBs and NSD was in 64 cases. There wasn't any statistically significant relationship between the presence of CB and NSD (p>0.05). No statistically significant differences found between types of CB and OMD (p>0.05) (Table 3).

Concha bullosa and mucosal thickening: Fifty of the right-sided CBs also presented right-sided MS. Twenty- four of the left-sided CBs also presented left-sided MT. In addition, 15 of the subjects with bilateral CB had bilateral MT. MT was seen more often in cases with lamellar type CB (73.3%). There wasn't any statistically significant relationship between MT and CB (p>0.05) (Table 3).

Nasal septal deviation and mucosal thickening: Thirty-three of the right-sided NSDs had ipsilateral MT. Twenty- seven left-sided NSDs, had also ipsilateral MT. There was no statistically significant difference between NSD and MT (p>0.05) (Table 3).

Osteomeatal complex disease and mucosal thickening: Aamong the 9 right-sided OMDs, 8 had ipsilateral MT. On the other hand, of 6 of 7 left-sided OMDs had ipsilateral MT. Additionally 9 of 10 bilateral OMDs also were presenting MT. There was a statistically significant relationship between OMD and MT (p=0.00) (Table 3).

Table 3. Statistical analyse of the evaluated parameters

	MT	CB	OMD	OL	SD
MT	-	0.31	0.000*	0.000*	0.37
CB	0.31	-	0.102	0.624	0.563
OMD	0.000*	0.102	-	0.906	0.104
OL	0.000*	0.624	0.906	-	0.268
SD	0.37	0.563	0.104	0.268	-

*Correlation is significant at the 0.01 level. MT: Mucosal thickening, CB: Concha bullosa, OMD: Osteomeatal complex disease, OL: Odontogenic lesion, SD: Septal deviation

Mucosal thickening and odontogenic lesion: Fourteen of the 16 right-sided OLs had right-sided MT, 7 of the 8 left-sided OLs had also left-sided MT, and additionally, 13 of the 15 subjects with bilateral OL had also bilateral MT. There was statistically significant relationship between MT and OL (p=0.00) (Table 3).

Discussion

MS is an inflammation of maxillary sinuses caused by mucociliary transport failure, related to numerous factors, such as anatomic malformations (NSD, CB), OLs, immune deficits, allergic reactions, smoking, atmospheric pollution, sinonasal polyposis, etc. (2-7,12,18,19). Maxillary sinus MT is the radiographical appearance of MS (20). Preoperative evaluation of these factors is important, as it helps to identify potential candidates for surgical procedure and the exact treatment of MS (21).

CB is the most common variation of the nasal cavity and is most often found in the middle concha (20,22). CB prevalence was reported in the earlier studies (13,20) to range from 14-53% to 35-53%. Tsai et al. (23) and Yiğit et al. (24) reported the CB ratio of 31.5% and 25%, respectively. Presence of bilateral CB reported to range from 45% to 61.5% in extant studies (22,25). Tunçyürek et al. (12) reported lamellar CB in 25.3% of the examined cases, bulbous CB in 6.2%, and extensive type of CB in 11.1% of the cases. Subramanian et al. (26) and Smith et al. (16) reported that CB was seen more frequently in females. This is in harmonious with our results, as most subjects with CB were female. In our study while the ratio of lamellar CB (25%) was similar with previous studies, the percentages of bulbous (12.12%) and extensive CBs (2.02%) were different.

NSD is the most frequent deformity of the nasal structures. In the extant literature, its incidence is varying from 40 to 45% (24,25). In our study, the ratio of cases with NSD (59.5%), is higher than the ones found in the study conducted by Smith et al. (16) (19.4%), and lower than those reported by Stallman et al. (13) (65%) and Subramanian et al. (26) (62.9%). Although some researchers indicated a relationship between NSD and large CB, the association between them is still unclear (14,25). The concomitant existence of NSD and CB was reported as 44.6% by Hatipoğlu et al. (20). On the other hand, Yiğit et al. (24) revealed the incidence of CB in to18.95% in non-NSD subjects and 45.34% in NSD subjects. With a ratio of 53.38%, our study revealed a higher incidence of concomitant presence of CB and NSD. Regional differences and genetic factors could be responsible for these paranasal variatons.

The relationship between NSD and MT is also still unclear (24). In CT studies, Tunçyürek et al. (12) and Smith et al. (16) could not establish an exact association between them. In a study, severe NSD was reported as a predisposing factor for MT (4). In another CT study, Hatipoğlu et al. (20) pointed out a relationship between the cases with severe NSD and existence of MT. In the present study, no statistically significant relationship was found between NSD and MT (p>0.05).

It is important to investigate the relationship of MT with the presence/absence of CB, reverse curvatured concha, and OMD. CB, especially when accompanied with pneumatization of the lower section, constitutes a predisposing factor for the development of OMD. The occurance of the sinus diseases by the block ventilation and mucociliary clearance of the maxillary sinuses caused by the pressure of OMD and CB to the middle meatus, infundibulum, uncinated process

was asserted (3,11,12). Also it was suggested that the presence of CB prevented appropriate airflow and predisposed the sinus diseases. Contrary findings were also reported (12,13,27-29). For example, Lee et al. (15) reported a case with massive CB which resulted in an OMD and caused right-sided MS. Yousem and David (14) pointed out that CB of a greater size could lead to MT. Contrary to this data, Stallman et al. (13) found no significant relationship between the size of CB and MT, as 78% of subjects had MT without CB (13). No relationship among NSD, CB and MT was stated in our study ($p>0.05$).

OMS is an inflammatory disease caused by the extent of apical inflammations due to proximity of tooth apexes into the sinus. Mucous membrane of maxillary sinus changes and adjacent OLs can be defined by CBCT images. OMS comprises about 10-12% of all MS cases and some studies determined its prevalence as ranging from 10% to 86% (21). Lu et al. (30), stated that 26% of subjects with maxillary sinus MT had also periapical lesions. In another study, 72.6 % of cases with MT also found in relationship with adjacent periapical infection and had changes in the maxillary sinus floor (31). Correspondingly, higher ratio of relationship between OL and MT was found in our study.

The diagnosis of MS should include clinical examination (dental examination, nasal endoscopic examination) and CT of paranasal sinuses (9,21). Cost and radiation dose from CBCT are substantially lower than the pertaining to CT, but are higher compared to traditional imagining techniques (31-33). CBCT provides advanced imagining opportunities of paranasal region with isotropic voxel size and high resolution images. Mucosal changes of sinuses, dimension of OLs, paranasal region abnormities and anatomic structures can be easily detected and evaluated by examining CBCT images (34).

Sinus membrane elevation or "sinus lift" is a significant procedure before dental implant placement (35). Preoperative CBCT examination allows evaluation of MT and residual ridge heights. Before sinus lift surgery, MT should be determined, because greater MT than 5 mm can be related with OMD as well as MS (36).

Study Limitations

Lack of clinical conditions and exact history of paranasal problems of the patients whose CBCT images were used are the limitations of our study. There is no previous CBCT study evaluated all parameter's relationships with each other. The main aim of this study is to give an idea about the utilization of the CBCT in the examination of the paranasal region.

Conclusion

Our results did not show statistically significant relationship MT and NSD, CB, OMD except OL. Maybe with future studies showing clinical conditions may give more accurate and compherensive statements about this relationship. Despite limitations, our findings showed that identification of MT by CBCT imaging could help to identify the reasons behind MS. CBCT examination enables diagnosis of mucosal changes in maxillary sinuses, as well as variations of paranasal structures and spread of OLs. Thus, CBCT imaging could also be a diagnostic tool for assessment of the NSD, CB, OMD, and OL and their potential relationship with MT.

Ethics

Ethics Committee Approval: The Ethical Committee of the Erciyes University (approval no: 2017/440, date: 15.09.2017).

Informed Consent: It was not taken.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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

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Original Article / Özgün Araştırma

Interactive Poster

İnteraktif Poster

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Abstract

Objective: The scientific presentations are usually in oral or poster form. Recently evolved methods are video and "e-poster" presentations. Video data and images can be presented in oral presentations but for a limited time. The presentation time is prolonged in poster technique and available for more people but video data can't be presented. The video presentations and e-posters are uploaded and ready to be selected through a menu in few computers because of the lack of available number of computers for every study. The interactive posters make the presentation of numerous images and video data to be accessible continuously during whole meeting period via its special screen. We aimed to increase sharing of knowledge and make it interactive during a limited period.

Materials and Methods: A 23-inch touch screen with a data processor was used for poster presentation. The special screen was provided for participants to read text, multiple images and video data continuously. The data processor was set to count the number of people to touch screen to read the presented study during the congress period.

Results: The interactive poster was set to be ready in 7th Congress of Surgical Research. The continuous presentation was lasted all through the 3-day Congress Period and 938 clicks were counted by the processor.

Conclusion: During a congress period, by touching the interactive screen it was possible to interchange between the menu topics. The software counting the clicks need to be developed to estimate the number of people evaluating the study as well as duration of interest and which image and video data were evaluated at most.

Keywords
Video poster, oral presentation, e-poster

Öz

Amaç: Bilimsel kongrelerde bildiriler genelde sözlü veya poster olarak sunulmaktadır. Son zamanlarda video sunumu ve "e-poster" de öne çıkmaktadır. Sözlü bildiride videolar, resimler gösterilebilir ancak sunum süreleri kısıtlıdır. Posterlerin sunum süreleri uzundur, daha çok katılımcıya ulaşabilir, ama video gösterilemez. Video sunumlar ve e-posterlerde ise her çalışma için ayrı bilgisayar olmadığından az sayıda bilgisayara yüklenmiş olarak menüden seçilmeyi beklerler. İnteraktif poster, özel ekranı sayesinde, kongre boyunca sürekli sunum yapabilir, sayısız resim ve film gösterebilir. Amacımız interaktif poster ile kongrelerde bilgi paylaşımını artırmak ve interaktif hale getirmektir.

Gereç ve Yöntemler: Bilgi işlemcisi ve 23 inç dokunmatik ekranlı poster hazırlandı. Özel ekranı ve sürekli sunum avantajıyla katılımcıların istedikleri zaman interaktif postere gelip yazıları okuma, yüzlerce resim ve videoyu izleyebilmelerine olanak sağlandı. Bilgi işlemci de kongre boyunca kaç kişinin çalışmayı okuduğunu, ekrana dokunduğunu belirleyecek şekilde ayarlandı.

Anahtar Kelimeler
Video sunumu, sözlü sunum, e-poster

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Bulgular: Yedinci Cerrahi Araştırma Kongresi'nde poster salonuna kurulan interaktif poster ile sunuma başlandı. Kongre süresince, yani üç gün boyunca çalışmanın kesintisiz sunumu gerçekleşti ve 938 tıklama olduğu saptandı.
Sonuç: Kongre süresince interaktif ekrana dokunarak menüler arasında geçiş yapılabilmesi mümkün oldu. Ekrana kaç kez dokunulduğunu saptayan programın geliştirilmesi halinde kaç kişinin ne kadar süreyle çalışmayı izlediği, hangi resimleri ve videoları incelediğini sayısal olarak saptamak da mümkün olacaktır.

Introduction

The presentations in scientific meetings are generally in two different methods: oral or poster (1-3). Video presentations and e-poster methods are also included recently. All of these methods have some advantages and disadvantages. Oral presentations are accepted relatively less in number but are better in presenting a topic with comparatively superior visual material (2,4-6). However, oral presentations need 10-15 minutes to go and limited to the number of audiences in the room. Video presentations are generally effective for discussion on surgical techniques. E-posters are presented in digital media. The last two presentation techniques need a specific monitor and operating system. Anyone choses a video presentation or e-poster within a topic list waiting in stand-by state. This method obviously requires an expensive technical infrastructure. Posters have no oral speech or video presentation but they are visually enriched by means of images and graphs. A poster is available throughout the meeting period and may reach comparatively higher number of participants if it is interesting enough (2-6). The advantages and disadvantages of various presentation methods are summarized at Table 1.

A new method of presentation arises when the advantages of these four methods are to be combined. Video poster method is designed to fulfill this need has more advantages than other presentation method (Table 1). Video poster method has found to be successful since 2007 (1,2). Encouraged by winning awards for presentation in National Congress, it was also used in other national and international meetings and found to be interesting and awarded (2). The portable DVD player in early presentations was replaced by a digital frame because it was assembled easier behind a poster (7). Later, mounting a digital frame in front of a poster was found to be even more easy and faster (8).

The interactive poster (IP) method is the last derivation of these methods. A data processor with touch screen monitor will be assembled on a poster. Digital data like text, many images, tables, graphs

Table 1. The properties of various presentation methods

	Poster	Video poster	Oral presentation	Video presentation	E-poster
Video play	-	+	+	+	-
Oral speech	-	+	+	+	-
Slide show display	-	+	+	+	-
Continuous presentation of study	+	+	-	-	-
Achievement of data anytime required during congress period	+	+	-	+/-	+/-
Continuous display of video presentation during congress period	-	+	-	-	-
Low cost of presentation for presenters	+	+	+	+	+
Low cost congress organizers	+	+	+	-	-
Interactive discussion with representative of the study	Whenever the representative is nearby the presentation	Whenever the representative is nearby the presentation	A few minutes after presentation of the representative	If the representative waits near the monitor for to be chosen	If the representative waits near the monitor for to be chosen

and video footages of a study will be uploaded to IP. Any participant will achieve any data, examine images and watch videos just by touching screen. If this presentation technique is widely accepted, more and various visual data will be included and common interest to poster area will be higher. This situation would serve to share and produce more knowledge.

Materials and Methods

A data processor with 23" touch screen monitor on a portable pole was prepared and placed in poster hall at the congress center. The written plates about the headlines of the study were mounted along the circumference of screen and placed in poster area (Figure 1).

The subject of the IP was the awarded innovations in 6th Surgical Research Congress. There were four main menus on the screen. First one was a slide show describing rules, evaluation and selection criteria of the jury of innovation contest. The other three menus were assigned to the awarded three innovations each. The specifications, technical drawings, images and videos of prototypes of the innovations to be used for patenting applications were uploaded. Any participant was free of achieving data of desired study by selecting menu on the screen. The data processor recorded the number of all clicks on the menu. Since this study is a work of presentation technique approval of local ethical committee and patient consent form was not necessary.

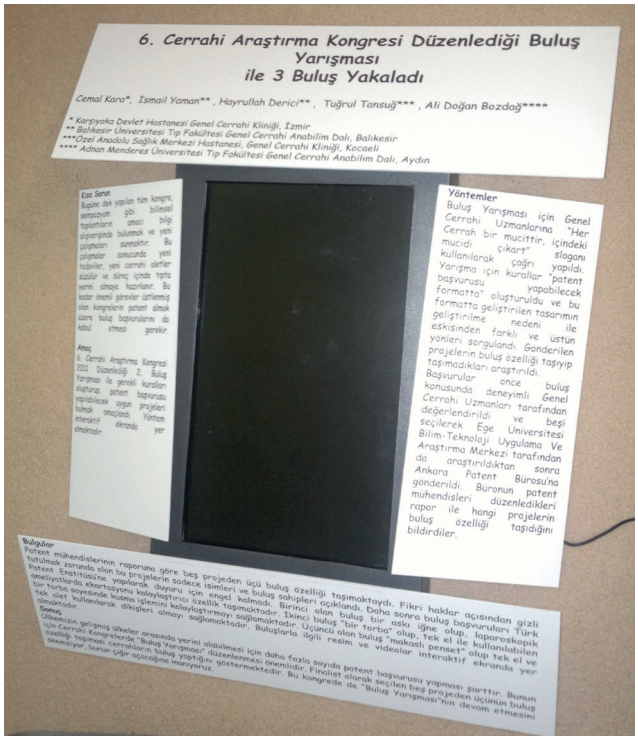

Figure 1. The mounting of written plates on interactive poster

Results

During November 7-9, 2013 period, the assembled IP was uninterruptedly presented at the 7th Surgical Research Meeting in Ankara (Figure 2, 3). All along the meeting period, the participants could reach the desired data. There were 938 clicks during this period.

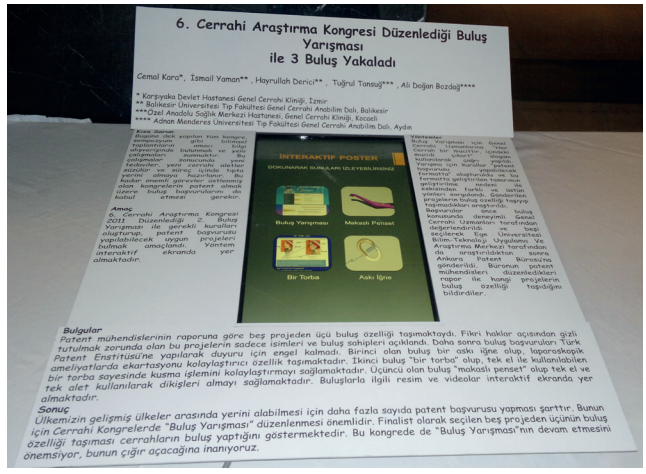

Figure 2. Placement of interactive poster

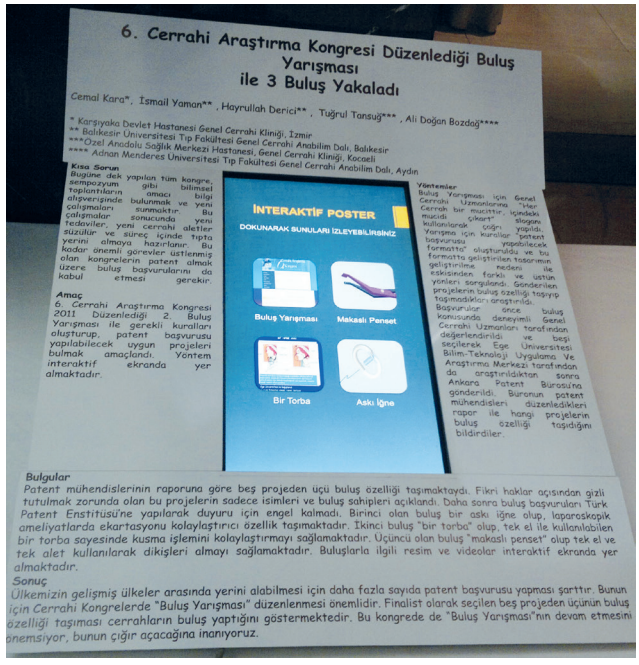

Figure 3. Main menu on the screen

Discussion

The poster presentation area is generally visited less relative to other activities of the meeting by participants. Limited visual contributions could make a poster monotonous and cause decreased attention (1). A video poster presentation with its enriched visual properties, continuity and low cost is an original work exposing dynamism (1,2,7). The presentation impact of a video poster is improved more by adding interactivity.

During this congress period, the number of clicks on this poster was counted as 938. Previously, there is no reported study to measure the rating of e-poster presentations. This improvement comes from technological improvements. When the software is upgraded, it will be possible to know the number of participants examining a specific text, image or video. Then, it would be easier to get feed-back of the participants and the researchers would be directed accordingly. We think that the IP presentation includes and enhances video and e-poster methods and the finding supported our idea. IP presentation will revive the poster areas of the meetings and may help to transfer more knowledge than previously. Additionally, the work inside the IP can easily be transferred via internet. In future, other presentation methods will be replaced by IP technique.

Conclusion

Since it's the early era of this technique, the published articles on this method can make IP gradually be acceptable more. Any knowledge increases when it is shared more and faster.

Ethics

Ethical Committee Approval: Since this study is a work of presentation technique approval of local ethical committee and patient consent form was not necessary.

Informed Consent: It was not necessary.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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

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Original Article / Özgün Araştırma

Effects of Glutamine, Arginine and Beta Hydroxymethybutyrate on Anastomotic Leakage in Experimental Colon Anastomosis

Deneysel Kolon Anastomozunda Glutamin, Arjinin ve Beta Hidroksibütiratın Anastomoz Kaçağına Etkisi

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Keywords
Beta-hydroxy-beta-methyl butyrate, anastomosis, nutrition

Anahtar Kelimeler
Beta-hidroksi-beta-metil butirat, anastomoz, nütrisyon

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Abstract

Objective: The studies on anastomotic leakage which is one of the leading serious complications of colonic anastomoses keep going as well as other surgical researches. This study was designed to investigate effects of hydroxy-ß-methyl butyrate (HMB) on anastomotic healing.

Materials and Methods: Forty rats were randomized into four groups. Group A (n=10) rats received chow food plus arginine+glutamine+HMB rich diet for 7 days before right colonic transection followed by an end-to-end anastomosis. Group B (n=10) rats received chow food plus arginine and glutamine rich diet for 7 days before the same surgical procedure. Group C (n=10) rats underwent the same procedure after a 7-day chow plus glutamin rich diet. Group D (n=10) rats had the surgery after a chow food only diet for seven days. All the subjects were fed accordingly to their groups for 7 days postoperatively. On the 7th day, all rats were sacrificed under anesthesia to measure anastomotic bursting pressure and evaluate hydroxyproline levels as well as histopathological scoring of anastomotic line.

Results: This study revealed significantly increased hydoxyproline level at the 7th day in group A ($p<0.002$) and group B ($p<0.001$) relative to group D. There were no significant differences among the groups for anastomotic bursting pressures and histopathological scores.

Conclusion: Enteral HMB support may result statistically significant increased biochemical anastomotic strength with an insignificant difference in biomechanical force. Although more studies are needed to delineate better efficacy, preoperative enteral HMB support may decrease postoperative morbidity and mortality.

Öz

Amaç: Kolon anastomozu ile ilgili kaçak nedenleri konusunda devam etmekte olan araştırmalar vardır. Bu çalışmada anastomoz dayanıklılığının ameliyat öncesinde beta-hidroksi-metil bütirat (HMB) ile artıp artmayacağı denetlenmiştir.

Gereç ve Yöntemler: Kırk rat 4 gruba randomize edildi. Grup A (n=10) ratları standart rat yemi ile birlikte arjinin+glutamin+HMB'den zengin diyetle 7 gün beslendikten

sonra sağ kolon transeksiyonu ve uç uca anastomoz uygulandı. Grup B (n=10) denekleri rat yemi ile birlikte arjinin ve glutaminden zengin diyet 7 gün beslendikten sonra aynı cerrahi işlem uygulandı. Grup C (n=10) 7 günlük rat yemi ile birlikte glutaminden zengin gıda ile beslendikten sonra cerrahiye alındı. Grup D (n=10) ratları sadece standart yem ile 7 gün beslendikten sonra ameliyata alındı. Tüm denekler postoperatif 7 gün boyunca beslendiler. Yedinci gün tüm ratlar anestezi altında sakrifiye edilerek anastomoz patlama basınçları ölçüldü. Hidroksiprolin değerleri ve histolopatolojik skorlama için anastomoz hattından örnek alındı.
Bulgular: Çalışmamızda A ve B grubunda hidroksiprolin düzeylerinde 7 günde belirgin ölçüde artmış hidroksiprolin düzeyleri saptandı. Gruplar arasında anastomoz patlama basıncı ve histolojik iyileşme parametreleri anlamında fark yoktu.
Sonuç: Enteral HMB desteği anlamlı artmış biyokimyasal anastomoz direnci sağlayabilir, ancak biyokimyasal güçte fark olmayabilir. HMB'nin yararını ortaya koymak için daha fazla çalışma gereksinimi olmasına rağmen preoperatif enteral HMB desteği postoperatif postoperatif mortalite ve morbiditeyi düşürebilir.

Introduction

Although anastomoses are an essential part of gastrointestinal (GI) surgery, they have carry a high risk for morbidity and mortality due to potential complications. Despite the current developments in GIS surgery, complications and particularly anastomotic leakage remains a major issue and post-operative anastomotic leakage occurs at a rate of 10-20% (1-3). The Various local and systemic factors influence on anastomotic recovery. Many investigators have tried many techniques and chemicals with regard to supporting anastomotic recovery and reducing post-operative risks (4-6). The one of the factors influencing wound healing and colon anastomosis is diet (5). The cases whose diet is inadequately planned are at higher risk for surgical complications, the wound healing in particular. Ensuring and maintaining appropriate nutritional supplementation are manifested as the important primary aim of perioperative care (7). Various amino acids influence wound healing. Glutamine, which is the most common amino acid, found in the body, accelerate mucosal growth and healing, reduce GIS-derived sepsis by inhibiting bacterial translocation and mediate nitrogen balance (8). Arginine, acts as L-arginine to improve immune system functions, accelerate wound healing and increase resistance to infections (9). A leucine metabolite named β-hydroxy-β-methyl butyrate (HMB) plays a significant role in wound healing and cachexia (10).

The aim of our study is to determine the role of perioperative glutamine, arginine and HMB-rich diet on anastomosichealing after elective colonic anastomosis in rats.

Materials and Methods

This study was conducted with approval of Local Animal Ethics Committee (approval number: 050.04/2011/099). Forty female Wistar Albino rats weighing between 170-220 g were used for the study. The rats were divided equally into four groups.

Group A (arginine+glutamine+HMB): Received 7 days of standard chow food plus arginine+glutamine+HMB rich diet preoperatively followed by right colonic transection and end-to-end anastomosis and received 7 days of standard chow food plus arginine+glutamine+HMB rich diet postoperatively before being sacrificed (n=10).

Group B (arginine+glutamine): Received 7 days of standard chow food plus arginine and glutamine rich diet preoperatively followed by segmental right colonic transection and end-to-end anastomosis and received 7 days of standard chow food plus arginine and glutamine rich diet postoperatively before being sacrificed (n=10).

Group C (glutamine): Received 7 days of standard chow food plus glutamine rich diet preoperatively followed by right colonic transection and end-to-end anastomosis and received 7 days of standard chow food plus glutamine rich diet postoperatively before being sacrificed (n=10).

Group D (control): Received 7 days of standard chow food preoperatively followed by right colonic transection and end-to-end anastomosis and received 7 days of standard chow food plus postoperatively before being sacrificed (n=10).

In addition to standard chow food, group A received 7 days of glutamine 1.44 g/kg/day, arginine 1.44 g/kg/day and HMB 2.6 g/kg/day (Abound Abbott Nutrition, Access Business Group, 19600 6th Street, Lakeview, CA92567-8403, USA) mixed in still tap water and continued at the same dose for 7 more days after the operation until sacrificed.

Group B received 7 days of standard chow food plus glutamine 1.5 g/kg/day and arginine 1.63 g/kg/day (Impact Glutamine, Nestle Health Care Nutrition

Germany) before the operation and continued at the same dose for 7 more days after the operation until sacrificed.

Group C received 7 days of standard chow food plus glutamine 1.3 g/kg/day (Glutamine Resource Nestle Health Care Nutrition Germany) mixed in still tap water and continued at the same dose for further 7 days after operation until sacrificed. Since it is an experimental study on nimals, there is no need for informed consent.

Surgical Procedure

Standard chow food was discontinued in all rats 12 hours before the operation and no colon cleansing was performed. However, there was no limitation for water in the control group and enteral nutritional solution in the other groups. The abdomen was entered by a 3 cm mid-line incision in sterile conditions. Following entering the abdomen, the caecum was found and complete transection of the colon segment 4 cm distal to the ileocecal junction was performed preserving the mesocolon. End-to-end anastomosis was established with 8 single-layer inverting sutures using atraumatic 4/0 polypropylene suture material (Figure 1). Abdominal incision was sutured with 3/0 silk material and operation was completed. The rats were started feeding relative to their groups 6 hours after the procedure.

Anastomotic Bursting Pressures Measurement

All rats were sacrificed 7 days after the operation by cervical dislocation under ether anesthesia. After the sacrification, the anastomotic segment was found at relaparotomy (Figure 2). Two umbilical 6-Fr catheters were inserted at 2 cm proximal and distal regions of the anastomosis using cut-down technique (Umbilical catheter–Bıçakçılar®). The catheters were fastened to the colon to ensure retention using 2/0 silk sutures. Thus, a 4 cm luminal segment including the anastomosic line in the middle was established (Figure 3). The intraluminal pressure was monitored electronically using a transducer (SS13L pressure transducer) and pressure line (Morton pressure resistant pressure line code: 441-Turkey) between the monitor (Biopac MP30 ultimate system Santa Barbara USA) and the catheter while saline fluid (SF) infusion at steady speed was introduced into the catheter through the distal end of the anastomosis. The highest pressure measured by the monitor just before the pressure reduction due to anastomosic leakage was recorded as the bursting pressure. The bursting pressure values were recorded in mmHg.

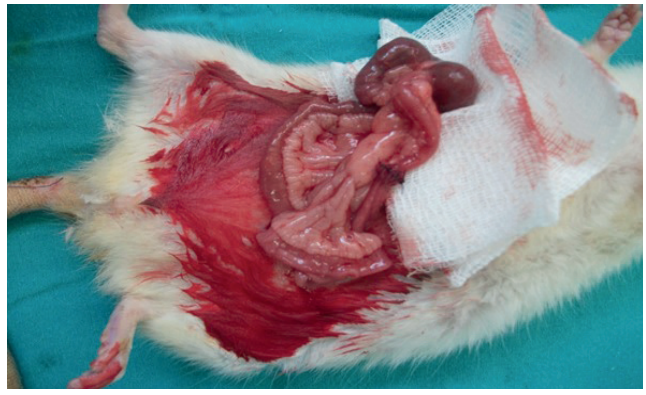

Figure 1. Anastomosis using 4/0 polypropylene suture material

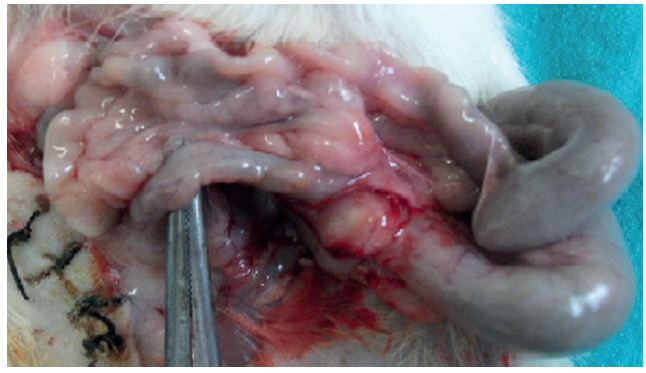

Figure 2. Relaparotomy-the anastomotic segment was found

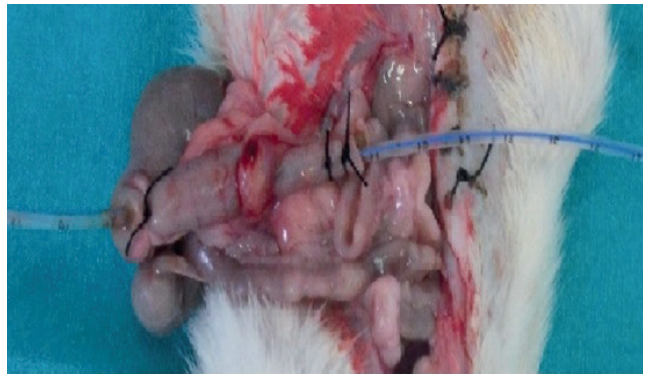

Figure 3. Anastomotic bursting pressure measurement model

Subsequently, tissue samples for histopathological and biochemical examinations were obtained from the anastomosic lines. The tissue hydroxyproline levels were measured. The neutrophilic infiltration scores were used for histopathological scoring (Table 1).

Table 1. The scoring for mucosal healing and neutrophilic infiltration. The mucosal healing was not prominent in groups, neutrophilic infiltration showed no significant difference (p=0.373)

Score	Mucosal healing	Neutrophilic infiltration	Macrophage, fibroblast and neovascularization
0	No healing	>75%	None
1	Surface epithelial healing	25-75%	Minimum
2	Submucosal healing	25-50%	Intense
3	Muscularis mucosa healing	<25%	-
4	Complete healing	None	-

Table 2. The anastomotic bursting pressures (mmHg). The difference was not significant among groups. The bursting pressure measurements were evenly distributed and there was no statistical difference between groups (p=0.54)

Rats	Group A (HMB)	Group B (glutamine+arginine)	Group C (glutamine)	Group D (control)
1	133.14	233.65	330.3	167.93
2	138.91	210.97	204.63	28.58
3	137.27	193.47	248.85	351.23
4	146.16	209.31	230.22	-
5	237.56	215.78	174.88	254.87
6	13.45	236.87	251.34	201.35
7	238.08	264.92	318.63	302.67
8	271.84	253.98	334.69	206.25
9	-	266.8	250.91	-
10	-	-	244.07	-
Mean value	**164.55±82.6**	**231.75±26.2**	**258.85±53.4**	**216.12±104.1**

HMB: Hydroxy-ß-methyl butyrate

Statistical Analysis

The results of the biochemical tests and the bursting pressure measurements were analyzed using One-Way variance analysis (ANOVA). Tukey's test and Bonferroni test were used as post-hoc tests. Kruskal-Wallis test was used to compare the histopathological scores. The difference was considered significant if p value is less than 0.05.

Results

Two rats from group A and 1 from group D died preoperatively and 1 rat from group D and 1 from group B lost at the postoperative period were not included in statistical analyses. One sacrificed rat from group D found to has already anastomosic leakage was also not included in statistical analyses. The bursting pressures and the group mean values at post-operative day 7 are presented in Table 2.

The bursting pressure measurements were distributed and there was no statistical difference between the groups (p=0.54)

The histopathological score evaluations were as follows; intense inflammatory cell infiltration, fibrin, granulocytes, fibroblasts and foreign body giant cells were detected at the anastomosic line in group A.

Prominent granulation tissue formation and almost chronic inflammation was found in group B. Abundant granulocytes, fibroblasts and foreign body giant cells were seen in group C. Minimal granulocytes, fibroblasts and foreign body giant cells were found in group D. Although mucosal healing was absent in all of the groups, statistical assessment for neutrophilic infiltration showed no significant difference (p=0.373).

The mean hydroxyproline levels as µg/mg wet tissue on day 7 after the operation are presented in Table 3. The difference was significant among the groups (p=0.0006).

Table 3. The tissue hydroxyproline levels (µg/mg wet tissue). The difference was significant among groups (p=0.0006)				
Rats	**Group A (HMB)**	**Group B (glutamine+arginine)**	**Group C (glutamine)**	**Group D (control)**
1	5.701710	6.224519	3.165495	4.307143
2	4.577269	5.996345	5.744869	3.869284
3	4.422086	5.154676	3.060069	3.194277
4	5.768745	4.230429	4.648631	3.207473
5	6.763813	9.149859	4.185815	2.982836
6	7.300578	4.685690	4.873407	3.755051
7	5.971772	6.108625	5.558381	3.304370
8	4.681921	10.757017	4.444135	3.161469
9	-	4.448646	3.798093	-
10	-	-	4.419914	-
Mean value	**5.64±1.0**	**6.30±2.2**	**4.39±0.8**	**3.47±0.4**

The hydroxyproline levels were higher in group A which included HMB administration compared to both the control group and group C which included glutamine administration (p=0.02). On the other hand, the hydroxyproline levels were higher in group B rats which received glutamine+arginine compared to both p and group C which included glutamine administration (p<0.01).

Discussion

Colon resections and anastomoses, particularly distal portion of the left colon, carry higher risk for anastomotic leakage and disintegration. Despite developments in surgical techniques and tools, morbidity and mortality rates related to anastomotic leakage after colon surgery remain high. Consequently, the studies on intestinal anastomosis focus on the colon (11). Thus, we preferred to investigate the effects of HMB on colonic anastomoses.

Wilson et al. (12) mentioned that the studied agent HMB used in our study shows its effect on anti-catabolic and protective mechanisms, thus enhancing protein synthesis. Manzano et al. (13) stated that HMB activates protein synthesis by activating the intracellular protein called mammalian target of rapamycin (mTOR). The mTOR signaling pathway is activated primarily when sufficient amount of intracellular glucose, amino acid and lipoproteins are available and enhances protein and amino acid synthesis by stimulating ribosomes. Insulin like growth factor-1 is the primary stimulus that activates mTOR in muscle cells while increasing HMB effect on mTOR (13,14).

The factors that have influence on wound healing can be classified under two main topics. The factors related to reduced collagen synthesis such as chronic nutritional disorders, diabetes, uremia, trauma, radiation injury, advanced age and the surgical factors such as tissue injury, infection of the tissue, poor vascularization or circulation of the tissue may influence wound healing (15).

The healing of intestinal anastomoses can be evaluated according to mechanical, biochemical or histopathological aspects. Mechanical evaluation includes assessment of bursting and breaking forces while biochemical evaluation includes rate, amount and structure of collagen synthesis at the anastomotic line (16). Croinin et al. (17) reported that the anastomotic bursting pressures gradually increased from the third day on and reached to maximum levels on day 10 while the hydroxyproline levels at the anastomotic area decreased by 40% within the first three days, approached to normal levels by approximately at the fifth day and raised above normal by days 10-14. In our study, the bursting pressure measurements were evenly distributed and there was no significant statistical difference between four groups (p>0.54).

Yıldız et al. (18) investigated the effects of cyanoacrylate on colonic anastomoses and measured bursting pressures and tissue hydroxyproline levels. The bursting pressures were found to be significantly

different between groups while there was no significant difference for the tissue hydroxyproline levels. Madden and Peacock (19) reported that in order to explain the role of collagen in the biology of wound healing, the collagen synthesis and lysis ratio should be considered as well as the available collagen amount. The investigators reported that collagen synthesis and disposition can be determined by applying labeled proline periodically and measuring hydroxyproline levels of the wound and they conducted a study for this purpose on rats. In our study, the hydroxyproline levels at the anastomotic line were significantly increased on day 7 in group A (HMB) compared to group D (control) ($p<0.002$). Similarly, hydroxyproline levels at the anastomotic line were significantly increased on day 7 in group B (glutamine+arginine) compared to group D (control).

Considering that the sutures maintain anastomotic endurance during the early period of reduced collagen concentration (20), the pressure resistance and suturing techniques appear to be correlated.

The tensile strength of the suture material is required for bridging between the newly formed collagen tissue and restoring the original tension of the intestinal wall. Therefore, improved collagen synthesis plays the primary role in wound healing and inhibition of regulation influences anastomotic tension. Additionally, fibrillary quality besides collagen mass determines tension and collagen type and stability of crosslinks are important for tension. The tensile strength depends on mechanical stability of the collagen fibrils and the formation of intermolecular crosslinks (16). In our study, although the mucosal healing was not observed in any of the groups, the statistical analysis for neutrophilic infiltration showed no significant difference.

Conclusion

In our study, the findings regarding tissue hydroxyproline levels were significant only while the anastomotic bursting pressures and the tissue histopathological results were insignificant. Although we believe this may be related with the late effects of HMB on tissue histopathology and tissue biochemistry, we suggest the preoperative enteral HMB supplementation could reduce the postoperative complications by improving anastomotic endurance.

Ethics

Ethical Committee Approval: Adnan Menderes University (ADU, Aydın, Turkey), Animal Ethical Committee (050.04/2011/099).

Informed Consent: Since it is an experiment on animals, there is no need for informed consent.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

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

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

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Original Article / Özgün Araştırma

Short and Medium Term Results of Posterior Segmental Instrumentation and Posterolateral Fusion in Female Patients with Spondylolisthesis: A Clinical Trial

Spondilolistezli Kadın Hastalarda Posterior Segmental Enstrümantasyon ve Posterolateral Füzyonun Kısa ve Orta Vadeli Sonuçları: Klinik Çalışma

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Keywords
Spinal fusion, spinal surgery, pain, disability, spondylolisthesis

Anahtar Kelimeler
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Abstract

Objective: Aim of this study was to short and medium term results of posterior segmental instrumentation and posterolateral fusion in female patients with spondylolisthesis.

Materials and Methods: Patients with lumbar sponylolisthesis who were performed convetional laminectomy, poliaxial secrew fixation and posterolateral fusion between the 2013 and 2015. The postoperative fusions of the female patients were evaluated through lumbar 3D computed tomography and X-ray imaging. The patients' back pain and lower extremities's pain were evaluated with Oswestry Disability index and visual analogue score. Patients' information was collected retrospectively from their files in May 2016.

Result: When the pain scores of the patients who underwent segmental instrumentation were compared in the preoperative, postoperative periods there was the significant decrease in pain scores ($p<0.001$).

Conclusion: The posterolateral fixation and fusion are an efficient treatment method in the medium and short term in female patients with spondylolisthesis. But, a secondary chronic disease such as osteoporosis associated with aging was added to impair bone quality, fusion success rate was seen to be decreased.

Öz

Amaç: Bu çalışmanın amacı, spondilolistezisli kadın hastalarda posterior segmental enstrümantasyon ve posterolateral füzyonun kısa ve orta vadeli sonuçlarının değerlendirmesidir.

Gereç ve Yöntemler: 2013-2015 yılları arası 50 spondilolistezisli kadın hastaya konvansiyonel laminektomi, poliaxial vida fiksasyon ve posterolateral füzyon yapıldı. Postoperatif dönemde hastaların füzyon durumları, 3D bilgisayarlı tomografi ve X-ray ile değerlendirildi. Hastaların bel ve alt ekstremite ağrıları sırayla Oswestry

Disability indeksi and vizüel analog skoru ile değerlendirildi. Hastaların bilgileri, hastaların dosyaları aracılığı ile retrospektif olarak taranak Mayıs 2016'da elde edildi.
Bulgular: Hastaların preoperatif ve postoperatif ağrı skorları karşılaştırıldığında ağrı skorlarında önemli bir azalama vardı (p<0,001).
Sonuç: Posterolateral fiksasyon ve füzyon, spondilolistezisli kadın hastalarda kısa ve orta vadede etkili bir tedavi yöntemidir. Fakat yaşlanmayla ilişkili osteoporoz gibi ikincil hastalıkların eklenmesinin füzyon oranını azalttığı görüldü.

Introduction

Spondylolisthesis is defined as subluxation of the spine on another spine in the sagittal plane (1). The etiological causes of spondylolisthesis were classified as dysplastic, ischemic, degenerative, traumatic pathologic, and iatrogenic by Wiltse et al. Rothman (2). Besides the etiologic classification, spondylolisthesis was divided into 5 groups depending on degree of slip by Meyerding (3). While grade 1 and 2 were defined as low-grade spondylolisthesis, grade 3, 4 and 5 were defined as high-grade spondylolisthesis (4). While the isthmic type whose etiological factors has not been fully revealed is often seen in young people, the degenerative type is often seen in the elderly group. It was reported that the incidence of spondylolisthesis in the lumbar region was 82% at L5-S1, 11% at L4-5, 0.5% at L3-4 and 0.5% at L2-3, respectively (5). Spondylolisthesis often causes clinically lower back pain which is worsened with motion and the cause of lower back pain is a weakness in the bone bridge connecting the upper and lower facet joints each other or instability associated with motion due to fracture (6). The increased instability may accelerate the disc degeneration in the affected level over time and this situation may cause that non-symptomatic discs become symptomatic. Moreover, it causes impairment of sagittal balance in the spine depending on the degree of slip (7,8).

The treatment options for spondylolisthesis include conservative treatment and surgical treatment. The conservative treatment is used frequently in patients with or without neurological deficit who have tolerable pain and improve with physical therapy. The surgical treatment is applied in patients with neurological deficit who do not benefit from physical therapy and conservative treatment (9). In the literature, many surgical techniques such as anterior interbody fusion, posterolateral fusion (PLF), posterior lumbar interbody fusion, circumflexial fusion, transforaminal interbody fusion and axial lumbar interbody fusion have been described (10-13). Although many different techniques have been described for the surgical treatment of spondylolisthesis, posterolateral lumbar fusion is regarded as the gold standard (14,15).

In the literature, there are many studies related to posterolateral fixation and PLF for treating spondylolisthesis. In nearly all of these studies, male and female genders were evaluated together. However, female patients have a hormonal disadvantage such as the development of osteoporosis secondary to menopause occurred after depletion of ovarian reserve or surgical ovariectomy compared to male patients. Our retrospective study includes 50 female patients with spondylolisthesis. The preoperative visual analogue score (VAS), body mass index (BMI) and Oswestry Disability index (ODI) scores of the patients were obtained and then the postoperative fusion, VAS and ODI scores of the patients were compared. Finally, we discussed our results in the context of the literature.

Materials and Methods

The Patients

This retrospective study was conducted with patients' file information and the last patients' operations were performed in December 2015. This retrospective study's information was collected in May 2016. This study included fifty female patients with the mean age of 58.14±13.32 (range: 34 to 79 years) who were operated by the same surgeon at Uşak State Hospital and Adnan Menderes University Medical Faculty Hospital between May 2013 and December 2015. The mean follow-up period was 22.06±9.12 months (range: 6 to 36 months). The grading of spondylolisthesis in the patients were made according to Meyerding classification. Twenty nine patients had grade 1 spondylolisthesis and 21 patients had grade 2 spondylolisthesis. Four patients had spondylolisthesis at L3-4 and above, 16 patients had spondylolisthesis at L4-5 and 17 patients had at L5-S1. Thirteen patients had spondylolisthesis at 2 or more levels. Twenty nine patients had single or multiple

chronic disease. The chronic diseases of the patients were hypertension, Chronic Obstructive Pulmonary disease, hypothyroidism, primary biliary cirrhosis, chronic kidney failure, osteoporosis, osteopenia, type 2 diabetes, rheumatoid arthritis, epilepsy, and ovarian carcinoma. Four (8%) patients had isolated radicular pain, 10 (20%) patients had isolated lower back pain, 36 (72%) patients had the combination of lumbar and leg pain. Fouteen (28%) patients had motor and sensory deficits, 11 (22%) patients had isolated sensory deficits, 25 (50%) patients had no any motor or sensory deficit. Eight (16%) had a 0-12 months follow-up period, 20 (40%) patients had a 13-24 months follow-up period, 22 (44%) patients had a 25-36 months follow-up period.

Surgical Technique

Informed concsent forms were obtained from the all patients and their relatives before the surgical procedure. The detection of the spinal level was made using fluoroscopy after the patients were prepared in the prone position. Then iliolumbar muscles were bilaterally opened up to the transverse processes via the traditional posterior midline approach. During surgery, automatic retractor was used in order to rule out skin, subcutaneous and muscle tissue. After the spinal level was determined again with fluoroscopy, secondary stenosis and foraminal stenosis were treated using Kerrison Rong or ultrasonic bone shaver and then total laminectomy and bilateral foraminotomy were performed. The disc fragment was removed if any. The areas for fusion were formed by scraping the surfaces of the facet joints and the transverse processes using a drill after discectomy. Then posterolateral fixation was made using fluoroscopy and bilateral rods were placed. After fixation, the bones which were removed after laminectomy were converted to chip-shaped. Then they were mixed with rifamycin and were placed between the transverse processes previously formed for PLF. The layers were closed in accordance with the anatomy by placing a hemovac drain on distance.

Intraoperative and Postoperative Complications

After 1 patient had the inc reased radicular complaints during PLFand fixation, a lumbar 3D computed tomography (CT) was performed. The patient was operated again upon the determination of the screw malposition on lumbar CT. The dural tear occurred in two patients during laminectomy but they were treated with primary suture and bed rest. Her complaints were started again after lifted a load of 25 kg on the 1st postoperative year. Then a lumbar CT was performed and the patient was operated again upon bilateral S1 rods were pull out on lumbar CT (Figure 1A-D). Unenhanced and contrast-enhanced lumbar magnetic rezonans imagining were performed upon 1 patient had fever on the 6th postoperative day. The antibiotic treatment was started and was given i.v. for 14 days upon there was a contrast enhancement in the area of operation on lumbar CT. The root anomaly was seen in 2 patients in the intraoperative period. One patient had unilateral fracture of S1 screw after 1 year but we recommended follow-up because the patient had no any complaint.

The Post-operative Medication of the Patients

The infusion of tramadol was administered to the patients for the first 3 days in the postoperative period. Tramadol 50 mg 2 times per day and amoxicillin-clavulanic acid 1000/125 mg 2 times per day were administered to the patients between the 3rd and 10th postoperative day. All patients were mobilized out of

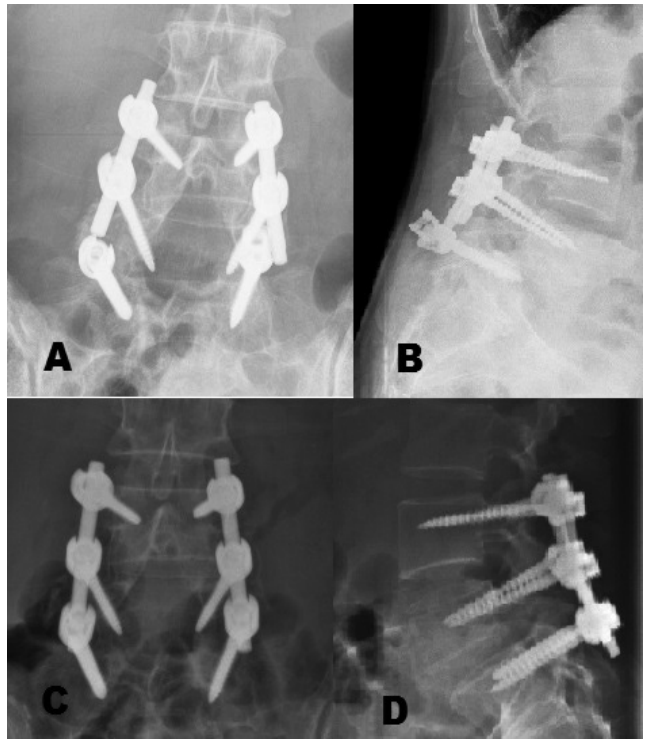

Figure 1. A and B; bilateral rod pull out owing to non-fusion after lifted a load of 25 kg (anteroposterior X-ray and lateral X-ray), C and D; anteroposterior and lateral X-ray after revison surgery

bed with brace on the 1st postoperative day. The drain was pulled out on the 4th postoperative day and the patients discharged on 4th or 5th postoperative day on condition that they use brace for 3 months.

The Evaluation Fusion and Pain

The patients were evaluated radiologically by lumbosacral X-ray and lumbar CT. On lumbosacral X-ray, fusion was evaluated as the formation of a solid bone bridge in the region including one upper and one lower segment of the spinal segment with spondylolisthesis and the transverse processes. On postoperative lumbar CT, fusion evaluated as the development of a solid bone bridge in the region including the transverse and superior articular processes in the spinal segment with spondylolisthesis (Figure 2A, 2B), (Figure 3A, 3B). The patients were evaluated for fusion by X-ray in the first one year and by 3D CT in later years. The patients were evaluated with the VAS and ODI pain scores in the preoperative and postoperative controls.

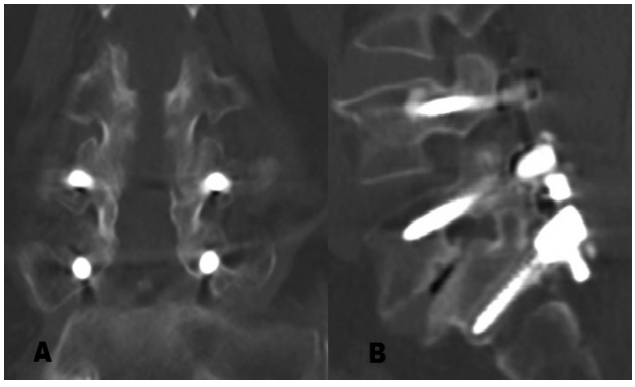

Figure 2. The expected fusion at coranal (A) and sagittal (B) 3D lumbar computed tomography view after 22 months later surgery

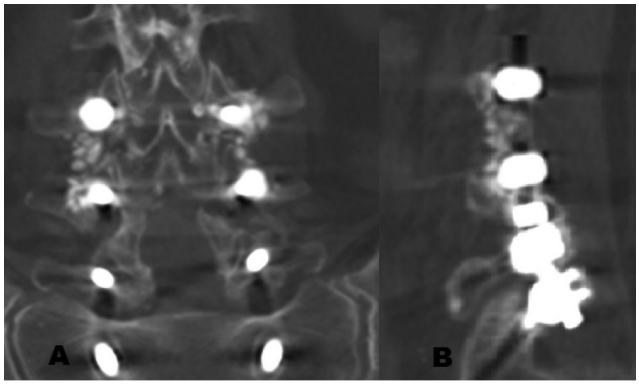

Figure 3. The unexpected fusion (pseudoarthrosis) at coranal (A) and sagittal (B) 3D lumbar computed tomography view after 12 months later surgery

Statistical Analysis

The Kolmogorov-Smirnov test was used to test whether the data were normally distributed. According to Kolmogorov-Smirnov test, preop ODI, postop ODI, preop VAS and postop VAS were non-normally distributed but age and follow up time were normally distributed. We used nonparametric test for non-normally distributed variables and median (25th-75th percentiles) were given for descriptive statistics. For normally distributed variables mean ± standard deviation were given. To evaluation of effect of posterolateral fixation on preop ODI, postop ODI, preop VAS and postop VAS we used the Wilcoxon test and to evaluation of effect of fusion on preop ODI, postop ODI, preop VAS and postop VAS. We used Mann-Whitney U test for effects of patients' preop BMI which was <30 or ≥30 on preop ODI and preop VAS scores. For fusion and chronic disease variables, descriptive statistics were given as frequency and percent. Chi-square test was used to determine whether there was a relation between fusion and chronic disease. P<0.05 was accepted significiantly.

Results

When the pain scores of the patients who underwent segmental instrumentation were compared in the preoperative and postoperative periods, there was the significant decrease in pain scores in the patients with posterior fixation (the ODI and VAS scores in the preoperative and postoperative periods), respectively, 84 (73-90), 18 (12-24) and 8 (8-9), 2 (1-3) (p<0.001) (Table 1). While fusion occurred in 66% of the patients (Table 2), it did not occur in 34% of the patients (Table 3). The ODI and VAS scores of the patients with fusion and non-fusion were compared in the preoperative and postoperative periods [the ODI and VAS scores in the patients with fusion (n=33)], respectively, 84 (78-89), 16 (12-24) and 9 (8-10), 2 (1-3) (p<0.001), the ODI and VAS scores in the patients with non-fusion (n=17), respectively, 74 (65-91), 22 (16-34) and 8 (8-9), 1 (1-4) (p<0.001), (Table 3). Screw loosening occurred most frequently at the S1 level. Moreover, when the impact of chronic disease was evaluated on the formation of fusion, fusion occurred in 17 (51.5%) of 29 patients with chronic disease but in 16 (48.5%) of 21 patients without chronic disease. This results showed that the presence of chronic disease had no negative effect on the formation of fusion (p=0.321)

(Table 4). When the average BMI was considered as 30 and the preoperative and postoperative pain scores were compared between the patients with a BMI <30 or ≥30 body, there was no a statistically significant difference between the two groups in terms of patient satisfaction. The average operation time was 3.5 hours, the average amount of blood used was 550 cc and the average length of stay in hospital was 5 days. There were no permanent motor and sensory deficits in patients after the surgery.

Table 1. Comprasion of preoperative and postoperative value of Oswestry Disability index and visual analogue score

	Preop	Postop	p
ODI	84 (73-90)	18 (12-24)	<0.001
VAS	8 (8-9.25)	2 (1-3)	<0.001

ODI: Oswestry Disability index, VAS: Visual analogue score

Table 2. Comprasion of preopoperative and postoperative value of Oswestry Disability index and visual analogue score in patient who developed fusion

	Preop	Postop	p
ODI	84 (78-89)	16 (12-24)	<0.001
VAS	9 (8-10)	2 (1-3)	<0.001

ODI: Oswestry Disability index, VAS: Visual analogue score

Table 3. Comprasion of preoperative and postoperative value of Oswestry Disability index and visual analogue score in patient who developed non-fusion

	Preop	Postop	p
ODI	74 (65-91)	22 (16-34)	<0.001
VAS	8 (8-9)	1 (1-4)	<0.001

ODI: Oswestry Disability index, VAS: Visual analogue score

Table 4. Comprasion of fusion rate in patient who have chronic diseases or not

Chronic disease	Fusion		p
	Yes	No	
Yes	17 (51.5%)	12 (70.6%)	0.321
No	16 (48.5%)	5 (29.4%)	

Discussion

When looking at the results of our study, posterolateral fixation and fusion which is one of the surgical treatments of spondylolisthesis was observed to be an effective treatment in the medium and short term period in female patients in terms of patient satisfaction.

In the literature, many different surgical procedures have been described in terms of surgical treatment of spondylolisthesis (10-13). The main purpose of these surgical procedures is to eliminate pain caused by instability, to remove compression causing neurological deficits in patients, to restorate normal spinal alignment by correcting the sagittal imbalance secondary to spondylolisthesis and to prevent the progression of spondylolisthesis by advancing the areas of fusion in medium and long term. The PLF is presented as a gold standard method in spondylolisthesis by some authors because pedicle screw fixation systems have advantages such as theoretically the reduction of spondylolisthesis and the correction of deformity and also have been reported to increase fusion rates of rigid fixation (7,14-16). However, the extra cost of treatment, a wide surgical area, the presence of neurological or vascular complications related to the pedicle screw, an increase in the degeneration of the adjacent level, and the speculations about the increased pseudarthrosis rate with the rigid fixation are reported as the disadvantages of this method (7). In analysis of the studies of the literature, when fusion was added to posterolateral fixation, fusion rate was seen in approximately 81%-100% and rate of clinical success was seen in 60%-98% (17). In our study, fusion rate has remained at 66% level. There can be many reasons for this. When our patient group is analyzed, the patient group with a low fusion rate often consisted of elderly patients with a multi-level spondylolisthesis. We think that the reason for low fusion rate in this patient group was the loosening of pedicle screws and the deterioration of the initial state of rigid fixation due to osteoporosis in these patients. The deterioration of rigid fixation can cause micro-instability in the spine. In this condition, fusion rate may fall further in patients with osteoporosis. Therefore anterior column-assisted surgical treatment options may be preferable to provide an increase in the rate of fusion

and to gain sufficient time for fusion in this patient group. The other possible factor is that the study group included the patients in the early postoperative period (1 year and not more). Moreover, another possible factor is that the differences in lifestyles, diet and genetic factors of the patients may have an effect on fusion.

The reason of lower back pain which is the most common complaint in patients with spondylolisthesis is instability associated with axial loading or rotation movement at the level of spondylolisthesis. When looking at the studies related to posterior fixation and fusion in the literature, a significant improvement was seen in VAS and ODI scores of patients in the early and medium term (18-20). In our study, when the ODI and VAS scores of the patients with fusion and non-fusion were compared in the preoperative and postoperative periods, the ODI and VAS scores were statistically significantly lower in the postoperative period than the preoperative period. Although the similar results in the patients with fusion and non-fusion seem like a paradox, in a study performed by Tsutsumimoto et al. (21) in a literature, when looking at short and medium term results of patients with fusion and non-fusion after PLF, there was no statistically significant difference between them. In the same study, they reported that age, comorbid conditions, fusion levels, gender, preoperative Japon Orthopedic Association score score and preoperative degree of slipping did not affect medium and short term clinical outcomes. A similar study was performed by Fischgrund (16), they reported 83% good results in patients who underwent instrumentation fusion and developed pseudarthrosis. These studies show that fusion status does not affect the clinical outcome of patients in the short and medium term in patients who underwent PLF without instrumentation. However, when clinical results of patients who underwent posterior fixation and fusion were compared with clinical results of patients who underwent laminectomy (without instrumentation) and fusion, the results were reported to be better in group with posterior fixation and fusion than group with laminectomy (without instrumentation) and fusion. In the same study, the poor clinical results of patients were shown to be an indicator of the progression of the degree of slip (17,22). These studies suggest that rigid fixation and fusion increase patient satisfaction. However, it was reported that pseudarthrosis may develop in 5-25% of patients who underwent PLF and 50% of patients with pseudarthrosis may be asymptomatic (23). Moreover, it was reported that short-term results of dynamic systems which have been started to be used in degenerative spondylolisthesis in recent times were successful and similar to the results of fixation and PLF (24,25). We think that short-term results of studies related to dynamic system may explain that patients develop pseudarthrosis but are asymptomatic patients. This is because dynamic fixation systems allow partial movement in the sagittal plane although they do not create instability as in the preoperative period. This situation occurs especially in patients who develop pseudoarthrosis around screw. This is because pseudarthrosis around screw may lead to a partial disruption of rigid structure of posterolateral fixation and so it may allow partial movement by acting as dynamic systems.

In the literature, it was reported that the success rates of fusion decreased and the risk of pseudoarthrosis increased in chronic diseases such as smoking, osteopenic bone structure, thyroid and growth hormone deficiency, chronic renal failure (26,27). When our case series are analyzed, it is observed that chronic disease did not affect the formation of spinal fusion. In fact, this may seem like a reverse situation with literature. However, when our case series are analyzed, the patients with low fusion rate had end-stage renal failure and osteoporosis. Fusion rate is observed to be increased in patients who were diagnosed with hypothyroidism and then treated with hormone replacement. Similarly, in our case series, although there is no any problem in the formation of fusion in diabetic patients with proper glycemic control, when a secondary chronic disease such as osteoporosis is added to chronic diseases in these patients, the success fusion rate is seen to be decreased. This situation suggests that fusion rate may be close to normal in patients with hormone replacement therapy. In our case series, fusion success rate is seen to be decreased in age-related osteoporosis in female patients.

Another result of our study is that there was no statistically difference between BMIs groups of the patients and preoperative and postoperative complaints of the patients. In the literature, a study performed by Rhin et al. (28), the average BMI was considered as 30 and also the preoperative and

postoperative results were compared between the patients with a BMI <30 and ≥30 body in terms of patient satisfaction. In this study, it was reported that a decrease in the complaints after spinal fusion in obese patients was similar to that in non-obese patients.

Conclusion

Consequently, it was demonstrated in this study that posterolateral fixation and fusion are an efficient treatment method in the medium and short term in female patients with spondylolisthesis in terms of patient satisfaction. Although fusion rates in female patients were similar to that in the literature, when a secondary chronic disease such as osteoporosis associated with aging was added to impair bone quality, fusion success rate was seen to be decreased in female patients with chronic diseases such as diabetes mellitus and hypothyroidism. Therefore, the different treatment strategies have been revealed to be required in patients with osteoporosis.

Ethics

Ethics Committee Approval: Retrospective study.

Informed Consent: Informed consent was obtained from the patients.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

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

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Original Article / Özgün Araştırma

The Comparison of Different Dimension Reduction and Classification Methods in Electroencephalogram Signals

Elektroensefalografi Sinyallerinde Farklı Boyut Indirgeme ve Sınıflandırma Yöntemlerinin Karşılaştırılması

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Keywords
Electroencephalogram, discrete wavelet transformation, Principal component analysis, Independent component analysis, Support vector machine, Linear discriminant analysis

Anahtar Kelimeler
Elektroensefelogram, ayrık dalgacık dönüşümü, temel bileşenler analizi, bağımsız bileşen analizi, destek vektör makinesi, doğrusal ayırma analizi

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Abstract

Objective: Electroencephalogram (EEG) signals have been broadly utilized for the diagnosis of epilepsy. Expert physicians must monitor long-term EEG signals that is sometimes difficult and time consuming process for epilepsy diagnosis. In this study, classification performances of support vector machine (SVM) and linear discriminant analysis (LDA), which are widely used in computer supported epilepsy diagnosis, were compared by using wavelet-based features of extracted from EEG signals which were derived in either normal or inter-ictal periods. In addition, principal component analysis (PCA) and independent component analysis (ICA) were used to determine the effects of dimension reduction on classification success.

Materials and Methods: The EEG data were sampled from the EEG laboratory of the Department of Neurology and Clinical Neurophysiology in Adnan Menderes University. Study was approved by Local Ethics Committee with protocol number 2016/873. Ten patients with epilepsy and 10 normal were the study group. EEG signals of patients with epilepsy were contains only seizure free- epochs. EEG signals were first decomposed into frequency sub-bands by using discrete wavelet transform (DWT) and then some statistical features were calculated from those to classify it's as normal or epileptic.

Results: In classification of the EEG signals, it's as normal or epileptic, we achieved 88.9% accuracy rate using SVM with radial basis function (RBF) kernel without dimension reduction.

Conclusion: Results showed that SVM was a powerful tool in classifying EEG signals if it's normal or epileptic.

Öz

Amaç: Bu çalışmada, epileptik ve epileptik olmayan elektroensefalografi (EEG) sinyallerinden elde edilen özniteliklerin boyutlarının temel bileşenler analizi ve bağımsız bileşenler analizi yöntemleri ile indirgenmesinin sınıflandırma başarısı üzerine etkilerinin belirlenmesi ve doğrusal ayırma analizi ile destek vektör makinesi (DVM) yöntemlerinin sınıflandırma performanslarının karşılaştırılması amaçlanmıştır.

Gereç ve Yöntemler: Çalışmaya 10 kontrol ve uzman hekim tarafından epilepsi tanısı konmuş 10 hasta olmak üzere toplam 20 kişi dahil edildi. Epilepsi tanısı konmuş

hastalardan alınan EEG kayıtları nöbet geçirmedikleri sırada alınan kayıtlardı. Epileptik ve epileptik olmayan sinyalleri sınıflandırmak için ayrık dalgacık dönüşümü ile sinyallerinin spektral analizi gerçekleştirildi ve sınıflandırmada kullanılacak olan öznitelikler elde edildi. Öncelikle özniteliklerin boyutu indirgenmeden, daha sonra temel bileşenler analizi ve bağımsız bileşenler analizi ile indirgenerek sınıflandırma yapıldı. Sınıflandırma doğrusal diskriminant analizi, lineer ve radyal tabanlı çekirdek fonksiyonlarının kullanıldığı DVM yöntemleri ile gerçekleştirildi.
Bulgular: EEG sinyallerinin epileptik ya da normal olarak sınıflandırılmasında radyal tabanlı çekirdek fonksiyonunun kullanıldığı DVM ile %88,9 doğruluk oranı elde edildi.
Sonuç: DVM yönteminin, epileptik ve normal sinyalleri ayırt etmede kullanılabilecek güçlü bir yöntem olduğu sonucuna varıldı.

Introduction

Electroencephalogram (EEG) is a recording of brain electrical oscillations from the scalp by using surface electrodes (1). Clinically, EEG is particularly helpful for epilepsy. In addition to clinical history and imaging studies, EEG findings help us to determine the diagnosis and the types of epilepsy (1,2). It is also helpful for the treatment of the disease (3). Epilepsy goes with seizures that occur in a sudden and unexpected nature. Mostly, seizures don''t observe during the event by the medical staff. When they are not observed or having described an atypical features, EEG helps us to reveal whether it is a seizure or not. In general, analysis of EEG signals done by expert physicians by visual analysis. Visual recognition of epileptic waveforms is sometimes difficult and time consuming for physicians who especially have not got enough expertise (4). There is also inter-reader differencesare also inter-reader differences during the visual analysis and it suggests that the visual analysis could be insufficient. With that reason, new computer evaluation techniques are developed and performed in healthy and diseased individuals (5-13). Most of these studies consist of two steps: feature extraction from the EEG signals and then classification of these features. In many study, extracted signals are derived at the time of seizure and at normal periods. So, classification performance of these studies were performance of these studies was quite high (9,11-21). In fact, visual assessment might be sufficient at that time. In that point, the question arise that whether there is an advanced statistical techniques clearly differentiate the normal and the patients with epilepsy while there is no seizure. In this study, our aim was to classify normal and epileptic patients by using their EEG data sets that derived from the archives of patients who known as epileptic (without a seizure activity at that time) and normal.

Materials and Methods

The EEG data were sampled from the EEG laboratory of the Department of Neurology and Clinical Neurophysiology in Adnan Menderes University. Study was approved by Adnan Menderes University Local Ethics Committee with (protocol approval number: 2016/873). The EEG data were recorded by Micromed EEG device (16 channels). Ten patients with epilepsy (5 male - 5 female, mean age 34±4 years) and 10 normal (5 male - 5 female, mean age 35±5 years) were the study group. EEG signals of patients with epilepsy were contains only seizure free- epochs. Nine mm, round, golden-cup electrodes were placed according to 10-20 international electrode placement system (Figure 1). Sampling frequency was 256 Hz. Reference montage (A1 and A2) measurements were derived from each channels and the duration of epoch was 30 seconds. In both group, each EEG datum was added one another and a single EEG data that was 300 seconds long were obtained. Thus each

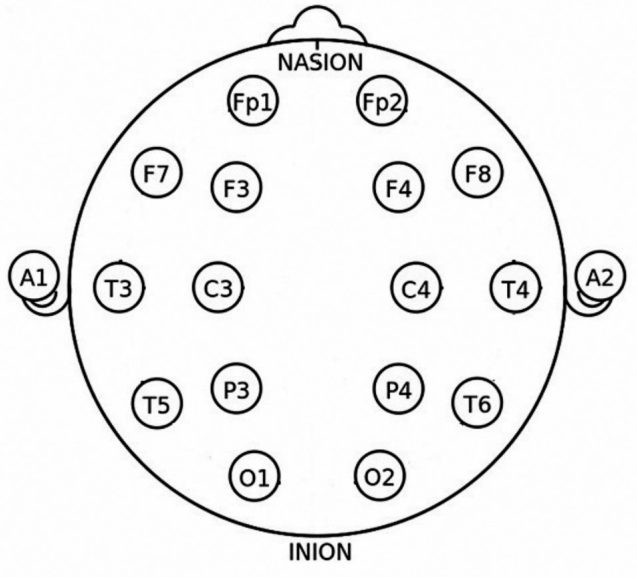

Figure 1. Electrode placement for 16 channels

channel consisted of total 76800 samples and then for each of those channels, 30 rectangular windows were formed which consists of 256 discrete data. Finally; total of 600 EEG segments, 300 epileptic and 300 normal, were obtained. EEG data was retrospectively collected.

2.1 Extracting Features with Discrete Wavelet Transform

Most of the biological signals like EEG are non-stationary signals. In other words, the amplitude, phase, and frequency of EEG signals are constantly changing signals. Various methods are used to analyse changes in the EEG signal (5). Wavelet transform (WT) is one of the most common methods which is used for time-frequency analysis of EEG signals. WT provides optimum time-frequency resolution over all frequency ranges (22). Therefore it has been widely used to provide a quantitative measure of the frequency distribution of the EEG and detect the presence of particular patterns (6).

WT analysis can be classified as two types: Continuous wavelet transform (CWT) and discrete WT (DWT). CWT is obtained by taking a projection of the signal to the functions created by scaling and shifting of a mother wavelet function. The mother wavelet function is a prototype function used to generate wavelets (23). According to the principle, the CWT is defined as:

$$CWT(s,\tau) = \frac{1}{\sqrt{s}} \int_{-\infty}^{+\infty} x(t)\psi\left(\frac{t-\tau}{s}\right) dt$$

where x(t), ψ, s and τ denote the signal to be processed, the wavelet function, scaling and shifting parameters, respectively. The different window functions used for the transformation are derived by shifting and scaling the mother wavelet. The shifting parameter τ changes the position of the window function on the signal. Hence the window moves on the signal. The scale parameter s expands or contracts the window function. Large values of s are suitable for general views and small values are suitable for detailed views. 1/√s is the normalization multiplier that ensures that the energy is the same for all values of s (13,22).

Calculation of the wavelet coefficients for every possible scale causes unnecessary information to be received from the signal. Moreover it takes a long time (24). If the scaling and shifting parameters are chosen as powers of 2, the analysis becomes more effective and faster (25). This method is called DWT and can be defined as:

$$DWT(j,k) = \frac{1}{\sqrt{2^j}} \int_{-\infty}^{+\infty} x(t)\psi\left(\frac{t-2^j k}{2^j}\right) dt$$

where the parameters s and τ are replaced by 2^j and 2^j k.

In the DWT, the signal is decomposed into approximation and detail coefficients at the first level by using low and high pass filters (Figure 2). Then the approximation coefficients are further decomposed into next level of approximation and detail coefficients (26,27).

It is very important to determine the appropriate wavelet function and the level of decomposition. The level of decomposition is chosen based on the dominant frequency components of the signal (13).

In this study DWT was employed to decompose the EEG signals into different frequency bands for different wavelet functions. Due to its high success the Daubechies wavelet order in 4 (Db4) was used to construct the feature vectors (13,15). Since the EEG signals do not have any useful frequency components above 30 Hz, the number of levels was chosen to be 6. After decomposition, D1-D6 details and A6 approximation coefficients were obtained (Figure 3).

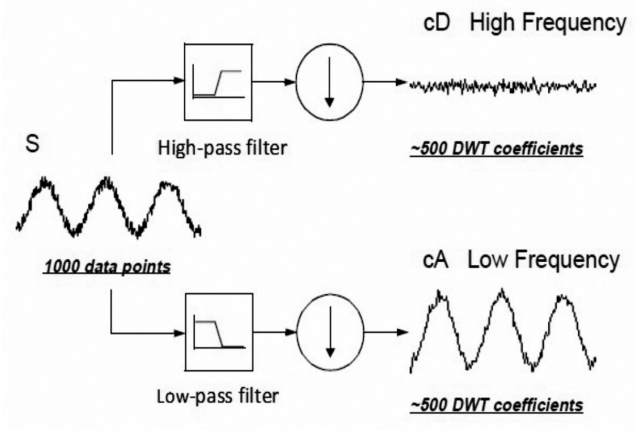

Figure 2. Decomposition of a signal into approximation and detail coefficients
DWT: Discrete wavelet transform

The extracted wavelet coefficients give a compact illustration that shows the energy distribution of the EEG signal in time and frequency. To characterize the EEG signals, statistics over the set of the wavelet coefficients were calculated (5, 6, 13, 27, 28). The following statistics were calculated from the wavelet coefficients:

1. Mean of the absolute values of the coefficients in each sub-band $\left(\frac{\sum|x_i|}{n}\right)$

2. The minimum value of the coefficients in each sub-band $\left(\min(x_i)\right)$.

3. The maximum value of the coefficients in each sub-band $\left(\max(x_i)\right)$.

4. Standard deviation of the coefficients in each sub-band $\left(s=\sqrt{\frac{\sum(x_i-\bar{x})^2}{n-1}}\right)$

5. Entropy of the coefficients in each sub-band ($S=\sum p_x \log p_x$).

6. Energy of the coefficients in each sub-band ($E=\sum|x_i|^2$).

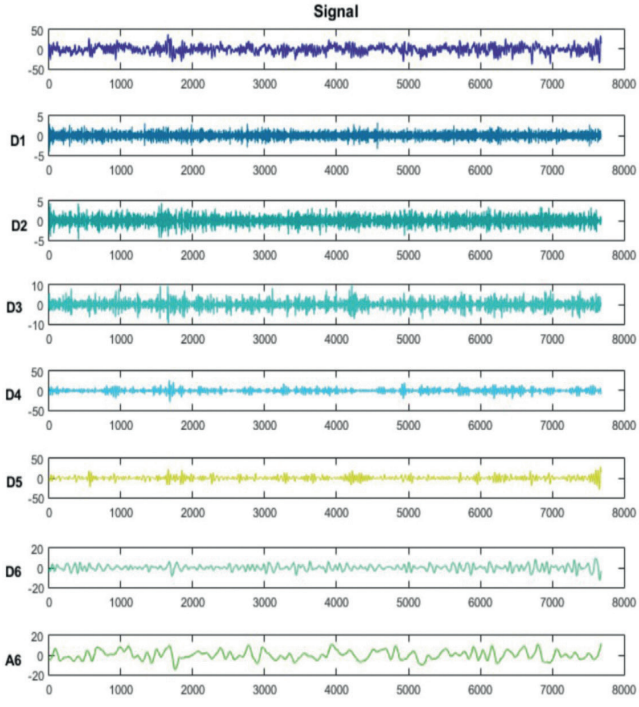

Figure 3. Approximate and detailed coefficients of epileptic electroencephalogram signal after decomposition

Since the frequency components above 30 Hz is lack of use in epilepsy analysis, the features were extracted from D4 (16-32 Hz), D5 (8-16 Hz), D6 (4-8 Hz) detail coefficients and A6 (0-4 Hz) approximation coefficients. Thus 24 statistical features were obtained from each channel. In total 384 features were obtained from 16 channels and normalized in [0,1].

Dimensionality Reduction of Features

Principal Component Analysis

Principal component analysis (PCA) is a transformation technique that reduces the dimension of p-dimensional data set containing correlated variables to a lower dimensional space containing uncorrelated variables while preserving the existing variability in the data set as much as possible. The variables obtained by the transformation are called the principal components of the original variables. The first principal component captures the maximum variance in the data set and the others capture the remaining variance according to decreasing order (29,30).

The number of principal components that can be obtained for p number of variables is at most p, and the principal components are formed as linear combinations of variables (29). A linear combination of any **x** random vector can be expressed as:

$$\mathbf{y_1} = \sum^{p} a_{k1}\, x_k = \mathbf{a}_1'\mathbf{x}$$

where $a_{11}, a_{21},\ldots,a_{p1}$ are the weighting coefficients of the weight vector $\mathbf{a}_1$ and $\mathbf{y}_1$ represents the first principal component. The variance of $\mathbf{y}_1$ depends on the norm and direction of $\mathbf{a}_1$. As the norm of a_1 increases, the variance of $\mathbf{y}_1$ will also increases. Therefore it is aimed to obtain the maximum variance by introducing a constraint such that the norm of $\mathbf{a}_1$ is 1. Under this constraint, the variance of the first principal component expressed as (29):

$$Var(\mathbf{a}_1) = E[\mathbf{y}_1^2] = \mathbf{a}_1' E[\mathbf{x}\mathbf{x}']\mathbf{a}_1 = \mathbf{a}_1'\mathbf{C}_x\mathbf{a}_1$$

where $\mathbf{C}_x$=E[**xx'**] denotes covariance matrix. The result that maximizes Var($\mathbf{a}_1$) is obtained by calculation of eigenvectors $\mathbf{v}_1,\ldots,\mathbf{v}_n$ corresponding to eigenvalues $\lambda_1,\ldots,\lambda_n$ ($\lambda_1 \geq \cdots\cdots \geq \lambda_n$) of $\mathbf{C}_x$ matrix. The first principal component is expressed as (29):

$$\mathbf{y}_1 = \mathbf{v}_1'\mathbf{x}$$

The second principal component with the constraint that is uncorrelated to the first principal component (E[$\mathbf{y_1 y_2}$]=0) is expressed as (29):

$$\mathbf{y}_2 = \mathbf{v}_2'\mathbf{x}$$

By this way, the mth principal component such that 1≤ m ≤ p and E[$\mathbf{y_k}$ $\mathbf{y_m}$]=0 (k≠m) is expressed as (29):

$$\mathbf{y}_m = \mathbf{v}_m'\mathbf{x}$$

PCA has been frequently used in studies on epilepsy diagnosis with EEG signals (13, 31, 32).

Independent Component Analysis

Independent component analysis (ICA) is a statistical method that tries to distinguish between multiple randomly mixed signals without knowing the mixing mechanism. ICA assumes that each measured signal is a linear combination of independent signals. It decomposes multidimensional data vector linearly to statistically independent components (5). The mixing model can be written as:

$$\mathbf{x=As}$$

where x denotes the random vector whose elements are the mixtures $x_1,\ldots,x_n$, and s denotes the vector of the original source signals with elements $s_1,\ldots,s_n$, and A denotes the mixing matrix with elements a_{ij} (33). In equation (8) neither A nor s are known. If a matrix W can be found as the inverse of of A, the original source signals can be estimated. The estimated signals can be expressed as:

$$\mathbf{y=Wx}$$

where y denotes the vector whose elements are the estimations of the original source signals (33). A number of algorithms have been developed for estimating W. One of these algorithms is the fast fixed-point algorithm (FastICA) developed by Hyvärinen (34). FastICA provides fast convergence, easy to apply and reliable results. In this study FastICA algorithm was used to estimate the W.

Classification of Features

Linear Discriminant Analysis

The goal of linear discriminant analysis (LDA) is to derive a discriminant function to maximize the difference between the groups. In LDA, the number of discriminant functions is determined according to the number of the groups. If there are two groups, then one discriminant function is used. A discriminant function consists of a linear combination of predictors. The weights of the predictors are calculated such that the ratio of the variance between classes to the variance within class is maximized. The discriminant function for two groups and p predictors expressed as:

$$D=w_0+w_1 X_1+w_2 X_2+\cdots\cdots+w_p X_p$$

where w_0, w_i and X_i (i = 1,…,p) denote the constant, the weights of the predictors and the predictors, respectively.

Support Vector Machine

In machine learning, support vector machines (SVMs) are supervised learning models with associated learning algorithms that analyse data used for classification and regression analysis (35).

SVM aims to find the best separating hyper plane (optimal hyper plane), with the maximum distance between observations in the two classes. The basic support vector classifier for linear separable data is shown in Figure 4. Where w is the normal of optimal hyper plane, b is bias and x is the features vector. The optimal hyper plane ($\mathbf{w^T x}$+b=0) divides the plane into two sets depending on the sign of $\mathbf{w^T x}$+b (36).

SVM maps the data that cannot be separated linearly into a higher dimensional space in which they can be separated linearly by using an appropriate kernel function (Figure 5) (9, 35, 37).

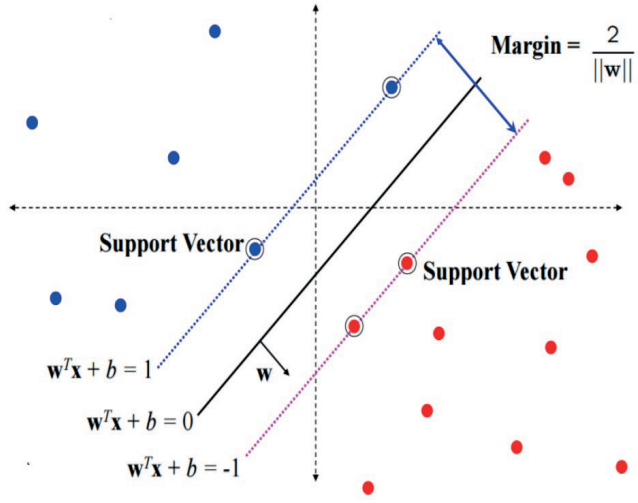

Figure 4. The linear support vector classifier

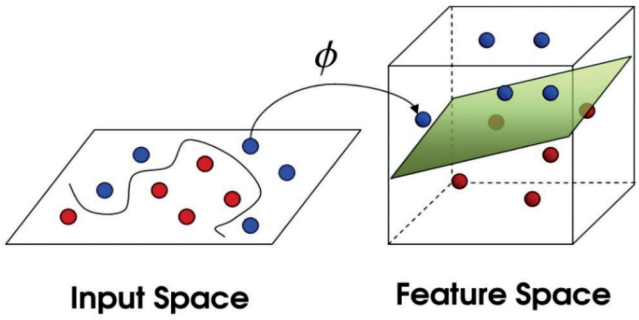

Figure 5. Non-linear support vector classifier

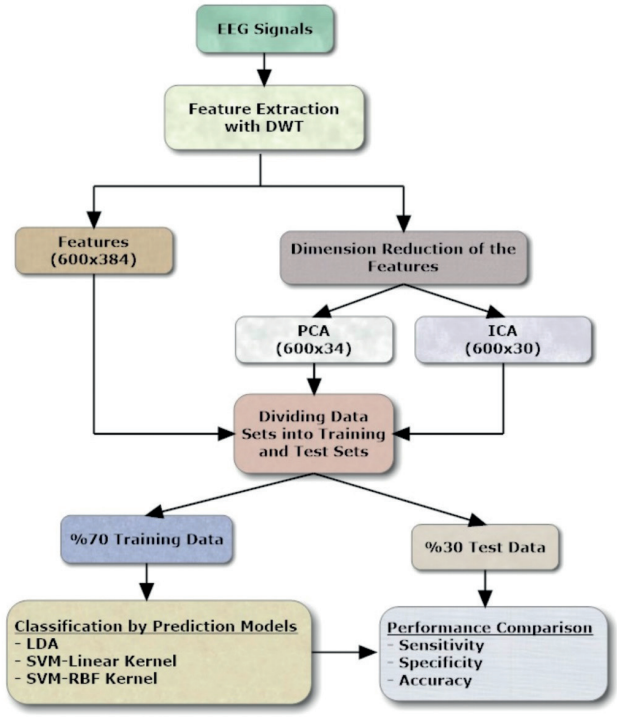

Figure 6. Diagram of the application process
EEG: Electroencephalogram, DWT: Discrete wavelet transform, SVM: Support vector machine, LDA: Linear discriminant analysis, PCA: Principal component analysis, ICA: Independent component analysis

In this study, the linear kernel: $K(x_i,x_j)=x_i.x_j$ and the radial basis function (RBF) kernel: $K(x_i,x_j)=\exp(-\gamma ||x_i-x_j||^2)$ were used as the kernel functions.

The hyper parameters C and γ of the SVM classifier were determined through a 10-fold cross-validation grid search performed and the parameters giving the highest accuracy were used in SVM classifiers.

Results

A comparison of both the effects of PCA and ICA on the classification performance and the classification performances of LDA and SVM with linear and RBF kernels was done on the EEG data that derived from the normal and patients with epilepsy. Classifications were performed for three different feature matrices: (a) features without dimension reduction (384 features), (b) features being reduced by PCA (34 features) and the and (3) features being reduced by ICA (30 features). The eigenvalues-greater-than-one rule proposed by Kaiser (1960) was used to determine for the number of components. These three feature matrices were classified by using LDA, SVM with linear kernel and RBF kernel. Sensitivity, specificity and accuracy rates were used for the performance measures of the classifiers. Additionally, for all feature matrices used in the classifications, training and test data sets were randomly divided into two parts: 70% training (n=420) and 30% test (n=180) data corresponding to the same points. The process performed in the application is given in Figure 6.

In training sets; among the classifiers, SVM with RBF kernel reached to the highest accuracy rate (96.2%) at the features without dimension reduction, SVM with linear kernel reached to the highest at the (94.7%) at similar to the previous; LDA reached to the highest rate (79.8%) at the features reduced by PCA (Table 1).

In test sets, the highest accuracy (88.9%) was obtained by SVM with RBF kernel without dimension reduction. The highest accuracy (82.2%) for SVM with linear kernel was obtained with the features being reduced by PCA. The highest accuracy (78.9%) for LDA was obtained for the features being reduced by both PCA and ICA (Table 2).

Discussion

A number of studies have been done to classify EEG signals for the diagnosis of epilepsy. A direct comparison of the previous studies that using EEG signals is hard due to the variety of EEG datasets, wavelet types, decomposition levels and also the variety of the statistical features used in the classification process (15). Previously, many researchers used the same EEG data which included five sets (named as A-E) described by Andrzejak, et al. (38). Set A and B were obtained from normal, set C-E were obtained from patients with epilepsy. Set C and D were included seizure-free interval while set E were include seizure-related interval. Wavelet-based

Table 1. The classification performances of prediction models in training sets

Training data sets	Classifiers	Sensitivity (%)	Specificity (%)	Accuracy (%)
Features without dimension reduction	LDA	69.0	83.3	76.2
	SVM-Linear kernel	91.4	98.1	94.7
	SVM-RBF kernel	93.3	99.1	96.2
Features reduced by PCA	LDA	73.8	85.7	79.8
	SVM-Linear kernel	80.0	88.6	84.3
	SVM-RBF kernel	83.8	90.9	87.4
Features reduced by ICA	LDA	70.5	86.2	78.3
	SVM-Linear kernel	80.0	85.2	82.6
	SVM-RBF kernel	74.8	93.8	84.3

SVM: Support vector machine, LDA: Linear discriminant analysis, PCA: Principal component analysis, ICA: Independent component analysis

Table 2. The classification performances of prediction models in test sets

Test data sets	Classifiers	Sensitivity (%)	Specificity (%)	Accuracy (%)
Features without dimension reduction	LDA	57.8	63.3	60.6
	SVM-Linear kernel	77.7	81.1	79.4
	SVM-RBF kernel	92.2	85.6	88.9
Features reduced by PCA	LDA	67.8	90.0	78.9
	SVM-Linear kernel	78.9	85.6	82.2
	SVM-RBF kernel	83.3	82.2	82.8
Features reduced by ICA	LDA	71.1	86.6	78.9
	SVM-Linear kernel	78.8	84.4	81.7
	SVM-RBF kernel	80.0	88.9	84.4

SVM: Support vector machine, LDA: Linear discriminant analysis, PCA: Principal component analysis, ICA: Independent component analysis

features that obtained from these sets (Set E and the others) were used to assess the performances of classifiers and to detection of a seizure activity. Nearly or exactly 100% accuracy rates were obtained with different classification methods. Among them, Xie and Krishnan (39) used k-nearest neighborhood method, Kumar et al. (11) used artificial neural network, Das et al. (9) used SVM method and all of these studies accuracy rates were 100%. In these sets, there were significant differences between the signals that were derived at the time of seizure and at normal periods, and no need to use of complicated statistical methods instead of using a simple threshold value or even visual assessment. It was also difficult to say that any classification method was superior to other methods. Different from these studies, we did not use an epileptic seizure activity in our EEG data set in patients with epilepsy. As a result, classification attempts of our data were more difficult than the previous data set and showed the real discrimination ability of these methods.

Orhan et al. (40) classified the wavelet-based features that obtained from data set A and D described by Andrzejak, et al. (38) and obtained 96% accuracy rate with a multilayer perception neural network model. Subasi and Ercelebi (41) used similar data sets that we used in this study. They classified the wavelet-based features and obtained respectively 93.0% and 89.0% accuracy rates for artificial neural network and logistic regression. In this study, we also classified some wavelet-based features and got respectively 88.9%, 82.2% and 78.9% accuracy rates for SVM with RBF kernel, SVM with linear kernel, and LDA. One of the most important reasons for different accuracy rates among the studies was the using of different data sets. The ratio of epileptic abnormalities in EEG data sets was variable and it made difficult to compare of the classification studies.

There is still a debate that linear or non-linear method has more successful than each others for classifying of EEG signals. Garrett et al. (42) classified five different mental states using EEG signals and achieved respectively 66.0%, 69.4% and 72.0% accuracy rates for LDA, artificial neural network and SVM with RBF kernel. They favoured that non-linear methods were more successful than linear methods for the classification of EEG signals. Lehmann, et al. (43) did the same classification of the EEG data that derived from the Alzheimer patients and normal, and they got 91.0% and 95.0% accuracy rates with LDA and SVM with RBF kernel. Supriya et al. (19) classified the EEG data sets A and E [described by Andrzejak et al. (38)] and achieved 86.87%, 99.25% and 100% accuracy rates with LDA, SVM with linear kernel and SVM with polynomial kernel. In this study, we achieved respectively 78.9%, 82.2% and 88.9% accuracy rates with LDA, SVM with linear kernel and SVM with RBF kernel. In this context, we have achieved similar results with previous studies.

Subasi and Gursoy (13) used PCA, ICA and LDA to reduce the dimension of the features that obtained from EEG signals and compared the performances on classification success. They used SVM with RBF kernel to classify the reduced features and achieved respectively 98.7%, 99.5% and 100% accuracy rates for PCA, ICA and LDA. We used SVM with RBF kernel to classify the reduced features and for ICA we found the highest accuracy rate 84.4% and for PCA the highest accuracy rate 82.8%. Hence we found similar result with Subasi and Gursoy (13). In addition, with dimensionally reduction, both the performance of LDA and SVM with linear kernel were increased. The highest classification performances in all data sets were got by using SVM with RBF kernel. It achieved the highest accuracy rate (88.9%) for the features without dimension reduction. It was noted that the dimension reduction methods were adversely affected the performance of its.

Conclusion

In this study, the classification performances of SVM and LDA, which are widely used for computer supported diagnose of epilepsy, were compared by using wavelet-based features extracted from EEG signals. In addition, PCA and ICA were used to determine the effects of dimension reduction on the classification success. Results showed that, SVM with RBF kernel achieved the highest accuracy rate (88.9%) for the features without dimension reduction. The dimension reduction methods PCA and ICA improved classification performances of LDA and SVM with linear kernel, but decreased the classification performance of SVM with RBF kernel. Consequently, with dimensionally reduction, LDA and SVM with linear kernel perform better classifications.

Ethics

Ethics Committee Approval: Adnan Menderes University Ethics Committee. (approval no: 2016/873).

Informed Consent: It was not taken.

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Authorship Contributions

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

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Original Article / Özgün Araştırma

Fractal Analysis of Temporomandibular Joint Trabecular Bone Structure in Patients with Rheumatoid Arthritis on Cone Beam Computed Tomography Images

Konik Işınlı Bilgisayarlı Tomografi Görüntülerinde Romatoid Artrit Hastalarının Tempromandibular Eklemdeki Trabeküler Kemik Yapısının Fraktal Analizi

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Keywords
Rheumatoid arthritis, trabecular bone, cone beam computed tomography, fractal analysis

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Abstract

Objective: The aim of this study was to evaluate the fractal dimension (FD) analysis in the temporomandibular joint for changes in trabecular bone structure on cone beam computed tomography (CBCT) images of patients with rheumatoid arthritis (RA).

Materials and Methods: In this study 17 female RA patients and 16 healthy female individuals who were underwent CBCT imaging with for diagnostic purposes such as pathologic lesion and implant treatment planning, were evaluated and fractal analysis was performed using Image J (National Institutes of Health, Bethesda, MD) program with box-counting method. The results were analysed using Student t-test with SPSS 20.0 (SPSS, Chicago; IL, USA) software program.

Results: According to the results, FD values of RA patients have wide range (for right side minimum: 0.919, maximum: 1.169 and for left side minimum: 0.958, maximum: 1.155). Statistical analysis of FD values showed significance between RA patients and the healthy individuals for both right/left sides (for the right side, the RA patients had a mean FD of 1.055, and the control group had a mean FD of 1.113; for the left side, the RA patients had a mean FD of 1.060, and the control group had a mean FD of 1.111) ($p<0.05$). There was no significant different between FD values of right and left side of mandibular condyle.

Conclusion: RA patients have lower FD values than healthy individuals for each side which means bone complexity of RA patients are less. The results recommend that FD is a promising tool for detection of bone-related bone changes.

Öz

Amaç: Bu çalışmanın amacı, romatoid artritli (RA) hastaların konik ışınlı bilgisayarlı tomografi (KIBT) görüntüleri üzerinde temporomandibular eklemin trabeküler kemik yapısındaki değişiklikleri fraktal boyut (FB) analizi ile değerlendirmektir.

Gereç ve Yöntemler: Bu çalışmada, patolojik lezyon veya implant tedavi planlaması gibi tanı amacıyla KIBT görüntülemesi yapılan 17 kadın RA hastası ve 16 sağlıklı kadın birey değerlendirildi ve Image J (National Institutes of Health, Bethesda, MD)

programı kullanılarak kutu sayma yöntemi ile fraktal analiz yapıldı. Sonuçlar SPSS 20.0 (SPSS, Chicago, IL, ABD) programı ile Student t-testi kullanılarak değerlendirildi.
Bulgular: RA hastalarının FB değerleri sağda minimum: 0,919, maksimum: 1,169 ve solda minimum: 0,958, maksimum: 1,155 olarak ölçüldü. FB değerlerinin istatistiksel analizi, sağ ve sol taraflar için RA hastaları ile sağlıklı bireyler arasında istatistiksel olarak anlamlı farklılık gösterdi (sağ taraf için RA hastalarının FB ortalaması 1,055, kontrol grubunun FB ortalaması 1,113, sol taraf için ise RA hastalarının FB ortalaması 1,060, kontrol grubunun FB ortalaması 1,111'dir) ($p<0,05$). Mandibuler kondilin sağ ve sol tarafı arasında ise FB değerleri istatistiksel olarak anlamlı fark göstermemektedir.
Sonuç: RA hastalarının her bir taraf için sağlıklı kişilere göre FB değerleri daha düşüktür, bu da RA hastalarının kemik yoğunluğunun daha az olduğu anlamına gelir. Sonuçlar FB'nin RA ile ilişkili kemik değişikliklerinin saptanmasında umut verici bir araç olduğunu düşündürmektedir.

Introduction

Rheumatoid arthritis (RA) is a systemic, chronic, autoimmune, inflammatory and erosive joint disease (1). Although RA usually affects the metacarpophalangeal, proximal interphalangeal and metatarsophalangeal joints, it can also affect other joints. Temporomandibular joint (TMJ) involvement is usually seen in advanced cases (2).

Fundamental changes in the process of bone remodeling potentially lead to changes in the structural and mechanical properties of bones (1). Rheumatic inflammation reduces the bone strength and toughness of the periarticular bone by disrupting bone quality and bone mass. In RA, periarticular osteoporosis is the first morphological sign associated with the disease and is seen before erosion and narrowing of the joint space (3).

Patients with RA are at risk for secondary osteoporosis. A change in bone architecture provides information for the diagnosis of osteoporosis and a possible fracture risk (4-6).

Because the trabecular bone has a higher metabolic activity than the cortex of the alveolar bone, it is more suitable for use in evaluating changes (7). The fractal dimension (FD) of the trabecular bone structure has been proven to have a significant correlation with the physical properties of the bone (8).

FD analysis is a statistical tissue analysis used to describe complex shapes and structural patterns based on fractal mathematics (9). A small FD value indicates a larger number of gaps in the bone, while a greater FD value indicates a smaller number of voids in the bone (4,10). It has been shown that an FD increases both after the experimental demineralization of bone and after changes in bone density due to age or disease (11,12).

Various studies have been conducted to apply different methods of analyzing bone structure with panoramic radiography (13-15). Only a limited number of studies have assessed bone structure using cone beam computerized tomography (CBCT) (16-18). While the detailed trabecular structure can not be achieved due to low resolution, the texture pattern in CBCT can still provide discriminative data to separate different trabecular patterns (16). It has been shown that fractal analysis can be applied to CBCT images for evaluation of bone quality (18). CBCT is used in dentistry in many different diagnoses such as assessing the bone quality before implant placement or identifying and following a variety of bone lesion types. Clinical applications in the field of dentomaxillofacial radiology have gained importance and become widespread (19).

In this study, CBCT data and the fractal analysis method were used to evaluate and compare the trabecular bone structure changes in condyle heads of patients in the RA group with those of the control group.

Materials and Methods

Subjects

In the present study, 17 female RA patients and 16 healthy female individuals who underwent CBCT imaging for various diagnostic purposes, such as pathologic lesion and implant treatment planning, were evaluated retrospectively. None of the study participants had any congenital TMJ disorder or surgical treatment for TMJ. The RA patients ranged in age from 20-63 years (mean of 44.4 years), and the healthy individuals ranged in age from 26-64 years (mean of 42 years). The control and RA patient groups were matched according to age and gender criteria. The CBCT examinations were performed at Atatürk University Faculty of Dentistry, Department of Dentomaxillofacial Radiology. The RA patients did not have any systemic disease other than RA, and

the control group did not have any type of systemic disease.

Cone Beam Computerized Tomography Data

CBCT images were obtained by using the NewTom 3G (Quantitative Radiology, Verona, Italy) which has a maximum output of 110 kV and 15 mA, a 0.16-mm voxel size, and an approximate 5.4s exposure time as scanner parameters. In the first instance, the TMJ was examined by viewing 0.5 mm axial slices; the coronal slices were selected from the axial views on which condylar process is the widest in mediolateral dimension. The coronal slices were taken in 0.3 mm thickness and parallel to the long axis of the condylar process on the selected axial image. All CBCT images were evaluated by a single observer.

Fractal Dimension Analysis

All the condyle slices were saved as JPEG images via the NNT Viewer software program. The regions of interest (ROIs) were selected as 20x20 pixels within each image with the use of Photoshop CS6 13.0 8.0 (Adobe Inc., San Jose, CA). The ROIs, located in the middle of the coronal TMJ slice (Figure 1), were cropped by using Adobe Photoshop and saved as JPEG images. The images were then transferred to the Image J (IJ) version 1.51 program (National Institutes of Health, Bethesda, MD @; https://imagej.nih.gov/ij/download.html) (20) in order to calculate the FDs. In this study, the images were converted to binary images by using the method created by White and Rudolph (21). The ROIs were duplicated and blurred by using a Gaussian filter with a diameter of 35 pixels. The blurring process aims to eliminate all fine- and medium-scale variations in image brightness. The blurred images were extracted from the original ROIs. To reflect each of the image's variations, such as trabecular bone structure and marrow spaces, 128 value was added to the obtained images at all pixel locations. The results were converted to binary with thresholding and a brightness value of 128. The binary images were eroded and dilated on time to minimize the noise. The final images were obtained after inverting and skeletonizing the binary images. On the skeletal binary images, the skeletal structure represents the bone pattern and the non-skeletal structure depicts the bone marrow (Figure 2). The FDs of the skeletonized images were calculated by using the box counting tool on Image J 1.51 program. The images were analyzed by covering it with a square grid of equally sized tiles. The widths of the square boxes were 2, 3, 4, 6, 8, 12, 16, 32, and 64 pixels. The number of the counted squares was plotted against the number of squares in the double logarithmic scale, and the FDs were calculated from the inclination of the line fitted on the data points (Figure 3).

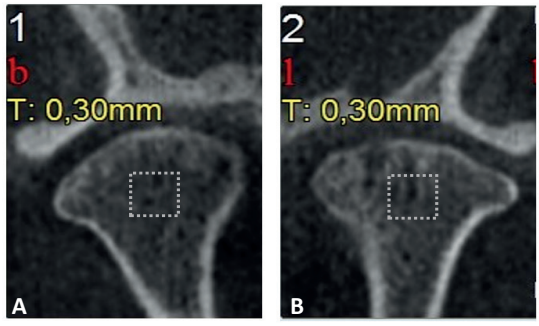

Figure 1. Coronal image of temporomandibular joint slice thickness is 0.3 mm. Regions of interest were taken from the mostly middle of the condyle and suitable place which has no cortical bone. A) right side, B) left side

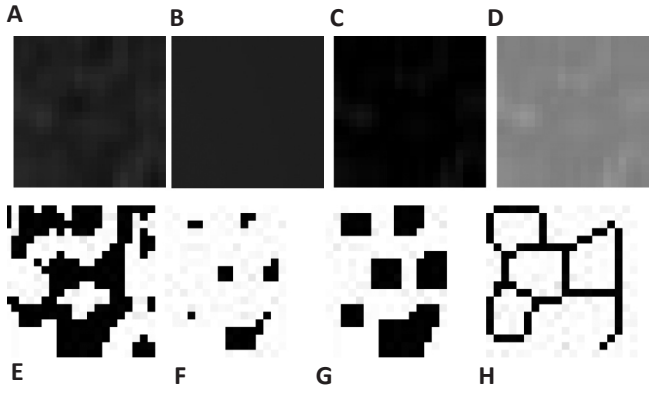

Figure 2. Image processing steps. A) Regions of interest 20x20 pixel, B) gaussian blurred image (diameter 35 pixel), C) image B was substracted from image, A D) 128 added, E) binary image, F) eroded image, G) dilated image, H) skeletonized

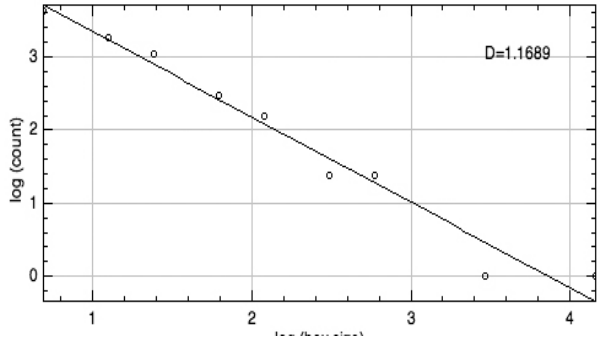

Figure 3. Fractal dimension calculation of the images with box counting method

Statistical Analysis

The FD values for both the RA patients and the control group are listed in Table 1. Statistical analyses were carried out using the SPSS 20.0 (SPSS, Chicago, IL) software program. The independent Samples t-test was used to determine the difference between FD values of the healthy individuals and the RA patients. Significance was defined as $p \leq 0.05$.

Results

Table 1 presents the FD values of every participant and Table 2 presents the mean values and standard deviations. The results indicated that RA patients had lower FDs than the healthy individuals for both the right and left sides of the condyle head, which means that RA patients had a lesser bone complexity. The FD values of the RA patients showed a wide range (for the right side, a minimum of 0.919 and a maximum of 1.169; for the left side, a minimum of 0.958 and a maximum of 1.155). A statistical analysis of the FD values showed significance between the RA patients and the healthy individuals for both right and left sides (for the right side, the RA patients had a mean FD of 1.068, and the control group had a mean FD of 1.113; for the left side, the RA patients had a mean FD of 1.060, and the control group had a mean FD of 1.111) ($p<0.05$). The FD values of the trabecular bone structure support the hypothesis of this study which is there's a significant difference between the individual trabecular bone complexity of female RA patients and females without RA.

Table 1. Presents fractal dimension values of right and left temporomandibular joints regions of interest

TMJ right side		TMJ left side	
RA patients FD	Healhty individuals FD	RA patients FD	Healhty individuals FD
1.063	1.223	1.036	1.112
1.083	1.097	1.084	1.201
1.117	1.140	1.049	1.165
1.041	1.139	1.074	1.028
1.074	1.051	0.958	1.047
1.077	1.142	1.001	1.112
1.073	1.142	1.045	1.162
0.976	1.114	1.155	1.148
1.070	1.173	1.119	1.141
1.169	1.132	1.015	1.074
1.027	1.130	0.967	1.169
1.122	1.019	1.087	1.148
1.143	1.151	1.085	1.186
1.077	1.028	1.033	0.955
1.077	1.031	1.069	1.010
0.919	1.109	1.135	1.128
1.050	-	1.112	-

TMJ: Temporomandibular joints, RA: Rheumatoid arthritis, FD: Fractal dimension

Table 2. Presents the means and standard deviations of the variables and paired Samples t-test results (p≤0.05) (group 1: Patients with rheumatoid arthritis, group 2: Healthy individuals)

Group	n	Mean	Standard deviation	Standard error of the mean	p (2-tailed)
TMJ right side					
1	17	1.06812	0.058683	0.014233	0.030
2	16	1.11381	0.056434	0.014109	
TMJ left side					
1	17	1.06024	0.055481	0.013456	0.026
2	16	1.11163	0.069962	0.017490	

TMJ: Temporomandibular joints

Discussion

RA is characterized by synovial membrane, cartilage and subchondral bone inflammation, and injury of joints that cause joint space and loss of function. Although radiographic changes occur in 60% of RA patients, these changes are not observable in the early stages of the disease (22). Periarticular osteopenia is an early and diffuse characteristic of RA and may be the first morphological change associated with the disease. At the same time, patients with RA are at risk for secondary osteoporosis (23).

Various studies have shown that fractal analysis, a quantitative tool for examining the morphology of complex structures. It can show the changes of trabecular bone structure in some diseases, such as sickle cell anemia, osteoporosis and hyperparathyroidism. According to these studies, the FD values of trabecular bone were related with differences between patient and healthy groups with respect to changes in trabecular bone architecture and bone density (21,24-26).

Alman et al. (27) and Demirbas et al. (25) reported that FD analysis was performed on spongeous bone of the second premolar and first molar teeth. Because trabecular bone has a higher metabolic activity than cortical bone, it determines bone structure changes (28). In this study, we also performed fractal analysis on the trabecular bone at the condyle head in the TMJ.

Alveolar bone shows changes in its internal structure, with a physiological adaptation capacity to accommodate the forces to which it is exposed. It is thought that the anisotropic feature of the trabecular bone and changes in the trabecular thickness cause different results in FD studies (5,25,29,30).

Although the literature states that as the FD increases, the complexity of the structure increases, there is no consensus on this subject. Differing results of FD studies are related to anatomical variations, the use of various techniques in obtaining FDs, and the examination of different regions in alveolar bone. It is also important to understand how the disease affects bone quality and how different sections of the body are affected by this condition. Additionally, it is critical to determine the criteria for choosing the ROI and the course of obtaining two-dimensional images (11,25,31) In our study, CBCT images were obtained with fixed exposure parameters by selecting standardized ROIs within the cortical borders of both mandibular condyles.

In contrast to the results of our study, some researchers, such as Ruttimann et al. (32) and Hua et al. (18) reported that FD is increased in some patients with diseases that cause osteoporotic effects on bone structure. However, Demirbas et al. (25), Southard et al. (10), Updike and Nowzari (31) and Ergun et al. (5) have reported decreased FD values. In this study, it was found that the right and left condyle head FD measurements in RA patients were significantly lower than those of the control group.

In their study of osteoporosis detection by fractal analysis of dental radiographs, Doyle et al. (33) reported that mandibular FD measurements in postmenopausal women were higher than those of premenopausal women.

Southard et al. (10) compared their own results with the results of Ruttimann et al. (32) and argued that the differences in the results may have been caused by the different analysis methods used. While there are many different approaches to the fractal analysis, the box-count is the most preferred method. (5,31) Therefore this method was chosen for this study.

Various studies have been conducted to apply several methods to analyze bone structure with panoramic radiography (9,34). Conventional radiography has disadvantages, such as limited sensitivity, in detecting early joint space narrowing and periarticular osteoporosis (35). Additionally, some studies have assessed the correlation between bone parameters obtained by CBCT or high-resolution micro-CT scans (36-40). CBCT allows direct visualization of the trabecular structure by eliminating superpositions, and the condyle head can be examined in three-dimensions. Given the clinical practice, previous studies have shown that the use of FDs in CBCT is giving promising results (17,41).

The preliminary results of Hua et al.'s (18) study showed that measuring bone area and fractal analysis had some potential for assessing bone quality in CBCT images. The results of Ling et al.'s (16) study support the use of CBCT for advanced trabecular analysis.

Study Limitations

The present retrospective pilot study has some limitations. Firstly, although CBCT provides superior diagnostic imaging for TMJ, it involves higher radiation exposure compared with conventional radiography and it is not routinely used in every patient in dentistry. Nevertheless, with consideration the disadvantage of CBCT, alternative studies can be carried out using a large number of patient data. Another limitation is that this study was carried out by CBCT images of RA patients that were stored at the Oral and Maxillofacial Radiology Department at the Ataturk University and the number of participant was limited.

Conclusion

In our study, structure of the trabecular bone in the condyle head was evaluated in RA and control group patients was evaluated by fractal analysis with CBCT data. The patients with RA included in our study were not admitted to our clinic with joint complaints. CBCT was required for various reasons. The results of this study demonstrate that osteoporosis-related changes in the condyle heads of RA patients can be identified by FD analysis on CBCT images.

Ethics

Ethical Committee Approval: Retrospective study.

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: S.Y., E.K., İ.S., Design: A.B.Y., Data Collection or Processing: E.K., S.Y., Analysis or Interpretation: S.Y., E.K., Literature Search: S.Y., Writing: S.Y., E.K., İ.S.

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

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Case Report / Olgu Sunumu

A Rare Cause of Empyema in Children: *Streptococcus pyogenes*

Çocuklarda Nadir Bir Ampiyem Etkeni: Streptococcus pyogenes

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Abstract

Empyema is the presence of purulent material in the pleural cavity, and it is a well-known and frequent complication of pneumonia in children. Despite the new antimicrobial treatment options and the different drainage techniques, parapneumonic pleural empyema still represents a significant cause of morbidity and mortality. *Streptococcus pyogenes* is an infrequent cause of empyema. In this article, we will present a four-teen years old girl with empyema who presented with severe additional clinical symptoms including hypotension and hypoxia. Streptokinase is five times table fibrinolytic therapy improved after implementation of broad-spectrum antibiotics, has been used up to six weeks. We wanted to present this case because of the challenges that we faced in the management of the disease.

Keywords
Empyema, *Streptococcus pyogenes*, fibrinolytic therapy, child

Öz

Ampiyem, plevral boşlukta pürülan sıvı varlığı olup çocuklarda pnömoninin bilinen ve sık rastlanan bir komplikasyonudur. Plevral ampiyemler, antimikrobiyal tedavi olanaklarının ve plevral boşluk drenaj seçeneklerinin artmasına rağmen çocuk hastalarda da yüksek oranda morbidite ve mortaliteye neden olmaktadır. *Streptococcus pyogenes* ampiyemin nadir görülen nedenlerindendir. Bu makalede hipotansiyon ve hipoksinin eşlik ettiği ağır bir klinik tabloda başvurmuş olan ve torakas tüpü ile kapalı sualtı drenajına rağmen düzelmeyen ampiyemli on dört yaşında bir kız olgu sunulmuştur. Beş defa fibrinolitik streptokinaz tedavisi uygulandıktan sonra tablosu düzelmiş olup, altı hafta kadar geniş spektrumlu ikili antibiyotik kullanılmıştır. *Streptococcus pyogenes*'in neden olduğu bu ampiyem tablosunu yönetimindeki güçlükler nedeniyle sunmayı amaçladık.

Anahtar Kelimeler
Ampiyem, *Streptococcus pyogenes*, fibrinolitik tedavi, çocuk

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Introduction

Pleural empyema is a purulent fluid presence in the pleural space and is a known and frequently encountered complication of pneumonia in children. In 40-50% of patients hospitalized with acute bacterial pneumonia, parapneumonic effusion is known to develop (1). Approximately 10% of these patients are in the complicated clinical table and effective drainage is required for resolution (2). Pleural empyemas lead to high rate of morbidity and mortality although antimicrobial treatment opportunities and pleural space drainage space choices are on the increase (3).

Streptococcus pneumonia, widely encountered sample of culture-positive pleural empyema, is known to be dependent on *Staphylococcus aureus* and *Haemophilus influenza*. *Streptococcus pyogenes* is another microorganism mostly giving rise to upper respiratory infections, however, rarely leading to pleural empyema. A 14 year-old female case was presented in this study in which *Streptococcus pyogenes* was determined to be an agent and followed in a difficult table that could cause hypotension and hypoxia.

Case Report

A 14 year-old girl referred to hospital with weakness, loss of appetite, fever, difficulty in breathing and cough complaints that were going on for 10 days. It was stated that the patient had used an antibiotic for 7 days due to her upper respiratory tract infection two weeks ago. No characteristic was identified in the personal background and family history of the case. In her physical examination; cardiac pulse: 108/min, respiratory rate: 40/min, oxygen saturation: 92%, blood pressure: 80/60 mmHg, weight: 50 kg (25-50 p), height: 160 cm, overall situation: mean, weak and dehydrated, conscious; dyspnea and tachypnea were present, oropharynx was hyperemic, mobile lymphadenopathy sized 1x1 cm was present in the right cervical. In the pulmonary auscultation, respiratory sounds reduced significantly in the right hemithorax and tuber was heard as murmur in the right hemithorax again. No pathological findings were identified in the other system examinations. In the laboratory analyses; in the memogram; leucocyte: 36,100/mm^3 (85% neutrophile, 10% banded neutrophil, 5% lymphocyte), hemoglobin: 11.8 g/dL, hematocrit: 33%, mean corpuscular volume: 83 fL, thrombocyte: 381.000/mm^3, C-reactive protein (CRP): 288 mg/L (0-6 mg/L), sedimentation: 80 mm/hour. In the blood biochemistry; sodium: 131 mmol/L, potassium: 5.5 mmmol/L, total protein: 6.9 g/dL, albumin: 2.9 g/dL, in the artery blood gas pH: 7.50, pCO_2 25 mmHg, pO_2 57 mmHg, HCO_3 22 mmol/L, BEB: -3, SaO_2 91.

In the PA lung graphy, diffuse opasity was observed in the medium and lower lobes of right lung (Figure 1). The patient was pre-diagnosed with pleural effusion secondary to pneumonia with anamnesis, examination and lung graphy findings; thoracentesis was performed after thorax computed tomography (CT) had been taken. The fluid obtained in thoracentesis appeared as liquid purulent. In the microscopic examination, massive leukocytes and gram (+) chains of cocci were seen.

In the thorax CT examination of the patient, however, free fluid accumulation was determined in consolidation area involving air bronchogram in lower lobe of right lung and amid pleural leaves in the right hemithorax and atelectatic area in the right medium lobe (Figure 2). In the pleural fluid culture conducted, group A beta hemolytic *Streptococcus* (*AGBHS*, *Streptococcus pyogenes*) multiplied. No reproduction was seen in the sputum culture, ASO value 288 IU/mL was found high (normal; 0-200). *Mycobacterium tuberculosis* could not be identified in the pleural fluid direct examination and culture. The patient was initiated oxygen with nasal cannula, IV fluid due to hypotension and dopamin 5 mcg/kg/day. Owing to her empyema and pneumonia, dual antibiotic treatment was commenced including ceftriaxone 100 mg/kg/day as 2 dose intravenous application and vancomycin 40 mg/kg/day as 4 isodose by intravenous infusion. Thoracostomy tube was affixed to right hemithorax of the case, 450 mL purulent, exudative fluid was drained, in the yellow-purulent material; leukocyte count 21,500/mm^3 (90% polymorphonuclear leukocyte), glucose 6 mg/dL, protein 3220 mg/dL and pH was determined as

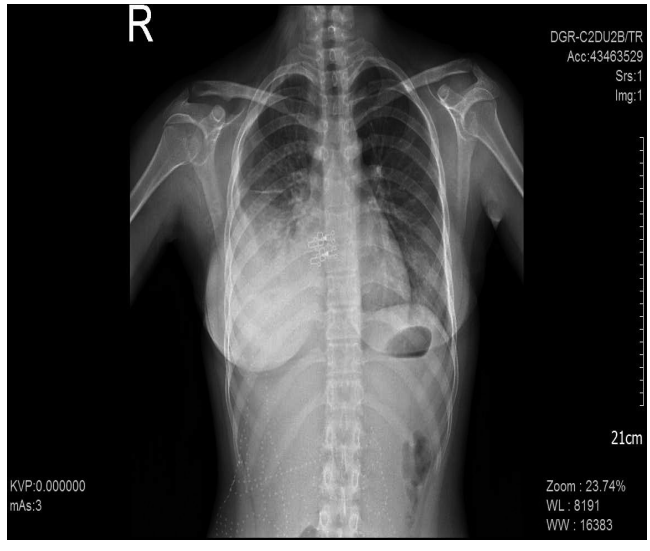

Figure 1. Opacity in the right lung middle-lower lobe (chest X-ray)

7.11. On the 3rd day of the treatment, the control lung graphy of the case whose respiratory complication and high fever were continuing was taken and it was seen that opacity significantly increased in her right hemithorax (Figure 3). The patient was evaluated by a pediatric surgeon and fibrinolytic treatment was recommended. 250.000 U streptokinase from thorax tube was applied in 100 mL serum physiologic as one dose. This application was repeated every two days and five times, approximately 100 mL purulent hemorrhagic fluid was attained after every application.

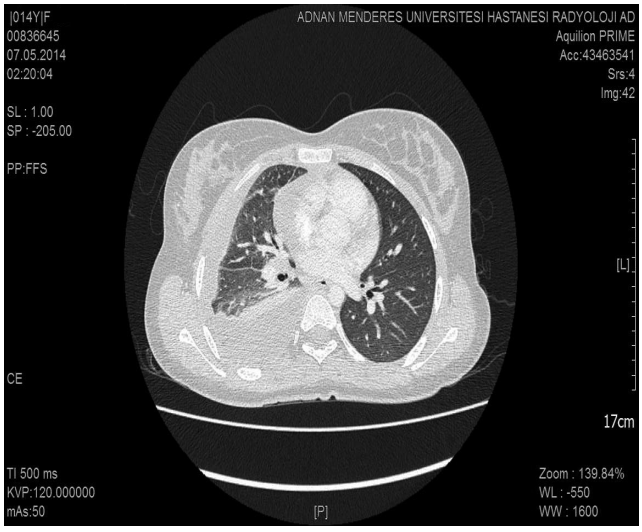

Figure 2. Consolidation site with air bronchogram in the right lung lower lobe, atelectatic area in the middle lobe and fluid accumulation in the right hemithorax (thorax computerized tomography)

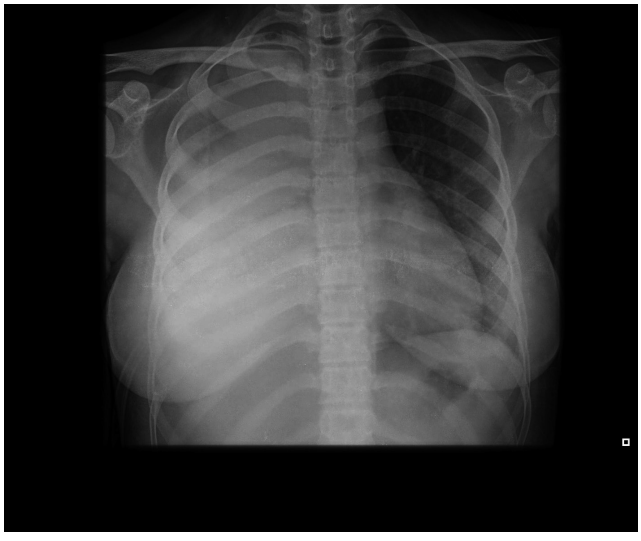

Figure 3. Significantly increased opacity in right hemithorax (control chest X-ray)

The antibiotic treatment of the case was discontinued by completing the treatment 4 weeks after her clinical findings and lung graphy ameliorated and she was discharged provided that she would come for the polyclinic control. Written and oral consent was acquired from the patient and her mother in terms of the study.

Discussion

Streptococcus is an immobile, asporous and gram-positive cocci shaped bacterium. *Streptococcus* types differ from each other in terms of hemolysis that they form in blood agar within themselves. If there is a hemolysis in the form of transparent zone around the colony in blood agar, it is called as beta; if there is a partial hemolysis with the greenish zone, it is called as alpha and if there is no hemolysis, it is named as nonhemolytic. Beta-hemolytic *Streptococcus* types are those responsible for most significant clinical tables in humans and this group is divided into more than 20 serogroups (A,B,C etc.) compared to C antigen on the cell wall. Among them, group A (GABHS) (*S. pyogenes*) is most commonly type composing infection in humans. They most frequently lead to clinical tables such as; pharyngitis, tonsillitis, red skin and soft tissue infections (impetigo, ecthyma, erysipelas), streptococcal toxic shock syndrome and rarely cause pneumonia, empyema, endocarditis, pericarditis, omphalitis, cellulitis and myositis. Empyema caused by *Streptoccus pyones* is rarely seen, on the other hand, it proceeds quite seriously. The bacterium creates an infection in the upper airways and then in the lung through airway and gives rise to empyema (4). Although *S. pneumonia, S. aureus* and *H. influenza* are most commonly encountered in the empyema etiology, group A *Streptococcus* types, gram-negative organisms, tuberculosis, fungi and malignancy are other rarely determined causes. In a study conducted by Celayir et al. (5), *S. pyogenes* was identified in only one out of 47 cases with empyema. GABHS could not be produced in 21 out of 85 cases with empyema that reproduction was determined in a study performed by Ertuğrul et al. (6). In a study published in 2014 by Sakran et al. (7), reproduction was identified in 32% of children with pleural empyema in 17-years of research, GABHS positivity remained at 1%. In another study where 602 cases were analyzed from

all ages, however, GABHS reproduction rate was found as 3% in 324 cultures cultivated in pleural fluid (8). Parapneumonic effusion and empyema are often seen in spring and winter months. Males are more affected by this condition than females. Although many pediatric patients were healthy previously, pediatric patients having underlying cerebral palsy, hypogammaglobulinaemia, chronic granulomatous diseases, Down syndrome, congenital heart disease, prematurity, cystic fibrosis, predisposing diseases such as tuberculosis and esophageal stricture were also reported (9). In the anamnesis of the patient, acute pharyngitis table ended 20 days ago was stated; ASO positivity was also a proof that she had GABHS infection. In addition, immunoglobulin and other primary immunological evaluations were also normal.

Parapneumonic effusion or the clinical course of empyema, causative organisms (aerob/anaerob), pus amount in pleural cavity, previous antibiotic treatment varied from medical condition of the patient. Most commonly seen symptom in patients with empyema is persistent fever and other popular findings are weakness, loss of appetite, mild non-productive cough, pleuritic chest pain, mild dyspnea and loss of weight (10). The patient with empyema has a toxic appearance. Empyema should be taken into consideration when pneumonia has a septic course or existing symptoms exacerbate. Antibiotic treatment can make clinical table less apparent and the differentiation of pneumonia and empyema can become difficult. Most encountered symptoms are short-interrupted breathing (82%), fever (81%), cough (70%) and chest pain (67%). All these symptoms are also seen in pneumonia. From time to time patients can show indications with severe breathing difficulty and sepsis findings involving hypotension (11). The patient brought to our emergency service by ambulance was found out to have had fever, cough, side pain, weakness and loss of appetite complaints for 10 days; a pharyngitis table ended approximately three weeks ago and was brought to our service after she had developed breathing difficulties and hypotension table. The patient with quite high leukocyte, CRP, erythrocyte sedimentation rate was also determined to have high ASO value when there was a *Streptoccus pyones* reproduction in pleural fluid culture.

Despite all diagnosis techniques, the presence of parapneumonic effusion and empyema and their distinctions (necessary for treatment) can be accurately disclosed by thoracentesis. 30-50 cc pleural fluid obtained by 18-19 G needles is examined in terms of macroscopic, microscopic, biochemical and microbiological parameters. If there is a clinical suspicion; tuberculosis, fungi culture and staining are performed. Purulent fluid is diagnostic for empyema. Cloudy fluid must be centrifuged to identify if the reason for cloudiness is due to leucocytes or lipids. After centrifuge, transparent fluid leucocytes are suggestive of cloudy fluid lipids. If pleural fluid is transparent in the first thoracentesis; the distinction of exudate or transudate should be carried out. Thoracentesis material of the case was dirty grey-green, and had a viscous consistent. Massive leukocytes were seen in microscopy and diplococcus that makes gram-positive chain was noticed but not acid fast bacilli and tuberculosis culture concluded as negative. Sputum and throat culture taken simultaneously resulted in as negative. Sputum and throat cultures turned out as negative were linked with the effect of empiric antibiotic that was used prior to hospitalization.

Although the treatment of parapneumonic empyema of children is similar to that of adults', treatment implementations of children are still contradictive. Numerous studies have defended that operative interventions are rarely necessary, on the other hand, some have suggested the benefits of early decortication in selected cases (12-15). Its specific treatment should be planned according to the phase of the disease at the time of diagnosis, the degree of lung pressure and overall condition of the patient. However, tube thoracostomy should be the first treatment in parapneumonic empyema of childhood despite everything because it has been determined that success rate was at 80-90% with only tube thoracostomy (15). Antibiotic selection must be conducted with regard to patient's culture outcome and/or his/her age and most likely microorganisms for predisposing situations.

Despite closed underwater tube thoracostomy and antibiotic treatment eligible for antibiogram, fibrinolytic agent from thoracostomy tube was applied to our case whose hypotensive and dyspneic table did not retreat. An important situation that drew our attention here was that empyema table caused by

Streptoccus pyones progressed quite severely and led to pleural cohesions and therefore, we had to apply streptokinase for five times.

Streptococcus pyogenes is one of the rare agents of empyema. It can give rise to complications such as severe clinical table and pleural cohesion. In the treatment, multidisciplinary approach is crucial as well as appropriate duration and antibiotic dose initially.

Ethics

Informed Consent: We received indormed consent from the patient.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

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

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Case Report / Olgu Sunumu

Confusion of Insulinoma's Neuroglycopenic Symptoms with Epilepsy, Two Case Presentation and a Review of Literature

İnsülinomada Nöroglikopenik Semptomların Epilepsi ile Karışıklığı, İki Olgu Sunumu ve Literatürün Gözden Geçirilmesi

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Keywords
Insulinoma, hypoglycemia, acute symptomatic seizure

Anahtar Kelimeler
İnsülinoma, hipoglisemi, akut semptomatik nöbet

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Abstract

Insulinoma is a pancreatic endocrine tumour which origins from beta cells and characterized by excessive insulin secretion and hypoglycemia due to this. Symptoms seen in hypoglycemia are due to brain's lack of glucose (neuroglycopenia) such as somnolence, irritability, confusion, abnormal behaviour, epileptic seizure and even coma. In this study, we presented two insulinoma cases who applied to our clinic with neuroglycopenic symptoms. Patients followed up with epilepsy diagnosis were referred to us because of low blood glucose rates. Hyperinsulinemic hypoglycemia in the prolonged fasting test and a mass in pancreas were detected in both patients and so they were diagnosed as insulinoma. The mass was excised by laparoscopic ways in surgical clinic and patients clinic were completely recovered post-operative. In conclusion, it is necessary to determine whether there is an underlying organic brain lesion or a metabolic condition in order to prevent long-term use of unnecessary antiepileptic in patients presenting with seizures.

Öz

İnsülinoma, aşırı insülin salınımı ve buna bağlı hipoglisemi ile karakterli, beta hücrelerinden köken alan endokrin bir pankreas tümörüdür. Hipoglisemide ortaya çıkan semptomlar beynin glikozsuz kalması (nöroglikopeni) sonucu, somnolans, irritabilite, konfüzyon, anormal davranış, epileptik atak ve hatta koma görülebilir. Biz, burada, nöroglikopenik semptomlarla başvuran iki insülinoma olgusunu sunduk. Epilepsi hastalığı ile takip edilen hastaların kan şekeri düşük saptanması üzerine kliniğimize refere edilmiş. Her iki olguda insülinoma tanısı, uzun açlık testinde hiperinsülinemik hipoglisemi, pankreasta kitle tespit edilmesiyle kondu. Cerrahi kliniğinde pankreastaki kitle laparoskopik olarak eksize edildi, post-operatif takiplerde hastaların kliniği tamamen düzeldi. Sonuç olarak nöbet ile gelen hastalarda uzun süreli gereksiz antiepileptik kullanımını önlemek amacıyla altta yatan organik beyin lezyonu ya da metabolik bir durum olup olmadığını belirlemek gerekir.

Introduction

Diagnosis of insulinoma is usually delayed because of non-spesific indications. Adrenergic and neuroglycopenic symptoms are seen when hypoglycemia occurs. There may be a long period between the onset of symptoms and the diagnosis. Because of neuroglycopenic symptoms, patients can be followed-up and treated with misdiagnoses such as epilepsy or psychosis in neurology or psychiatry clinics (1).

In this case report we wanted to remind and emphasize the existence of two insulinoma cases who applied to the neurology and psychiatry clinics before diagnosis and who used long term un necessary antiepileptic drugs due to misdiagnosis of epilepsy or psychiatric disorders.

Case Reports

Case 1

Sixty-six year old female patient, she had the complaints of getting fat, sweating, palpitation, seizure and fainting. The patient began to use antiepileptic drugs with the diagnosis of epilepsy, three years ago. The patient's seizure frequency had increased in the last 3-5 months and seizures become uncontrollable with the drugs. The patient was referred to our clinic with the prediagnosis of insulinoma after they measured fingertip blood glucose 26 mg/dL in the emergency service when she applied with neuroglycopenic symptoms. Her fasting blood glucose was measured as 60 mg/dL after biochemical tests. At the 4th hour of the prolonged fasting test the insulin value was determined as 28.4 (6-27) IU/dL and c-peptide: 5 (1.1-5) IU/dL while blood glucose was measured as 30 mg/dL. These findings were biochemically suitable with insulinoma. Pancreas ultrasonography, magnetic resonance imaging (MRI) and tomography were performed to the patient and tumour could not be localized in the pancreas. Endoscopic ultrasonography reported 11x7 mm mass in the tail zone of the pancreas (NET?). Patient under went to operation in our general surgery clinic. Immunohistochemical studies in the pathological examination of the fragment revealed that the resulting tumour was a well differentiated endocrine tumour insulinoma carrying low-grade malignancy potential. Post-operative follow-up showed a blood glucose level of 110-150 mg/dL and there was no epileptic attack in the last 3 months. Informed consent was obtained from the patients.

Case 2

Fifty-seven year old female patient was admitted to the emergency center clinic with the complaints of palpitation and weakness. She was referred to our endocrinology clinic because of low blood glucose level (45 mg/dL) during the examinations. About three years ago patient was diagnosed as epilepsy in the neurology center due to the complaints of fatigue, loss of concentration, excessive nervousness, trembling in hands, glazed eyes, cold sweating, fainting, palpitations and treated with levatiracetam 2x500 mg, lamotrigine 1x25 mg. There was no decrease in complaints and seizure frequency of the patients on the follow-up. We made prolonged fasting test to the patient. We detected insulin: 68 uIU/mL, c-peptide: 5.1 mg/mL, cortisole: 15.3 mg/dL, at the third hour of the test while blood glucose level was 45 mg/dL. These findings were biochemically compatible with insulinoma. Pancreas MR showed approximately 15x13 mm hypervascular mass lesion in the tail section of the pancreas extending partially out of the pancreas contour, which was hypointense in T1 weighted series. Pancreatic mass was laparoscopically excised in general surgery clinic. In tissue pathology tumour was defined as neuroendocrine tumour grade 1, immunohistochemically positive for chromogranin and synoptophysin, proliferation index was 1%. There were no hypoglycemia and seizures in post op follow-ups. Informed consent was obtained from the patients (Figure 1).

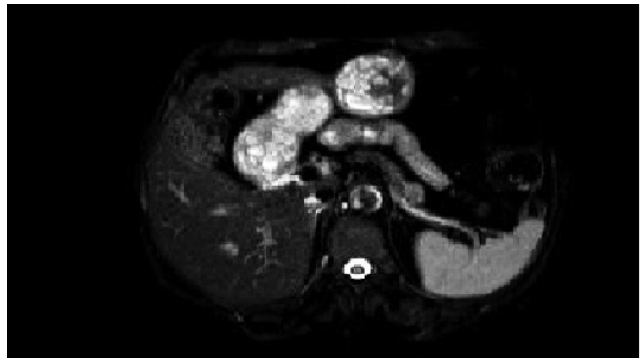

Figure 1. The pancreas MRI shows a hypervascular mass of 15x13 mm in size, hypointense in T1-weighted series, hyperintense in the T2-weighted series in the tail of the pancreas, extending partially out of the contour of the pancreas.
MRI: Magnetic resonance imaging

Discussion

Insulinoma is a rare neuroendocrine tumour originating from the Langerhans islet cells of the pancreas (2). Epileptic attacks due to hypoglycemia can be seen in these patients. Acute symptomatic seizure is associated with a systemic or neurological condition. These seizures are not as repetitive as epilepsy. The recurrence of acute symptomatic seizures is not expected unless the underlying event is repeated (3). Both two cases were followed-up in neurology and physchiatry clinics due to epileptic seizures.

In insulinoma, clinical symptoms ocur due to hypoglycemia, sweating, tremor, anxiety, starvation and palpitation feelings due to hypoglycemia after exercise or eating, determine the clinical picture. Central nervous system findings occur when blood glucose falls below 50 mg/dL. Confusion, seizure, abnormal behaviours can be seen in patients (4). Patients may be followed up and treated with false diagnoses such as epilepsy or physchosis in clinics due to non-spesific symptoms, like our cases.

In our cases, hypoglycemia is probably masked by frequent eating for about three years, neuroglycopenic symptoms may come out after hypoglycemia. But in last months the number of referrals to the doctor because of uncontrolled seizures has increased although high doses of drugs. Patients were referred to our clinic because of the low blood glucose levels observed during seizures in emergency. We made prolonged fasting test to the patients with a close follow-up and the serum glucose, insulin, c-peptide and insulin glucose ratio detected at the third and 4^{th} hours of the test were biochemically suitable with insulinoma.

Insulinomas are usually small (<2 cm), solid, 90% benign tumours; they are small and it is hard to localize preoperatively. Conventional computerized tomography (specificity 12-73%) and transabdominal ultrasonographic examinations (spesificity 62%) are relatively insensitive methods in the localization of these tumours and using dual phase computerized spiral tomography can be used to show smaller insulinomas not seen with other radiological methods (6-18 mm). First studies with MR are not promising. Pancreatic Endoscopic ultrasound (EUS) is a non-invazive technique for the localization of pancreatic neuroendocrine tumours and reported sensitivity of 75-96% (5). We performed pancreatic tumour localization with MRI in one of the cases and with pancreatic EUS in other case.

As a result seizures associated with acute metabolic disturbance can be separated from epilepsy by lack off the tendency to recurrence and an identifiable cause of seizure. Forth is reason in a patient applying with seizure we should determine if there is a metabolic status or an organic brain lesion underlying to avoid long term unnecessary antiepileptic drug use.

Ethics

Informed Consent: Informed consent was obtained from the patients.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: M.H.A., Concept: R.Ç., Design: F.G., Data Collection or Processing: F.G., M.H.A., Analysis or Interpretation: F.G., R.Ç., Literature Search: M.H.A.

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Case Report / Olgu Sunumu

Very Late Onset "Trichotillomania": A Case Report

Çok Geç Başlangıçlı "Trikotilomani": Bir Olgu Sunumu

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Abstract

Trichotillomania is a chronic psychiatric disorder that repetitive hair pulling which leads to marked hair loss, negative effects on all areas of the person's functioning. Although trichotillomania has been known for many years, it has attracted less attention than other psychiatric disorders. Therefore, the information about its epidemiology, etiology, clinical feature and therapeutic approaches are limited. Trichotillomania usually begins in early childhood or adolescence. It is more common in women than men. Cases are frequently referred to the first dermatology outpatient clinics due to marked hair loss. Trichotillomania is one of the common issues of psychiatry and dermatology. In patients with trichotillomania, comorbid psychiatric disorders often accompany the clinical picture. The most common psychiatric comorbidities are mood disorders and anxiety disorders. In this study, we report a male patient who was treated with a diagnosis of very late onset trichotillomania with comorbid depression. The fact that trichotillomania started at an advanced age is a feature that makes our case interesting.

Keywords
Trichotillomania, very late onset, comorbidity

Öz

Trikotilomani, yineleyen saç yolmalar sonucu belirgin saç kaybına yol açan, kişinin işlevselliğinin tüm alanlarını olumsuz etkileyen süregen seyirli bir ruhsal bozukluktur. Trikotilomani uzun yıllardır bilinmesine rağmen diğer ruhsal bozukluklardan daha az ilgi çekmiştir. Bu nedenle epidemiyolojisi, etiyolojisi, klinik görünümü ve tedavi yaklaşımları ile ilgili bilgiler sınırlıdır. Trikotilomani genellikle erken çocukluk ya da ergenlik döneminde başlar. Kadınlarda erkeklere göre daha sık görülmektedir. Belirgin saç kaybı nedeni ile olgular ilk kez dermatoloji polikliniklerine başvururlar. Trikotillomani psikiyatri ile dermatolojinin ortak konularından biridir. Trikotilomanili olgularda eş tanılı psikiyatrik bozukluklar da sıklıkla klinik tabloya eşlik eder. En yaygın görülen psikiyatrik eş tanılar duygudurum bozuklukları, anksiyete bozukluklarıdır. Bu çalışmada eştanılı depresyonu da olan çok geç başlangıçlı trikotillomani tanısı ile tedavisi düzenlenen bir erkek olgu sunulmuştur. Trikotillomaninin ileri yaşta başlamış olması olgumuzu ilginç kılan bir özelliktir.

Anahtar Kelimeler
Trikotilomani, çok geç başlangıç, eştanı

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Introduction

Trichotillomania is a psychiatric disorder that repetitive hair pulling behavior leading to marked hair loss, severe, progressive and difficult to treat. As Hautmann and others (1) reported, even though trichotillomania has been known since the 12th century, it was described for the first time in the late 19th century by a French dermatologist Francois Hallopeau. Trichotillomania lexically means uncontrolled hair pulling in ancient Greek (2). Even though it has been known for a long time, epidemiology, etiology and treatment approaches of trichotillomania are still not known exactly today. In other words, it took less attraction in comparison with other psychiatric disorders although it has been known for many years. As a result of trichotillomania, complete or partial alopecia occurs in the scalp in most cases. According to basic clinical data in recent terms trichotillomania is seen more common than it was believed to occur in the past. This disorder starting in infancy and adolescence period and typically appearing in critical developmental periods may reoccur in other parts of the life as a result of unwanted and distressing situations (2,3). Although trichotillomania can be seen in every age, starting age is average 12-13 (4,5). It generally starts before the age of 20, while it rarely starts after 20. It is mostly seen between the ages of 11 and 15 (2,6). It is reported that in the early type which starts before 6 the rate of male and female equals to each other and this type gives a good respond to the treatment with a better clinical course. It is also reported that late start trichotillomania which is seen after 13 is more seen on girls tend to becoming chronic and reveal comorbidity and resistance to the treatment (7,8). The studies related to *etiology* of *trichotillomania* and treatment have been increased in the last 20 years. Especially, new factors such as relaxation training, making patients raise awareness on the habit, self monitoring, and training on reversing the habit as treatment approach (2). Even though the reasons taking role in etiology of trichotillomania are not definitely known, it is thought that biological, psychological and social factors are effective. The findings which refer to that hair pulling behaviour is more seen in the families of the cases indicates the importance of the genetic predisposition on which many various genes take role (9-11). In this study a very late start trichotillomania patient, not defined in literature before and accompanied by depressive symptoms is presented under literature knowledge.

Case Report

The case is 72 years old, male, married, retired, primary school graduate and has five children. He was born in a village of Rize. He still lives with his family in the same village. He applied to our outpatient clinic at the recommendation of his family and also voluntarily as he had the habit of pulling hair and other hairs on his body.

History: This behaviour of the patient who started to pull his hairs on his body as far as his hand reach about 4 years ago emerged mostly when he had a trouble. The patient said that in those times he felt itching in hair and hair roots and overwhelmingly wanted to pull his hair. He also added that he enjoyed the sound which he heard while pulling his hairs because this relaxed him and during that he did not feel any pain. The patient who threw his hairs around randomly after pulling them said the feeling of itching was lost and he felt relaxed afterwards. He also reported that he sometimes regretted pulling hairs but he could not stop doing this. He repeated this 4 or 5 times a day and almost everywhere. Sometimes he suddenly felt this while he was performing prayer, he could not beat this and started to pull his hairs and discontinued his prayer. His 41 year old son died 18 months earlier. After he lost his child, he had complaints such as adductive, forgetfulness, sleeplessness, not enjoying the life, but there was increase in the behaviour of the patient who did not have any treatment during that time.

Developmental History

The patient who led all his life in the village where he was born was born by normal delivery. He had no problem during the growth and development periods. He had umbilical hernia operation 15 years earlier and cataract operation 6 years earlier. He had had a regular benign prostatic hyperplasia treatment for 3 years. There was no neurologic disease story in the family. Any change was not defined in the sleep and appetite habits of the patient who was outgoing and had a lot of friends before the illness. On the other hand, after he got the illness, he did not go out much and did not see his friends.

Physical Examination and Laboratory Findings

In the physical examination of the patient, apparent hair loss was observed especially on the arms and legs of the patient. It was also seen that there were scar tissues and colour changes on the skin as a result of hair pulling. On the other hand, there were different lengths and areas not affected by hair pulling and seen normal in the alopecia part as a classical finding of trichotillomania. Also there was apparent localized hair loss in the frontotemporal part of the patient (Picture from 1 to 3). Hair pulling was undulatory from a central part. The patient was wearing a hat and had a beard to hide the hair loss. The patient was directed to dermatology polyclinic for his skin lesions and his necessary topical dermatological treatment was started. His electroencephalography (EEG) included normal findings. In the magnetic resonance imaging, slight explicitness was determined in the hemispheric cortical sulcus. In the other parts his age compatible changes were reported. Any pathological findings were not obtained from the patient who was examined in terms of dementia. Hemogram value, thyroid and parathyroid hormones, ferritin, vitamin B12, folic acid and syphilis tests were done and any pathological findings were not seen. The Mini–Mental State Examination (MMSE) was evaluated as 28 points.

Mental State Examination

Apparent hair loss in the body of the male patient, looking in his age, having compatible clothing to socio-demographic level, respectful, having eye contact, and with partly reduced self care was remarkable. During the interview, he gave brief and clear short answers to the questions. The tone of his voice was normal. Especially, he had a rising anxiety when hair pulling was mentioned. His sensitivity was depressed and his sensation was troublesome. His consciousness was open, oriented and cooperative. No pathological finding was detected in the perception. Memory capacity, intelligence level, the ability to assess reality and judgement were normal. His connotations were in regular thinking content and his disease, his case and his treatment, and the problems which he had with his family were related to insignificance and guilt feelings. His self esteem was reduced.

Findings

Following the psychiatric examination, the patient was diagnosed with major depressive disorder and trichotillomania and treated with fluoxetine 20 mg/day (12).

Hamilton Depression Rating Scale (HDRS) was 18 points and Hamilton Anxiety Assessment Scale (HAAS) was 12 points. The patient and his family were informed about the disease and the treatment. After the eighth week of the treatment, apparent improvement in the symptoms of depression anxiety of the patient was observed. Also HDRS and HAAS points decreased. Especially raising the awareness for hair pulling was emphasized during patient interviews. The patient said that he felt discomfort because of hair pulling. In the next phase the treatment was carried on with cognitive behavioural therapy (CBT) with the patient whose awareness was raised. The patient was asked to record his feelings and thoughts while pulling hairs, but he could not comply with it. On the other hand, it was observed that the patient who communicated in a difficulty when he first applied to the clinic expressed himself better in later interviews. He gained new skills to control his behaviours. Apparent improvement in hair pulling behaviour of the patient was determined in the fourteenth week of the treatment even though hair pulling seldom iterated from time to time with stress factors. His treatment is still going on. The patient was informed before the study and his written and verbal informed consent was obtained.

Discussion

Trichotillomania may cause serious worsening on the life quality by affecting the relationships between people, avoiding society and social activities, losing self control, feeling low respect with disappointment and affecting productivity negatively. Hair was pulled mostly from the vertex and then from the temporoparietal, occipital and frontal regions on the scalp (1,5). In the presented case hair pulling from vertex and temporal region complies with the literature information. Some positive or negative reinforcers about hair pulling take role in the continuation and the irritation of the action (5,6). In this case satisfaction and pleasure can be seen as positive reinforcers, while an unwanted affection or avoiding and recession from a situation can be seen negative reinforcers. It is known that patients tend to hide hair pulled parts (1,2,13). The fact that the patient came to the interview proves this. A

view which supports that trichotillomania is seen in both genders and the rate of females' increases in advancing ages has a command. On the other hand, that trichotillomania is seen in males less than in females can be males' hiding hair pulling, decreasing hair pulling, and their preference to have their hair cut to struggle against hair pulling, or explaining this with male type alopecia. In addition another reason is that male patients apply to clinics less than female patients or they go to a clinic very much later (2-5). Our patient's being male and applying to the clinic 4 years after his disease started supports these results. It is stated that feelings mostly expressed before the action are anxiety, tension, boredom and guilt. When feelings before and after the action are compared, it is seen that the biggest change is in the feelings of guilt, boredom, comfort, sadness and anger (14,15).

Tension which occurred before hair pulling and comfort which was felt after the action in DSM-IV-TR criteria were determined in our case. However, tension before the action and comfort and pleasure after the action taking place among previous diagnostic criteria in newly published DSM-5 diagnose and classification system were removed and instead iterating reducing hair pulling and stopping it criterion was placed (16). Also, it was reported that there was stressful events and trauma stories which started the illness in our case (2,17). It is remarkable in the case that increase in the complaints of the patient was observed after his son suddenly died. Even though the knowledge related to an efficient treatment is quite limited, prevalence and possible effects on life quality of trichotillomania were defined. In the studies for adult sample group with trichotillomania Axis I disorder existence during and before diagnosis was reported 82% (18). Most frequently seen togetherness is mood and anxiety disorders. Swedo and Leonard (19) (1992) determined the unipolar depression rate as 39% for a mixed sample group formed with children, teenagers, and adults with trichotillomania. In the presented case comorbid depression complies with those results. As in other psychiatric disorders, that trichotillomania is together with other psychiatric disorders makes diagnosis in the patients difficult, and also negatively affects the treatment and the prognosis of the disease. Questioning the disorders in routine psychiatric examinations and semi structured interviews will be beneficial for early diagnosis. Although there is not enough evidence, firstly with selective serotonin reuptake inhibitors (SSRIs), clomipramine and other antidepressants there are CBT as effective therapies (20-28). Also, fluoxetine was preferred for the treatment of our patient. As a result of controlled studies, CBT, mainly Habit Reversal Training (HRT), arranged to raise the awareness on hair pulling are more effective in automatic pulling, whereas the strategies based on cognitive and adoption are more effective in focused pulling (29). For our case firstly awareness for hair pulling was provided and then the treatment was carried out with cognitive behaviourist techniques. Hiding hair pulling behaviours or limited or late application to the treatment due to embarrassment restrains wide controlled studies to be done. In our study similarly it is obvious that our case who had a late standard trichotillomania applied to the treatment very late and there was functionality.

In conclusion, it should not be forgotten that although trichotillomania is a psychiatric disorder accepted to start in early ages, it is also possible for it to be seen in old ages. To understand trichotillomania better, wide scale epidemiologic studies, arrangement of objective criterion of functionality disorder, searching pain tolerance in detail, and controlled studies which can compare psychological (supporting) and pharmacological treatments with CBT peculiar to young and old patients are required.

Ethics

Informed Consent: The patient was signed an informed consent form. He was photographed with plaque zones for comparison before and after the treatment.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: M.A., Ç.H., N.D., Concept: M.A., Ç.H., D.Y., Design: Ç.H., N.D., Data Collection or Processing: M.A., D.Y., Analysis or Interpretation: Ç.H., N.D., Literature Search: M.A., D.Y., Writing: M.A., Ç.H., N.D.

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