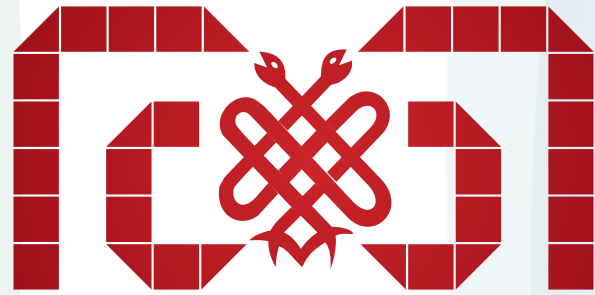


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# MEANDROS

## MEDICAL AND DENTAL JOURNAL



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ORCID ID: orcid.org/0000-0002-2360-3942



**Editorial Office**

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Phone: +90 256 444 12 56 | +90 256 214 64 95

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E-mail: yuziar\_12@yahoo.com

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E-mail: gokhancesur@hotmail.com

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ORCID ID: orcid.org/0000-0001-9461-5812

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ORCID ID: orcid.org/0000-0001-9976-8797

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ORCID ID: [orcid.org/0000-0003-4850-5094](https://orcid.org/0000-0003-4850-5094)

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ORCID ID: [orcid.org/0000-0002-5055-1551](https://orcid.org/0000-0002-5055-1551)

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ORCID ID: [orcid.org/0000-0001-9139-8752](https://orcid.org/0000-0001-9139-8752)

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**Başak Bıyıkoglu**

Altınbaş University Faculty of Dentistry, Department of Periodontology, İstanbul, Turkey  
ORCID ID: [orcid.org/0000-0001-8830-9835](https://orcid.org/0000-0001-8830-9835)

**Berza Yılmaz**

Bezmialem Foundation University Faculty of Dentistry, Orthodontics Department, İstanbul, Turkey  
ORCID ID: [orcid.org/0000-0002-7961-0535](https://orcid.org/0000-0002-7961-0535)

**Meltem Mert Eren**

Altınbaş University Faculty of Dentistry, Department of Restorative Dentistry, İstanbul, Turkey  
ORCID ID: [orcid.org/0000-0002-5903-6636](https://orcid.org/0000-0002-5903-6636)

**Gülsüm Sayın Özel**

İstanbul Medipol University Faculty of Dentistry, Department of Prosthodontics, İstanbul, Turkey  
ORCID ID: [orcid.org/0000-0001-8833-5259](https://orcid.org/0000-0001-8833-5259)



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Adnan Menderes University Faculty of Medicine,  
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Phone: +90 256 4441256  
Fax: +90 256 214 64 95  
E-mail: [dryaseminturan@gmail.com](mailto:dryaseminturan@gmail.com)

#### Prof. Dr. Senem YİĞİT ÖZER

Adnan Menderes University Faculty of Dentistry,  
Department of Endodontics, Aydın, Turkey  
Phone: +90 256 2136347  
Fax: +90 256 2151918  
E-mail: [senem@adu.edu.tr](mailto:senem@adu.edu.tr)

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# Oral Precancerous Lesions in Childhood: Attention to the Pediatricians and Pediatric Dentists

## *Çocuklarda Oral Prekanseröz Lezyonlar: Pediatri ve Pedodontistlerin Dikkatine*

Şaziye Sarı<sup>1</sup>, İlhan Kaya<sup>2</sup>, Akif Demirel<sup>1</sup>

<sup>1</sup>Ankara University Faculty of Dentistry, Department of Pediatric Dentistry, Ankara, Turkey

<sup>2</sup>Uşak University Faculty of Dentistry, Department of Oral and Maxillofacial Surgery, Uşak, Turkey



### Keywords

Precancerous lesion, oral cancer, oral lichen planus, leukoplakia, erythroplakia

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### Address for Correspondence/Yazışma Adresi:

Akif Demirel DDS,  
Ankara University Faculty of Dentistry,  
Department of Pediatric Dentistry, Ankara,  
Turkey

Phone : +90 506 287 33 80

E-mail : akifdemirel@ankara.edu.tr

ORCID ID: orcid.org/0000-0002-1433-0452

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### Abstract

Precancerous lesions of oral mucosa, known as premalignant disorders, have greater malignant transform risk concerning dysplastic changes, carcinoma in situ or oral cancers. Oral lichen planus, oral leukoplakia, oral erythroplakia, oral submucous fibrosis, dyskeratosis congenita and epidermolysis bullosa are the most common precancerous lesions in varying frequency in children. Although the etiological factors of these lesions are not entirely understood; the main factors are smoking, alcohol consumption or addictive habits such as areca nut chewing. Moreover; these harmful habits are increasing dramatically in children in early ages and it is challenging regarding public health. Despite the oral cancers or precancerous lesions are rarely seen in childhood, early diagnosis and treatment of these lesions are critical regarding differences in treatment procedures in children, elimination of harmful habits that may cause these lesions and providing regular follow-ups. Therefore, it is thought that specialist in different disciplines which involved pediatric patients need to be informed and raised awareness. The aim of this review is to summarize the clinical features, pathogenesis, etiological factors, current treatment approaches and preventive procedures of the most common oral precancerous lesions in pediatric patients.

### Öz

Premalign hastalıklar olarak da bilinen oral mukozanın prekanseröz lezyonları; displastik değişimler, karsinoma in-situ ya da oral kanserler konusunda ciddi malign dönüşüm riskine sahiptir. Oral liken planus, oral lökoplaki, oral eritroplaki, oral submüköz fibrozis, diskeratosis kongenita ve epidermolizis büllosa çocuklarda değişken görülme sıklığı ile beraber, en çok görülen prekanseröz lezyonlardır. Bu lezyonların etiyolojik faktörleri tam olarak anlaşılamamış olsa da; ana faktörler sigara kullanımı, alkol tüketimi ya da areca nut çiğneme gibi bağımlılık yapan alışkanlıklardır. Dahası; bu zararlı alışkanlıklar çocuklarda özellikle erken yaşlarda dramatik olarak artmakta ve halk sağlığı konusunda sorun olmaktadır. Çocukluk çağında oral kanserlere ya da prekanseröz lezyonlara nadiren rastlanmasına rağmen, bu lezyonların erken tanı ve tedavisi; çocuklarda tedavi prosedürlerindeki farklılıklar, bu lezyonlara neden olabilecek zararlı alışkanlıkların giderilmesi

ve düzenli takiplerin sağlanması açısından önemlidir. Bu nedenle, pediyatrik hastaları konu alan farklı disiplinlerdeki uzmanların bilgilendirilmesi ve farkındalığının artırılması gerektiği düşünülmektedir.

Bu derlemenin amacı, pediyatrik hastalarda yaygın görülen oral prekanseröz lezyonların klinik özelliklerini, patogenezi, etiyolojik faktörlerini, güncel tedavi yaklaşımlarını ve önleyici prosedürlerini özetlemektir.

## Introduction

Oral cancers have become an important and growing problem in many parts of the world. Oral and pharyngeal cancers are the sixth most common lesions all over the globe (1,2). In some cases, oral cancers derive from precancerous lesions which can be detectable clinically. The most frequently observed precancerous lesions are; leukoplakia, erythroplakia, oral submucous fibrosis, which have higher potential for malignancy risk (2).

Squamous cell carcinomas (SCCs), one of the most common types of oral cancers, often develop from precancerous lesions. Despite the current developments in the treatment procedures of SCCs, 5-year survival rate is only about approximately 50% due to the late diagnosis of SCCs and precancerous lesions. Early diagnosis of precancerous conditions will not only increase the success rate, but also will significantly reduce mortality and morbidity (3).

Although oral cancers are usually seen in the middle and upper age group, younger patient populations have been reported to be affected in recent years (4-6). Despite the oral cancers are increased in children and adolescents, most of the oral cancer cases have reported without any risk factors in younger patients (7). Therefore, because of the high mortality rate of oral cancers, early diagnosis should not be overlooked, especially in children. The ulcerations, leukoplakia, erythroplakia, swelling and high-risk areas (especially tongue and floor of the mouth) in the oral cavity should be carefully examined and evaluated. In this regard, diagnosis of premalignant lesions is at least as important as diagnosis of oral cancers. Additionally, because the frequency of precancerous lesions is rare in pediatric patients, there are not many prevalence studies in the literature, except for a few case reports.

The main etiological factors of oral cancers and premalignant lesions are use of tobacco products, addictive harmful substances and alcohol consumption. Epidemiological studies have reported that the risk of oral cancers is 5 times higher in smokers than in non-smokers. In addition, even in the

cases of treated oral cancer, the risk of occurring and developing secondary malignancy increases when smoking is continued. Likewise, alcohol consumption increases the risk 3-9 times (8,9).

Oral precancerous lesions, risk of future malign changes and their clinic sequelae lead to severe impairments in quality of life and treatment procedures are costly for especially developing countries. Primary prevention of precancerous lesions is possible by eliminating risk factors which will reduce the incidence of premalignant lesions. Unfortunately, informing general public regarding etiological risk factors, prevention and potential complications of precancerous lesions remains inadequate. Hence, early diagnosis is essential to increase treatment success and survival rates (2). In this respect, since pediatricians, pediatric dentists and oral maxillofacial surgeons may be the first physician in diagnosing oral precancerous lesions, they should be informed about these lesions.

The prevalence of smoking, alcohol consumption and other addictional habits are increasing all over the globe (10,11). Smoking causes deaths worldwide, especially in developing countries (12). Moreover, dependence of tobacco products increases in children and adolescents and emerges as a major threat in populations (12-14). In a study conducted in primary school children in Turkey (15), it has been reported the mean initiation age of smoking was 11.7 and smoking abuse occurred mostly due to curiosity and imitation. In another study conducted in Turkey (14), it was reported that 43% of children in the study group of smoking in any way, while 24% of regular smokers. In the same study, it was stated that most of the smoker children (86%) had no difficulty in providing tobacco products and the smoking age was 8.3-10.5 in males and females, respectively. Starting smoking at an early age is also closely related to other risky behaviors such as substance and alcohol use (16). Alcohol use and addiction in children and adolescents remains a significant problem (17). As a matter of fact, studies have proved that alcohol use is started at a

much earlier age (18). MacKintosh et al. (19) reported that 48% of children between the ages of 12-13 had alcohol consumption. Regular use of alcohol products among children is reported 24% in Turkish population (20). The fact that both smoking and alcohol use are the etiological factors of oral precancerous lesions (8) and the reducing age of these habits in developing countries show that children are at severe risk. On the other hand, it is observed that substance addiction such as areca nut which is etiological factors of precancerous lesions of oral mucosa, especially in Asian societies, increases in children aged 5-12 years (21). The reduce of age of initiation of these harmful habits in children leads to the occurrence of potential oral precancerous lesions. Thus, carefully examination of oral precancerous lesions by medical doctors, pediatricians, pediatric dentists and oral maxillofacial surgeons and early treatment in case of diagnosis is life saving.

The aim of this review is to present the clinical aspects and appereances, pathogenesis, etiological factors, current treatment approaches and preventive procedures of the most frequently observed oral precancerous lesions in children

#### **Oral Lichen Planus**

Oral lichen planus (OLP) is one of the chronic inflammatory diseases of oral mucosal membranes. Although OLP is commonly seen in adults, it can be rarely observed in pediatric patients (22-24). In OLP examination in the oral cavity, lesions are generally located bilaterally and symmetrically. OLP lesions are most seen in buccal mucosa, gingiva and dorsum of tongue. On the other hand, clinical appereance of OLP lesions can often seen in the forms of reticular, papular, plaque-like, atrophic, erosive or bullous (24,25). In erosive and atrophic form of OLP, patients complain burning sensation in the mouth (24,26). OLP lesions are mostly diagnosed by clinical and histopathological examination. The lesions are histopathologically characterized by hyperkeratinized epithelium, atrophic epithelium with shortened retepegs (saw tooth), and band-like lymphocytic infiltration in epithelial and connective tissue junction (27,28).

The etiology of OLP is multifactorial; autoimmunity, stress, genetic factors (human leukocyte antigen antigen), systemic diseases, viruses [*Helicobacter pylori*, *Epstein-Barr*, Human Papilloma virus (HPV),

Hepatitis C virus], dental restorative materials and some drugs are considered that trigger this disease. (24,29). In etiopathogenesis of the disease, there is an autoimmune reaction in which autocytotoxic CD8+ T cells induce apoptosis of basal cells of the oral epithelium (30). On the other hand, OLP may also be associated with other autoimmune diseases such as Lupus Erythematosus, Pemphigus, Sjögren's syndrome (23).

In children, OLP is more prevalent than some precancerous lesions such as leukoplakia and erythroplakia. OLP seen in children is generally asymptomatic or minimally symptomatic (31). However, in the case reports presented, clinical findings such as bilateral white lineations in the buccal mucosa, white discoloration under the tongue and burning sensation were reported (23,32). OLP, is seen in the form of reticular pattern in many children patients and does not require active treatment. Treatment applications are recommended when the lesions are symptomatic, erosive or ulcerative (31). Local regimens are usually applied to prevent side effects of systemic treatment in children. Hereof, the agents such as topical steroids, topical anesthetics, and topical tacrolimus are used. On the other hand, 0.12% chlorhexidine irrigation is recommended for plaque control and prevention of Candidiasis lesions. In children with OLP, if the symptoms are severe topical steroids should be combined with systemic steroids (0.5-1 mg/kg) for 3-6 weeks. In addition, for OLP treatment in children; medications and methods such as retinoids, cyclosporine irrigations, psoralen and ultraviolet therapy, dapson and cryotherapy have also been reported (24,33).

One of the most controversial points related to OLP is whether these lesions are precancerous. The World Health Organization has categorized OLP as a precancerous condition, although there are disagreements between authors in this respect (34). Malathi and Thappa (31) reported that erosive form of OLP usually has a malignant transformation risk in children. Although ulcerative form is known to have malignant tendency in adults, no cases have been reported in children up to the present (35). In case of malignancy, biopsy should be performed on suspicious lesions and cases should be followed at periodic intervals.



Elimination of the etiological factors such as anxiety and depression, mechanical trauma, dental treatment procedures, harmful oral habits, excessive consumption of spicy foods and irritation of the dental plaque is recommended in prevention of development of OLP and in treatment process in children (31).

### **Oral Leukoplakia**

Oral leukoplakia (OL) is the most common premalignant lesion of oral mucosa and it was first described by the World Health Organization in 1978 as "a white patch or plaque that cannot be characterized clinically or pathologically as any other disease" (36,37). In 1994, Uppsala, Sweden, it has been reported that this lesion, which is not associated with any physical or chemical cause other than smoking, is highly likely to become an oral cancer (37). OL generally involves all intraoral regions, but common sites of involvement include the dorsum and lateral areas of tongue. The tongue and floor of the mouth, are considered as high-risk zones because these areas exhibit dysplastic transformation for SCC formation (38), however; although the tongue and oral cavity are dangerous zones for malign transformation, it may vary and all the suspicious cases should be monitored carefully regarding oral cancer (36).

Smoking and use of tobacco products are shown in the etiology of OL, however, malignancy potential may vary with the severity of these habits. Even though it has been suggested that HPV is also considered in the etiology of the disease, there are conflicting results in the literature (36,37). It has also been reported that in addition to mentioned etiologic factors alcohol consumption, chronic irritation, trauma and oral candidiasis may be causative factors of OL (39).

OL is generally divided into 2 main groups as homogeneous and non-homogeneous. While homogeneous OL is in the form of thin white flat layer on the mucosal membranes, non-homogeneous type of OL lesions may be seen as speckled, nodular or verrucous form. The speckled type may be white-red clinical appearance and verrucous type of OL lesions may be elevated, proliferative or corrugated clinical appearance (40). It has been reported that the proliferative form is generally more aggressive, malignant and recurrent than the others (41). Clinical and histopathological examination is required in accurate diagnosis of OL lesions as in OLP. One of the most important criteria for histopathological

appearance of OL is "epithelial dysplasia". van der Waal et al. (36) reported that epithelial dysplasia is the most critical factor for malignant potential of OL, but emphasizes that lesions without epithelial dysplasia may become malignant. Therefore, all OL patients should be carefully monitored for the risk of oral cancer. Treatment options for OL include chemotherapeutic agents (vitamins A, C, E), fenretinide, carotenoids, bleomycin, protease inhibitors and antiinflammatory drugs and elimination of risk factors. In addition, conventional excisions, cryosurgery and laser applications are the main surgical approaches for OL (42).

The incidence of classical OL in pediatric population is very rare, however in children with HIV+, hairy leukoplakia (HL), a form of leukoplakia, is found at high rates (43-45). The etiological factor of HL is known as *Epstein-Barr* virus and in clinical examination, HL lesions show flat, corrugated or hairy appearance and are located on the lateral or ventral surfaces of the tongue (45). HL lesions are not only seen in HIV+ children but also in other immunosuppression cases. These include leukemia and organ transplantation cases (46,47). In children with HL, treatment is usually provided by the elimination of the predisposing pathologies (48). In addition, although the OL is not common in children, dyskeratosis congenita (DC) associated OL is more prevalent in children. OL is the most primary oral findings of DC. OL in DC patients should be carefully examined and early treatment options should be performed. In this manner, the presence of OL findings may be beneficial to the clinician in DC diagnosis.

### **Oral Erythroplakia**

Oral erythroplakia (OE) is defined as "a fiery red patch that cannot be characterized clinically or pathologically as any other disease". OE lesions are well-defined, clinical appearance shows irregular in outline, bright red velvety surface and sometimes granular (49). The red color of the OE lesions is due to the appearance of the underlying microvascular area of the epithelium. Heavy alcohol consumption and smoking are the main etiological factors of OE (50). The most common location of OE is soft palate and accurate differential diagnosis should be made with other erythematous oral lesions by histopathological examination (49-51). In this manner, as with the other lesions, OE lesions should be diagnosed by clinical and

histopathological examination. Histopathologically, epithelium of oral mucosa is mostly atrophic, non-keratinized and sometimes hyperplastic. Biopsy and surgical excision are recommended in surgical treatment of OE (52,53). In some cases, OE can be seen with OL, grouped together, and it is termed erythroleukoplakia. The most common sites of involvement include floor of the mouth, soft palate, ventral surface of the tongue and tonsillas. Although this lesion is usually asymptomatic, sometimes patients may complain of burning sensation or pain (50). OE is rarely seen in children (54), nevertheless child patients should be carefully assessed and followed regarding the formation of these lesions and high malignancy risk (7). Otherwise, lesion showing rapid dysplastic formation is at risk of carcinoma in situ or invasive carcinoma (49-51).

#### **Oral Submucous Fibrosis**

Oral submucous fibrosis (OSF); is a premalignant oral mucosa lesion characterized by chronic inflammation and progressive fibrosis of the oral submucosal tissues including lamina propria and deeper connective tissues. The main findings of OSF are marked rigidity in oral tissues, leathery mucosal texture, loss of fibroelasticity and severe limitation of mouth opening (55,56). It has been reported that the OSF may even affect the pharynx in some cases in the oral region (56). Clinical examination of OSF reveals white discoloration of oral mucosa, pigmentation or loss of papilla, sunken cheeks and limited mobility, horizontal bands along the soft palate, deformation in the uvula and mucosal burning sensation (49,57). However, it is stated that the clinical findings in OSF are quite variable and the severity of disease can be measured by the limitation of mouth opening (49).

Areca nut chewing are the dominant factor in OSF etiology and it is used as fourth harmful habit in the world after tobacco, alcohol and caffeine (58). The use of Areca nut is widely preferred, especially in India, Western Pacific, South and Southeast Asia (21,59). The sale of Areca nut products in attractive and coloured packages in general public areas in recent years has led to an increase prevalence of OSF in young populations. The addiction of areca nut and the increased risk of OSF especially in children aged 5-12 cause great concerns (21). Gupta et al. (21), in a case study of two children aged 10 and 11 years, reported

that children were chewing areca nut for 6-7 years and main clinical findings were limitation of mouth opening, burning oral mucosa and poor oral hygiene.

In case of OSF diagnosis; providing regular nutrition, avoidance of spicy foods, maintenance of oral hygiene, mouth opening exercises to increase mouth opening and follow-ups are recommended. Gupta et al. (21) reported that healing could be achieved by providing these proposals. It is stated such harmful habits that are risky in quality of life are generally acquired in childhood and this results in addiction. In this respect, it is recommended that pediatric specialists should be aware of this issue, informed about early diagnosis practices such as detailed clinical examination, biopsy and active preventive approaches (21).

#### **Dyskeratosis Congenita**

DC is a rare hereditary bone marrow failure syndrome and DC patients have increased risk of malignancy and morbidity (49,60,61). DC disease characterized by a triad which include dystrophic nails, reticular skin pigmentation and leukoplakic patches in mucous membranes. Pulmonary fibrosis and liver diseases are also accompanied by the syndrome. DC disease is thought to originate from early telomeric shortening and it is inherited autosomal dominant, autosomal recessive or X-linked genetic pattern (60-62).

OL is a common oral finding in 65-80% of the patients with DC (49,62). OL is generally seen in the dorsum of tongue and buccal mucosa, similar to classic OL (63). Tongue is affected at a young age and in most cases, especially in children under 15 years of age (49,64). Therefore, OL which seen in DC differs prevalence from classical OL in children. Ogden et al. (65) reported a 10-year-old patient had OL and all the triad of DC with a high malignant risk. Bongiorno et al. (62) also reported that DC can be transformed to many types of cancer, especially oral SCC in the head and neck region. In addition, oral gingivitis, gingival bleeding, dental caries and periodontitis are seen as oral findings in patients with DC in addition to OL (66). Due to the potential malignancy risk, diagnosis of DC should be provided at an early stage, the treatment process should be initiated and other dental treatments should be completed. Moreover, DC should be considered in all cases in which white lesions exists in oral cavity.

### Epidermolysis Bullosa

Epidermolysis bullosa (EB) is characterized by fragility of the skin and mucous membranes resulting in painful blisters and erosions (67). Intraoral findings such as ulcerations, scarring and microstomias are seen in all subtypes of EB (49). In some subtypes of EB, lesions are tended to be transformed into SCCs (68-70). As EB is found in children (69,71-73), early diagnosis is mostly important because of malignancy risk. In addition, it has been reported that in children with EB showed common dental caries and delay of tooth eruption. In childhood, dental treatments must be completed rapidly and the comfort of life should be provided (69,71,73).

Treatment of EB is usually based on vital support and symptoms. Perforation of the blisters accelerates the healing process and prevents the spread of the lesions. However, open wounds should be covered with non-adhesive materials such as sterile gauze or hydrogel (74). In addition, gene and cell therapy, recombinant protein infusions, intradermal injections of allogenic fibroblasts and stem cell transplantation are known as the current treatment options. The developing treatment modalities aim to improve wound healing and better quality of life for EB patients (71,72,75). Above all, as with other precancerous lesion, all EB cases should be detailed examined and monitored since it may be future malign changes.

### Conclusion

The decrease of initiation age of smoking, alcohol consumption and harmful addictional habits increases the risk of occurrence oral precancerous lesions in children especially in developing countries. However, despite these concerns, oral cancers and premalignant lesions are not seen commonly in children. The low prevalence rate of oral precancerous lesions in children compared to adults can be attributed to the need for a certain amount of time for the occurrence of destructive effects even if children are started these harmful habits at an early age. Therefore, pediatric patients who having these etiological factors should be monitored and followed up for the risk of development of oral precancerous lesions. These social concerns are the future troubles of the most part of the world and require immediate intervention. In this respect; parents, school teachers, children and adolescents should be carefully informed. On

the other hand, the probable complications of the precancerous lesions, decreased the quality of life and high mortality rates are require early diagnosis and treatment. Additionally, early diagnosis, carefully evaluating of the cases, monitoring and periodic follow-ups, evaluating the clinical and histologic status provide lower morbidity and mortality rates. Therefore, extraoral and intraoral examination of the head and neck region in pediatric patients have an important effect in diagnosis of the changes affecting oral mucosa and oral cavity. In this respect, all the findings showing premalignancy in pediatric patients should not be overlooked by medical doctors, pediatric specialists, pediatric dentists or oral and maxillofacial surgeons and multidisciplinary approaches should be provided.

### Ethics

**Peer-review:** Externally peer-reviewed.

### Authorship Contributions

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# Effect of Poly(Methyl Vinyl Ether-co-maleic Anhydride) Copolymer on Bond Strength of Experimental Dental Adhesive

## *Poli(Metil Vinil Eter-ko-maleik Anhidrit) Kopolimerinin Deneysel Dental Adeziv Bağ Dayanımına Etkisi*

Özge Çeliksöz<sup>1</sup>, Özgür Irmak<sup>1</sup>, Zeynep Dikmen<sup>2</sup>, Batu Can Yaman<sup>1</sup>, Vural Bütün<sup>2</sup>, Füsün Özer<sup>3</sup>

<sup>1</sup>Eskişehir Osmangazi University Faculty of Dentistry, Restorative Dentistry, Eskişehir, Turkey

<sup>2</sup>Eskişehir Osmangazi University Faculty of Science, Polymer Science and Technology, Eskişehir, Turkey

<sup>3</sup>University of Pennsylvania School of Dental Medicine, Preventive and Restorative Sciences, Philadelphia, PA, USA



### Keywords

Adhesive, bond strength, experimental adhesive, micro-tensile, PVM/MA

### Anahtar Kelimeler

Adeziv, bağ dayanımı, deneysel adeziv, mikro-gerilim, PVM/MA

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### Address for Correspondence/Yazışma Adresi:

Özge Çeliksöz DDS,  
Eskişehir Osmangazi University Faculty of  
Dentistry, Restorative Dentistry, Eskişehir,  
Turkey

Phone : +90 506 964 75 05

E-mail : ozgee\_ozdil@hotmail.com

ORCID ID: orcid.org/0000-0003-3095-6815

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### Abstract

**Objective:** The contents of adhesive systems directly affect their bond strength. The purpose of this *in vitro* study was to evaluate the bond strength of experimental two-step self-etch (SE) adhesive containing Poly(methyl vinyl ether-co-maleic anhydride) (PVM/MA) Copolymer (Gantrez S-97 HSU Solution, USA) and to compare its bond strength with those of commercially available SE adhesives and experimental two-step SE adhesive free of PVM/MA copolymer.

**Materials and Methods:** Fifty extracted human molar teeth were randomly divided into 5 equal group as follows (n=10); Clearfil SE Bond (SEB) (Kuraray Co., Osaka, Japan), Clearfil Protect Bond (PrB) (Kuraray Co., Osaka, Japan), Clearfil Universal Bond (UniB) (Kuraray Co., Osaka, Japan), experimental adhesive (ExpA), ExpA containing PVM/MA (ExpA-G). After application of the adhesives to dentin, resin composites were bonded. Specimens were stored in distilled water for 24 h, and sectioned into beams followed by subjection to microtensile bond forces. Data were analyzed with ANOVA followed by Holm-Sidak multiple comparison test ( $\alpha=0.05$ ).

**Results:** ANOVA results revealed a significant difference between the mean bond strength values of the adhesives ( $p<0.0001$ ). Multiple comparisons showed that mean bond strengths of all of the adhesives were significantly different from each other ( $p<0.05$ ). Highest bond strength was obtained in commercially available two-step SE adhesive, SEB ( $15.93\pm6.84$  MPa); while the lowest bond strength was obtained in the ExpA containing PVM/MA, ExpA-G ( $6.08\pm2.85$  MPa). The ExpA containing PVM/MA (ExpA-G) showed lower bonds strength values than the ExpA without PVM/MA (ExpA) ( $p<0.05$ ).

**Conclusion:** Addition of PVM/MA into the experimental two-step self-etch adhesive led to lower bond strengths.

### Öz

**Amaç:** Adeziv sistemlerin içeriği bağlanma dayanımını doğrudan etkiler. Bu çalışmanın amacı, deneysel olarak hazırlanan iki aşamalı kendinden asitli dental adeziv materyale Poly(methyl vinyl ether-co-maleic anhydride) Copolymer (Gantrez S-97 HSU Solution, USA) kopolimerinin ilavesinin dentin bağ dayanımına etkisinin, piyasada bulunan kendinden asitli diğer bağlayıcı ajanlarla karşılaştırılmasıdır.



**Gereç ve Yöntemler:** Deneyisel olarak hazırlanan dental adeziv içeriğine %10 oranında PVM/MA ilave edildi. Elli adet çekilmiş insan dişi rastgele 5 eşit gruba ayrıldı (n=10). Deney grupları şu şekildedir: Clearfil SE Bond (SEB) (Kuraray, Osaka, Japan), Clearfil Protect Bond (PrB) (Kuraray, Osaka, Japan), Clearfil Universal Bond (UniB) (Kuraray, Osaka, Japan), deneyisel adeziv (ExpA), PVM/MA kopolimeri eklenmiş deneyisel adeziv (ExpA-G). Restorasyonu tamamlanan dişler 24 saat distile suda bekletildikten sonra kestiler alınarak mikrogerilim bağ dayanımı testi uygulandı. Veriler tek yönlü varyans analizi ve Holm-Sidak çoklu karşılaştırma testiyle analiz edildi ( $\alpha=0,05$ ).

**Bulgular:** Varyans analizi sonuçları test edilen adezivlerin ortalama  $\mu$ GBD değerleri arasında istatistiksel olarak belirgin bir fark olduğunu ortaya koydu ( $p<0,0001$ ). Çoklu karşılaştırma sonuçları ise tüm grupların ortalama  $\mu$ GBD değerlerinin istatistiksel olarak birbirlerinden farklı olduğunu gösterdi ( $p<0,05$ ). En yüksek  $\mu$ GBD iki aşamalı SE adeziv olan SEB grubunda [ $15,93\pm6,84$  megapascal (MPa)] gözlenirken, en düşük değer iki aşamalı SE deneyisel adeziv olan ExpA-G grubunda ( $6,08\pm2,85$  MPa) tespit edildi. PVM/MA içeren ExpA-G ( $6,08\pm2,85$  MPa) grubunun  $\mu$ GBD değerleri, PVM/MA içermeyen deneyisel adeviz olan ExpA ( $9,46\pm2,68$  MPa) grubundan anlamlı olarak düşük bulundu ( $p<0,05$ ).

**Sonuç:** Deneyisel adezive PVM/MA kopolimeri ilavesi bağlanma dayanımını olumsuz yönde etkilemiştir.

## Introduction

Despite the advancements in dental adhesive and restorative systems, polymerization contraction of composite resins and resulting micro-gaps at the tooth-restoration interface is still a major problem in restorative dentistry. Cariogenic bacteria may leak through these gaps and lead to secondary caries (1-3). Another cause of formation of secondary caries is residual caries and bacteria (4). Even if a leakproof restoration is built; residual bacteria can live more than 1 year and grow (5). Secondary caries is one of the main reasons for the need of replacing the composite restorations (6,7). Therefore, besides providing a good seal, it is necessary to reduce the amount of residual bacteria for a long-lasting restoration. In this manner, the most essential and promising strategy is to add antibacterial agents to resin composites or adhesives.

The first adhesive system in which an antibacterial group added was Clearfil Protect Bond (PrB) (Kuraray Co., Osaka, Japan). It is a two-step self-etch (SE) adhesive system containing antibacterial methacryloyloxydodecyl pyridinium bromide (MDPB) monomer, in the acidic primer of the system (8). This MDPB containing adhesive has antibacterial effect in case of contact, even if it does not release any antibacterial agent when polymerized (9-12).

Poly(methyl vinyl ether-co-maleic anhydride) (PVM/MA) is a biodegradable polyanhydride which is known as 'Gantrez' in the market and is used widely for pharmaceutical purposes. This molecule is a suitable copolymer for the preparation of dosage forms with nanoparticles having bioadhesive or mucoadhesive properties (13). It can be used for treatment of toothache, bacterial and fungal infections, aphthous ulcers, lichen planus, inflammation and stomatitis, which occur in the mouth cavity (14). The PVM/MA

copolymer has the effect of reducing the adhesion of bacteria to the enamel surface. This effect may be attributed to the prevention of calcium accumulation on the enamel surface of the carboxylate side chains that chelate calcium ions (15). There is a commercial toothpaste (Colgate Total, Colgate-Palmolive Company) containing a combination of triclosan, PVM/MA and fluoride. PVM/MA enhances the effect of triclosan and its antibacterial properties and allows it to stay longer on the tooth surface (16). A mouthwash containing 3% triclosan and 0.25% PVM/MA has been shown to significantly reduce dental plaque when used before or after brushing (17).

Taking advantage of antibacterial properties by adding PVM/MA copolymer into a dental adhesive system could be promising. Therefore, it is essential to know the bonding performance of the adhesive systems with antibacterial agent. In this study, it was aimed to evaluate the bond strength of an experimental SE adhesive system containing antibacterial agent, PVM/MA copolymer. The first null hypothesis tested was that the bond strength of experimental adhesive would not be different from the bond strength values of the commercial adhesives tested. The second null hypothesis was that addition of PVM/MA to the experimental adhesive would not affect the bond strength of the experimental adhesive.

## Materials and Methods

In this study, 50 extracted human molar teeth were used. Teeth were stored at room temperature in distilled water until the time of experiment. Teeth were embedded in acrylic blocks 2 mm below the cemento-enamel junction. Occlusal surfaces of the teeth were cut horizontally at low speed under water cooling with diamond cutting disc (Isomet, Buehler

**Table 1. Grouping of samples**

Group	Adhesive system	Composite
Group 1 (SEB)	Clearfil SE Bond	Z550
Group 2 (PrB)	Clearfil Protect Bond	Z550
Group 3 (UniB)	Clearfil Universal Bond	Z550
Group 4 (ExpA)	Experimental Adhesive	Z550
Group 5 (ExpA-G)	Experimental Adhesive with PVM/MA	Z550

SEB: SE Bond, PrB: Protect Bond, UniB: Universal Bond, ExpA: Experimental Adhesive, ExpA-G: Experimental Adhesive with polymethyl vinyl ether-co-maleic anhydride, PVM/MA: Polymethyl vinyl ether-co-maleic anhydride, SE: Self-etch

Ltd., Lake Bluff, IL, USA) to obtain deep dentine tissue. Exposed dentin surfaces were polished with 600 grit silicon carbide paper under running water for 30 seconds to obtain a uniform smear layer.

The study were approved by the Eskişehir Osmangazi University of Local Ethics Committee (date: 18.04.2016, no: 13).

#### Grouping of Samples and Restorative Procedures

Teeth were randomly and equally divided into 5 groups according to the adhesive systems (n=10) (Table 1).

After application of the commercial adhesive systems [SE Bond (SEB), PrB and Universal Bond (UniB)]

**Table 2. Adhesive systems and restorative materials used in the study**

Material	Type	Content	Lot number	Manufacturer
<b>Clearfil SE Bond</b>	Two step self-etch adhesive	<b>Primer:</b> 10-methacryloyloxydodecyl dihydrogen phosphate, 2-hydroxyethyl methacrylate, hydrophilic aliphatic dimethacrylate, dl-camphorquinone, N, N-diethanol-p-tolidine, water <b>Bond:</b> 10-methacryloyloxydodecyl dihydrogen phosphate, bisphenol A diglycidyl methacrylate, 2-hydroxyethyl methacrylate hydrophobic aliphatic dimethacrylate, dl-camphorquinone, N, N-diethanol-p-tolidine, colloidal silica	000181	Kuraray Co., Osaka, Japan
<b>Clearfil SE Protect Bond</b>	Two step self-etch adhesive	<b>Primer:</b> 10-methacryloyloxydodecyl dihydrogen phosphate, 12-methacryloyloxydodecylpyridinium bromide, 2-hydroxyethyl methacrylate hydrophilic dimethacrylate, water <b>Bond:</b> 10-methacryloyloxydodecyl dihydrogen phosphate, bisphenol A diglycidyl methacrylate, 2-hydroxyethyl methacrylate hydrophobic aliphatic dimethacrylate, dl-camphorquinone, N, N-diethanol-p-tolidine, colloidal silica, sodium fluoride	000015	Kuraray Co., Osaka, Japan
<b>Clearfil Universal Bond</b>	One step self-etch universal adhesive	10-methacryloyloxydodecyl dihydrogen phosphate, bisphenol A diglycidyl methacrylate, 2-hydroxyethyl methacrylate, hydrophilic aliphatic dimethacrylate, dl-camphorquinone, colloidal silica, silane coupling agent, ethanol, water	000019	Kuraray Co., Osaka, Japan
<b>Experimental Adhesive</b>	Two step self-etch adhesive	<b>Primer:</b> 40% 2-methacryloyloxy ethyl phosphate, ethanol <b>Bond:</b> HEMA, triethylene glycol dimethacrylate, bisphenol A glycerolate dimethacrylate, dimethylaminoethyl methacrylate, camphorquinone	-	-
<b>Experimental adhesive with Polymethyl vinyl ether-co-maleic anhydride</b>	Two step self-etch adhesive	<b>Primer:</b> 40% 2-methacryloyloxy ethyl phosphate, ethanol <b>Bond:</b> HEMA, triethylene glycol dimethacrylate, bisphenol A glycerolate dimethacrylate, dimethylaminoethyl methacrylate, camphorquinone, polymethyl vinyl ether-co-maleic anhydride	-	-
<b>Z550</b>	Nano hybrid universal composite resin	-	N728631	3M/ESPE, St. Paul, MN, U.S.A

SE: Self-etch, HEMA: hydroxyethyl methacrylate

according to the manufacturer's instructions, resin composite build-ups (Z550, 3M Espe, St. Paul, Mn, U.S.A.) were constructed in double 2 mm increments, with each increment being light-cured for 20 s. A light emitting diode curing device (Starlight S, Mectron, SPA, Loreto, Italy) with a light intensity of 800 mW/cm<sup>2</sup> was used for polymerization of the adhesives and resin composites. All adhesive systems and restorative procedures were performed by a single operator. The contents of adhesives and restorative materials used in the study are given in Table 2.

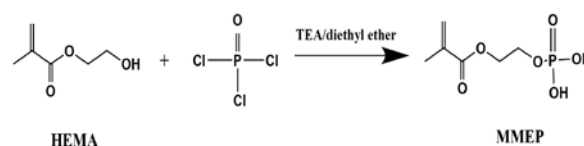
### Preparation of Experimental Adhesive

Two different two-step SE experimental adhesive systems were used in this study. Only difference between these adhesive systems were that one of the experimental adhesive system contained PVM/MA in its adhesive bottle.

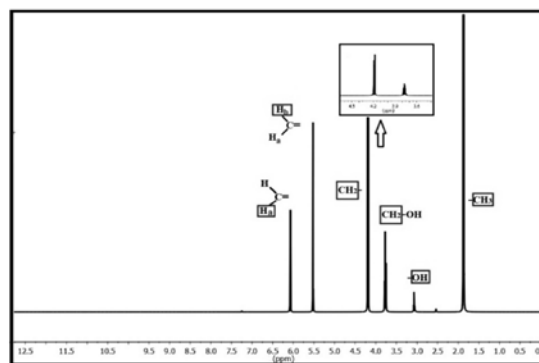
### 2- Methacryloyloxy Ethyl Phosphate (Methacryloyloxyethyl Dihydrogen Phosphate) Synthesis

Eighteen mL (0.148 mol) of 2-hydroxyethyl methacrylate (HEMA), 0.01 g of hydroquinone monomethyl ether inhibitor was dissolved in 175 mL diethyl ether with a magnetic stirrer in a three-neck round-bottom flask in an inert gas atmosphere. Twenty-two mL of triethyl amine (0.157 mol) was then added to remove the HCl acid which would form in the medium as a salt. A mixture of 13.82 mL of POCl<sub>3</sub> (0.148 mol) (nHEMA/nPOCl<sub>3</sub>:1:1) and 50 mL of diethyl ether at -40 °C was slowly added to the medium using a dropping funnel. When the addition of the POCl<sub>3</sub> solution was completed, flask was kept at room temperature nightlong. After stirring nightlong at room temperature, the triethylamine hydrochloride salt was removed from the medium by filtration. The organic phases were washed twice with 150 mL of saturated sodium chloride solution adjusted to a pH of about 1 with 1 N hydrochloric acid (HCl) and then the mixture was washed twice with an aqueous solution of which by weight 10% is 150 mL of NaHCO<sub>3</sub>. By extraction method, the aqueous phase and the diethyl ether phase were separated. The diethyl ether phase contains organic structures, while the aqueous phase contains salt forms of unreacted reactants. After drying the diethyl ether phase with anhydrous sodium sulphate, the solvent was evaporated under vacuum by means of an evaporator to a constant weight to yield 5 mL of light yellow-colored liquid (Figure 1) (18).

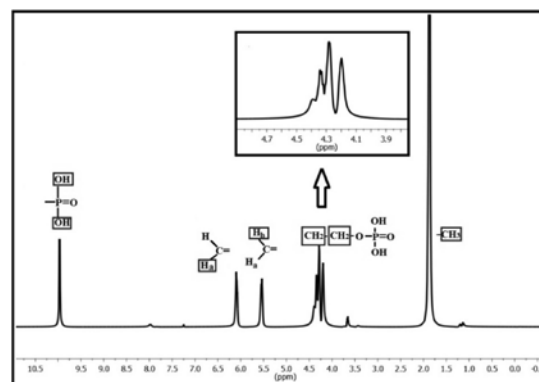
<sup>1</sup>H nuclear magnetic resonance (NMR) spectrum of the output molecule and the synthesized molecule in CDCl<sub>3</sub> are exhibited. In the after-synthesis NMR spectrum, protons of -OH groups due to phosphonic acid were observed at δ=10 ppm. Besides, the -OH group of the output molecule at δ=3 ppm disappeared. An upward chemical shift was observed in the CH<sub>2</sub> groups observed at δ=3.75 ppm bound to the OH group and the CH<sub>2</sub> groups observed at δ=4.15 ppm at the β position, and peaks at ~4.25 ppm were observed to coincide (Figure 2, 3).



**Figure 1.** 2-methacryloyloxy ethyl phosphate synthesis scheme  
HEMA: Hydroxyethyl methacrylate, MMEP: Methacryloyloxy ethyl phosphate



**Figure 2.** <sup>1</sup>H nuclear magnetic resonance spectrum (CDCl<sub>3</sub>) of 2-hydroxyethyl methacrylate output material  
CDCl<sub>3</sub>: Deuterated chloroform



**Figure 3.** The synthesized 2- (phosphonoxy) ethyl methacrylate molecule <sup>1</sup>H nuclear magnetic resonance spectrum (CDCl<sub>3</sub>)  
CDCl<sub>3</sub>: Deuterated chloroform

### Preparation of the Experimental Primer Mixture

The primer mixture was obtained by dissolving the freshly synthesized methacryloyloxy ethyl phosphate (MMEP) monomer in ethanol in 10%, 20%, 30%, 40% proportions, and used as the self-etching agent. In these samples prepared at different concentrations, the solutions prepared at 10%, 20%, 30%, could not be used because their demineralizing effects were not sufficient as complete premature failures of the specimens occurred during the sample preparation for the bond strength test.

### Preparation of Experimental Bond Mixture

A total of 10 g of adhesive material was prepared using 30% HEMA (3 g), 10% (1 g), triethylene glycol dimethacrylate and 60% (6 g) bisphenol A glycerolate dimethacrylate (BISGMA). 0.1 g dimethylaminoethyl methacrylate (1% of the total mass) was added as an accelerator. 40 mg camphorquinone (CQ) (0.4% of the total mass) was added as photoinitiator. Mixture was mixed at 1200 rpm for an hour.

### Adding PVM/MA into the Experimental Adhesive

2 g PVM/MA (Gantrez S-97 HSU Solution) (10% of total mass) was added to the bonding agent.

### Application of Experimental Adhesive Systems

Acidic primer (first bottle) of the experimental adhesive system without PVM/MA was applied to the dentin surface by rubbing for 40 s followed by 5 s air-drying. Adhesive (second bottle) was then applied to the dentin surface for 10 s and was light-cured for 40 s (Starlight S, Mectron Dental). Experimental adhesive material containing PVM/MA was applied exactly the same was described.

### Micro Tensile Bond Strength Test

After completing of the adhesive protocols, two 2 mm thick resin composite increments were bonded to the dentin surfaces. Each increment was light cured for 20 s. Restored specimens were kept in distilled water at 37 °C for 24 h. The samples were then cut along the long axis with a cutting device (Isomet, Buehler Ltd., Lake Bluff, IL, USA) at 300 rpm under water cooling to obtain test sticks of 1.00 mm<sup>2</sup> bonded cross-sectional surface area. Each obtained sample was glued to the test apparatus the microtensile test device (MOD Dental MIC-101, Esetron Smart Robotechnologies, Ankara, Turkey) with a cyanoacrylate adhesive, and was subjected to tensile force at a cross-head speed of 0.5 mm/min. The tensile force of each sample was

measured in Newtons. The bond strength of each sample was then recorded in megapascals, obtained by dividing the fracture force by the cross-sectional surface area.

### Statistical Analysis

The fitness of the obtained data to the normal distribution was examined by D'Agostino & Pearson omnibus normality test. One-way analysis of variance and the Holm-Sidak multiple comparison tests were performed after the data were determined to be normally distributed. Statistical significance was set at 0.05. A commercial statistical analysis software (Prism 6.0, GraphPad Software, La Jolla, CA, USA) was used for the analysis.

### Results

The variance analysis results showed a statistically significant difference between the mean micro tensile bond strength ( $\mu$ TBS) values of the tested adhesive systems ( $p < 0.0001$ ). Multiple comparison results showed that the mean  $\mu$ TBS values of all groups were statistically different ( $p < 0.05$ ). Mean  $\mu$ TBS values and standard deviations are given in Table 3. The highest  $\mu$ TBS was observed in the two-step SE adhesive system, SEB ( $15.93 \pm 6.84$  MPa), while the lowest value was observed in the two-step experimental SE adhesive system, Exp-G ( $6.08 \pm 2.85$  MPa). The mean  $\mu$ TBS of the experimental adhesive system containing PVM/MA, ExpA-G ( $6.08 \pm 2.85$  MPa), was significantly lower than that of the experimental adhesive system without PVM/MA, ExpA ( $9.46 \pm 2.68$  MPa) group ( $p < 0.05$ ).

**Table 3. The mean and standard deviation values of the groups for the micro-voltage bond strengths test**

Groups	Mean $\pm$ standard deviation (MPa)*
SEB	$15.93 \pm 6.84^a$
PrB	$13.45 \pm 4.22^b$
UniB	$12.56 \pm 4.47^c$
ExpA	$9.46 \pm 2.68^d$
ExpA-G	$6.08 \pm 2.85^e$

SEB: SE Bond, PrB: Protect Bond, UniB: Universal Bond, ExpA: Experimental Adhesive, ExpA-G: Experimental Adhesive with polymethyl vinyl ether-co-maleic anhydride, SE: Self-etch

\*Different letters indicate statistical differences between groups ( $p < 0.05$ )



## Discussion

In this study; bond strengths of experimental two-step SE adhesive systems with or without the addition of antibacterial PVM/MA copolymer were with that of 3 commercial adhesives were compared. As the bond strength of experimental adhesive systems was found to be significantly lower than that of commercial adhesives, the first null hypothesis that the bond strength of experimental adhesive systems would be similar to that of commercial adhesive systems was rejected. Furthermore, the addition of PVM/MA to experimental adhesive system significantly reduced the bond strength of the experimental adhesive system; therefore, the second hypothesis of the study was rejected.

Polymerizable phosphate monomers are widely used in the production of SE adhesive systems. These hydrophilic phosphate monomers are dissolved in solvents like pure water, ethanol-water or acetone-water mixtures (19). The MMEP monomer is one of these acidic phosphates and is present in the content of some commercial SE adhesive systems (19,20). In this study, we synthesized and added MMEP monomer in the experimental adhesive systems. Micro-shear bond strength of with MMEP containing adhesive system (Self & Etch Bond, Vigodent) was found to be very low compared to MDP containing two-step SE adhesive system (Clearfil SE Bond, Kuraray Co., Osaka, Japan) (21). Mentioned study was conducted in human enamel, so, it is impossible to know how this monomer will perform in the dentin. It was reported that the MDP monomer is more stable than the MMEP monomer (19). In our study, primer mixture of the experimental adhesive systems was obtained by dissolving synthesized MMEP in ethanol at 10%, 20%, 30%, 40% concentrations. All the samples bonded with the experimental adhesive systems having MMEP concentration below 40% in the primer solution, showed premature failures during sample sectioning for the  $\mu$ TBS test. This could indicate lower demineralization potential of MMEP at lower concentrations than 40% on human dentin. Correspondingly, the  $\mu$ TBS of the experimental adhesives synthesized in this study were lower compared to the commercial adhesives; Clearfil SE Bond, Clearfil PrB and Clearfil UniB.

The addition of the PVM/MA copolymer into the adhesive bottle of the SE experimental adhesive system negatively affected the  $\mu$ TBS to dentin. Two studies have investigated the effect of PVM/MA copolymer on dental adhesives. In the first study, 50 mg/mL PVM/MA copolymer (Gantrez S97 BF form) in powder was added to the primer and adhesive bottles of Clearfil SE Bond and to Prime & Bond NT, and 24 h fracture toughness tests were performed on the extracted human teeth. According to the results of the study, the addition of PVM/MA copolymer to the primer of Clearfil SE Bond or to the adhesive did not change the bonding values statistically. However, the addition of PVM/MA to Prime & Bond NT, which is a total etch (TE) adhesive system, has greatly reduced the bonding strength (22). The researchers noted that PVM/MA copolymer did not completely dissolve in the acetone-based Prime & Bond NT and formed microscopic clusters in the mixture. The reason for this might be that PVM/MA possesses carboxylic acid and anhydride side chains which may be more easily soluble in ethanol and water than in acetone. In the second study, 50 mg/mL PVM/MA copolymer (Gantrez S97 BF form) in powder form was added to the 2-step SE adhesive systems Clearfil SE Bond, Fluorobond II and TE adhesive system Adper Single Bond Plus. Twenty-four h micro-shear bond strength results indicated stated that the PVM/MA copolymer positively affected the bonding performance of Clearfil SE Bond and Adper Single Bond Plus, while Fluorobond II were not effected by the addition of PVM/MA (23). In the two studies mentioned above and in the present study, quite different methods were followed. While, in previous studies, PVM/MA in powder form was added to the commercially available adhesive systems; in the present study, liquid PVM/MA was added into the experimental adhesive during the production phase. PVM/MA copolymer has anhydride functional groups which are very reactive and can easily react with various mono and bifunctional reagents. These reactive anhydride structures can be easily degraded with water hydrolytically or with protic solvents. For example, reaction of water with anhydride groups results in a carboxylic acid containing polymeric structure. These groups confer weak acid quality to the polymer. The -OH end groups and carboxylic acid groups increase the ability of polymers to form hydrogen bonds with

-OH, -NH functional compounds such as water and alcohols. If bifunctional crosslinkers such as diamines and diols are used, PVM/MA chains can be cross-linked by amidation and esterification reactions, respectively. In addition, after converting anhydride groups to carboxylic acid groups, the carboxylic ends can be crosslinked via ring opening and esterification reactions with bis-epoxy compounds (24). Because of the sensitive properties mentioned above, the use of the PVM/MA copolymer during production may allow chemical bonding to the adhesive component, preventing possible side reactions with components such as moisture in the air.

## Conclusion

The addition of PVM/MA copolymer in experimental adhesive system with MMEP monomer negatively influenced the  $\mu$ TBS to dentin. MMEP containing experimental two-step SE systems showed lower bond strengths than that of the commercially available two-step and one-step SE adhesive systems. Further studies investigating the effect of PVM/MA on long-term bond strength of SE adhesives are needed.

## Ethics

**Ethics Committee Approval:** The study were approved by the Eskişehir Osmangazi University of Local Ethics Committee (date: 18.04.2016, no: 13).

**Informed Consent:** Consent form was filled out by all participants.

**Peer-review:** Externally peer-reviewed.

## Authorship Contributions

Concept: F.Ö., Ö.I., V.B., Z.D., Design: Ö.I., V.B., Z.D., Data Collection or Processing: Ö.Ç., Ö.I., Analysis or Interpretation: Ö.I., Ö.Ç., B.C.Y., Literature Search: Ö.I., Ö.Ç., Z.D., Writing: Ö.I., Ö.Ç., Z.D.

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# Effect of Initial Periodontal Treatment on Cardiovascular Risk Markers in Patients with Severe Chronic Periodontitis

## *Şiddetli Kronik Periodontitisli Hastalarda Başlangıç Periodontal Tedavinin Kardiyovasküler Risk Belirteçleri Üzerindeki Etkisi*

© Meltem Karşıyaka Hendek<sup>1</sup>, Ebru Olgun<sup>1</sup>, Üçler Kısa<sup>2</sup>

<sup>1</sup>Kırıkkale University Faculty of Dentistry, Department of Periodontology, Kırıkkale, Turkey

<sup>2</sup>Kırıkkale University Faculty of Medicine, Department of Biochemistry, Kırıkkale, Turkey



### Keywords

Cardiovascular disease, initial periodontal treatment, asymmetric dimethylarginine, endothelial nitric oxide synthase, homocysteine, monocyte chemoattractant protein-1

### Anahtar Kelimeler

Kardiyovasküler hastalık, başlangıç periodontal tedavi, asimetrik dimetil arjinin, endotelial nitrik oksit sentaz, homosistein, monosit kemoatraktan protein-1

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### Address for Correspondence/Yazışma Adresi:

Meltem Karşıyaka Hendek DDS, PhD,  
Kırıkkale University Faculty of Dentistry,  
Department of Periodontology, Kırıkkale, Turkey  
Phone : +90 318 224 49 27  
E-mail : mlmkrkyk@yahoo.com

ORCID ID: orcid.org/0000-0003-1518-4159

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### Abstract

**Objective:** The aim of the study was to determine the influence of initial periodontal treatment in patients with severe chronic periodontitis on inflammatory markers related to risk for cardiovascular diseases.

**Materials and Methods:** A total of 80 non-smokers with systemically healthy, including 40 patients (29 female, 11 male) with severe chronic periodontitis (test group) and 40 periodontally healthy participants (21 female, 19 male) (control group) were included into the present study. The probing depth, clinical attachment level, plaque index, gingival index and blood samples were collected at baseline and at the 3<sup>rd</sup> months after treatment and the serum levels of asymmetric dimethylarginine (ADMA), endothelial nitric oxide synthase (eNOS), homocysteine (Hcy), monocyte chemoattractant protein-1 (MCP-1) were determined with enzyme-linked immunosorbent assay.

**Results:** At baseline, all clinical periodontal parameters were significantly higher in the chronic periodontitis group than in the periodontally healthy group ( $p<0.05$ ). After the initial periodontal treatment, in the test group, all of the clinical periodontal parameters showed a significant decrease compared to the baseline values ( $p<0.05$ ). At baseline, ADMA, Hcy and MCP-1 levels were significantly higher in the test group than in the control group ( $p<0.05$ ), and after treatment ADMA and MCP-1 levels showed a significant decrease whereas eNOS level showed significant increase ( $p<0.05$ ).

**Conclusion:** It was observed that initial periodontal treatment in patients with severe chronic periodontitis has positive effects on cardiovascular risk markers.

### Öz

**Amaç:** Bu çalışmanın amacı, şiddetli kronik periodontitisli hastalarda kardiyovasküler hastalık ile ilişkili enflamatuvar belirteçler üzerinde başlangıç periodontal tedavinin etkisini belirlemektir.

**Gereç ve Yöntemler:** Bu çalışmaya, sistemik olarak sağlıklı, şiddetli kronik periodontitisli 40 hasta (29'u kadın, 11'i erkek) (test grubu) ve periodontal olarak sağlıklı 40 birey (21 kadın, 19 erkek) (kontrol grubu) olmak üzere toplam da 80 sigara içmeyen birey dahil edildi. Sondalama derinliği, klinik ataşman seviyesi, plak indeksi, gingival indeks ve kan örnekleri tedavi öncesinde ve tedaviden sonraki 3.

ayda toplandı ve asimetrik dimetilarginin (ADMA), endotelial nitrik oksit sentaz (eNOS), homosistein (Hcy) ve monosit kemoatraktan protein-1 (MCP-1) serum seviyeleri enzim-bağımlı immüno-sorbent analiziyle belirlendi.

**Bulgular:** Başlangıçta, tüm klinik periodontal parametreler kronik periodontitisli grupta periodontal olarak sağlıklı gruba göre anlamlı derecede yüksek bulundu ( $p<0.05$ ). Başlangıç periodontal tedaviden sonra, test grubunda, tüm klinik periodontal parametreler, başlangıç değerleri ile karşılaştırıldığında anlamlı bir düşüş gösterdi ( $p<0.05$ ). Başlangıçta test grubunda ADMA, Hcy ve MCP-1 düzeyleri kontrol grubuna göre anlamlı olarak yüksekti ( $p<0.05$ ), tedavi sonrası ADMA ve MCP-1 düzeylerinde anlamlı azalma, eNOS seviyesinde anlamlı artış saptandı ( $p<0.05$ ).

**Sonuç:** Şiddetli kronik periodontitisli hastalarda başlangıç periodontal tedavinin kardiyovasküler risk belirteçleri üzerinde olumlu etkileri olduğu gözlemlendi.

## Introduction

Periodontitis is a chronic infectious disease caused by the microbial dental plaque. The microorganisms in the subgingival environment and their products can enter the periodontal tissues and circulation through the epithelium, which is mostly ulcerated and impaired in integrity. While periodontal tissues give an immunoinflammatory response to these bacteria and their products, the systemic effects of these agents also result in a major vascular response (1). Recently, there has been an increased relevance between periodontitis and cardiovascular conditions. Bacteraemia caused by chronic infections and host-induced inflammatory products may cause to susceptibility to coronary heart disease by damaging of vascular endothelium. The damaged vessel endothelium is unable to function normally and endothelial dysfunction occurs (2). The clinical significance of endothelial dysfunction has been emphasized in study with increased risk of cardiovascular disease (CVD) in patients with endothelial dysfunction in coronary and peripheral arteries (3). Abundant evidence supports that periodontal disease-induced infections are potential risk factors for CVD (2,4). It has been shown that low-level chronic systemic inflammation has associated with undesirable cardiovascular outcomes (5). It has been shown that periodontal pathogens induce platelet aggregation, foam cell formation and atheroma development (6). Inflammation induces atheroma formation in major elastic arteries by damaging endothelial function. It leads to susceptibility to thrombotic and embolic conditions by disrupting the integrity of the arterial plaque and creating unstable plaque vascular spaces (7). Therefore, periodontitis, like many infectious diseases, is considered a risk factor for CVDs (8-10). Interventional trials suggest that periodontal therapy, in general, results in significant reduction of systemic

marker levels (11,12), especially in patients with systemic diseases such as CVD (12).

Nitric oxide (NO) has some functions in cardiovascular system, such as regulation of vasomotor tonus, modulation of myocardial contraction, platelet activation with control of cell proliferation, inhibition of adhesion and aggregation. NO is synthesized by nitric oxide synthases (NOS), which have three isoforms. Endothelial nitric oxide synthase (eNOS) enzyme, which is constitutively formulated from endothelial cells, has important roles in the cardiovascular system (13). Asymmetric dimethylarginine (ADMA) inhibits NO synthesis and causes vasospasm and endothelial dysfunction and is considered a risk factor for the development of coronary artery disease (14). Homocysteine (Hcy) is a sulfur-containing amino acid generated as an intermediate product in methionine metabolism. Increased levels of Hcy have been associated with a number of vascular complications, and due to this fact hyperhomocysteinemia has been classified as an independent risk factor for atherosclerosis and CVDs (15-17). Monocyte chemoattractant protein (MCP)-1 is one of the chemokines that contributes to the inflammatory process in atherosclerosis. It provides monocyte migration to the region of atherosclerotic lesions (18).

There is limited data on the possible effect of initial periodontal treatment on the systemic biomarkers of CVDs in systemically healthy individuals with severe chronic periodontitis (CP). Therefore, the objectives of the study were (1) to determine the levels of serum ADMA, eNOS, Hcy and MCP-1 in periodontal health and disease; (2) to assess the effect of initial periodontal treatment on these markers in patients with severe chronic periodontitis.

The hypothesis of this study was that severe chronic periodontitis adversely affects the levels of cardiovascular risk markers in serum in systemically



healthy individuals and initial periodontal treatment modifies these levels, favorably.

## Materials and Methods

### Study Population

40 patients (29 female, 11 male) with severe chronic periodontitis as a test group and 40 patients (21 female, 19 male) with periodontally healthy as a control group were enrolled in the study from Kırıkkale University Faculty of Dentistry, Department of Periodontology, Kırıkkale. Patients were excluded who: 1) had any systemic disease; 2) received any periodontal treatment within the previous year; 3) received any drugs such as non-steroidal anti-inflammatory drugs, systemic steroids, immunosuppressants, contraceptives, hormone drugs, anticoagulants, cholesterol regulating drugs, systemic antibiotics, antioxidants within the previous 3 months; 4) were pregnant or lactating; 5) consumed alcohol; or 6) were smoker. For consecutive patients fulfilling the inclusion criteria, the purpose and procedures were fully explained and they were asked to participate in the study. Patients were entered into the study only after verbal consent was obtained from each subject. The study was approved by the Ethics Committee of Kırıkkale University Kırıkkale, Turkey. (date: 06.07.2015, no: 19/07)

### Study Groups

At the screening visit, a full-mouth periodontal evaluation was performed in order to assess the inclusion/exclusion criteria. Patients with severe chronic periodontitis had teeth with alveolar bone loss,  $\geq 2$  non-adjacent sites per quadrant with clinical attachment level (CAL)  $\geq 6$  mm and bleeding on probing. The individuals with CAL  $\leq 2$ , no history of gingival inflammation and radiographic alveolar bone loss were included as the periodontally healthy group. The total number of teeth for each participant was  $\geq 20$ .

### Clinical Periodontal Parameters

One masked examiner (E.O.) assessed the periodontal status of each patient at baseline and three months post-treatment. Clinical periodontal measurements including plaque index (19), gingival index (20), probing depth (PD) (measured from gingival margin to pocket bottom) and CAL (measured from cemento-enamel junction to pocket bottom)

were taken from six sites of the teeth. Intra-examiner calibration was performed twice, before and during the study, by assessing PD and CAL in duplicate, with a degree of agreement within  $\pm 1$  mm higher than 85% at both tests. Under local anesthesia, within 14 days, initial periodontal treatment (scaling + root planing + polishing) was performed in patients with CP with manual and ultrasonic devices and standardized oral hygiene instructions including methods of tooth-brushing and interdental cleaning were also given to each one. During the study period, professional plaque control was performed monthly. Scaling and oral hygiene were carried out in periodontally healthy groups. All periodontal clinical measurements were repeated at baseline and the 3<sup>rd</sup> month after the treatment in the test group and at baseline in periodontally healthy group.

### Collection and Analysis of Blood Samples

Five mL venous blood samples were drawn in biochemical tubes including gel to prevent the potentiality of mixture of serum and plasma. The samples were centrifuged at 5.000 rpm for 10 minutes and stored at  $-80^{\circ}\text{C}$  until analysis. Serum samples were taken in the test group at baseline and at the 3<sup>rd</sup> month after treatment and in the control group at baseline. ADMA, eNOS, Hcy and MCP-1 in serum samples were measured by enzyme-linked immunosorbent assay using commercial kits according to the manufacturers' instructions.

### Statistical Analysis

To achieve 99% power and detect differences between groups, 40 participants in each group were required. At the end of the study, a post hoc analysis was also performed and 98% power was detected with a sample size of 28 for test group. The Shapiro-Wilk test was used to examine the normality of data distribution. The median (interquartile range) values were used for the non-normally distributed data. Differences between groups were analyzed by Mann-Whitney U test and intra group differences were analyzed by Wilcoxon test. Software was used for statistical analysis. A p value  $< 0.05$  was considered significant.

## Results

### Demographic and Clinical Findings

Initial periodontal treatment was performed on 40 individuals with CP. At the 3<sup>rd</sup> month, twelve

participants were lost during follow-up and 28 patients with CP completed all visits of the study. The demographical and periodontal parameters of the study participants are presented in Table 1. Age and gender distributions were not significant between the groups ( $p>0.05$ ). All clinical periodontal parameters were significantly higher in the chronic periodontitis group than in the periodontally healthy group ( $p<0.05$ ). In the test group, all of the clinical periodontal parameters after the initial periodontal treatment showed a statistically significant decrease compared to the baseline values ( $p<0.05$ ) (Table 1).

### Laboratory Findings

The ADMA, eNOS, Hcy and MCP-1 levels of the groups are shown in Table 2. ADMA, Hcy and MCP-1 levels in the test group were significantly higher than the control group, and ADMA and MCP-1 levels after treatment showed a significant decrease whereas eNOS level increased significantly ( $p<0.05$ ).

### Discussion

In this study, the effect of initial periodontal treatment on cardiovascular risk markers was

evaluated in patients with severe chronic periodontitis. According to this study, serum ADMA and MCP-1 levels significantly decreased with initial periodontal treatment and eNOS level significantly increased.

ADMA is an amino acid derivative synthesized endogenously by the methylation of arginine residues in proteins (21). ADMA has increasingly become a prevalent molecule, which is being intensively studied for use in clinical diagnosis. ADMA reduces ventricular contraction and heart rate, and its levels increase in cardiac failure (22). The mechanism of endothelial dysfunction caused by ADMA is due to the increase in vascular superoxide levels and decrease in the availability of vascular NO (23). Oxidative stress reduces the activity of the enzyme responsible for ADMA catabolism and thus increases the blood level while ADMA degradation is reduced (24). Therefore, in many degenerative diseases that increase oxidative stress, ADMA level is found to be increased. There are studies showing that ADMA plays a role not only in CVDs but also in the pathogenesis of renal diseases, type 2 diabetes mellitus and preeclampsia (25,26). It has shown that an increase in reactive oxygen

**Table 1. Demographic and clinical periodontal parameters in the study groups [median (interquartile range)]**

	Control group (n=40)	Test group (n=40)	Test group (post-treatment; n=28)
Age (year)	36 [6]	38.5 [7]	-
Gender (F/M)	21/19	29/11	-
PD (mm)	2.25 [0.84]	5.70 [1.10] <sup>a,b</sup>	3.68 [0.76]
CAL (mm)	0.00 [1.08]	6.26 [1.17] <sup>a,b</sup>	4.24 [0.36]
PI	0.33 [0.18]	2.19 [0.46] <sup>a,b</sup>	0.51 [1.01]
GI	0.33 [0.26]	2.07 [0.33] <sup>a,b</sup>	0.66 [0.10]

PD: Probing depth, CAL: Clinical attachment level, PI: Plaque index, GI: Gingival index, F: Female, M: Male  
<sup>a</sup>,  $p<0.05$ , difference between test and control groups  
<sup>b</sup>,  $p<0.05$ , difference before and after treatment

**Table 2. Serum asymmetric dimethylarginine, endothelial nitric oxide synthase, homocysteine, monocyte chemoattractant protein-1 concentrations in the study groups [median (interquartile range)]**

	Control group (n=40)	Test group (n=40)	Test group (post-treatment; n=28)
ADMA (pg/mL)	89.34 [65.13]	158.24 [147.74] <sup>a,b</sup>	104.54 [49.19]
eNOS (pg/mL)	183.58 [90.19]	197.45 [129.97] <sup>b</sup>	286.29 [80.23]
Hcy (nmol/mL)	15.28 [4.32]	17.09 [8.68] <sup>a</sup>	17.22 [5.91]
MCP-1 (ng/mL)	26.44 [18.97]	34.32 [23.22] <sup>a,b</sup>	28.06 [24.66]

ADMA: Asymmetric dimethylarginine, eNOS: Endothelial nitric oxide synthase, Hcy: Homocysteine, MCP: Monocyte chemoattractant protein  
<sup>a</sup>,  $p<0.05$ , difference between test and control groups  
<sup>b</sup>,  $p<0.05$ , difference before and after treatment

species (ROS) may cause endothelial dysfunction (27). The activation of endothelial cells induced by pro-inflammatory cytokines produces ROS that inactivate NO. Therefore, increase in NO inactivation and decrease in antioxidant system caused by overproduction of ROS may cause endothelial dysfunction in patients with periodontitis. Antoniadis et al. (28) have shown that oxidative stress and pro-inflammatory cytokines increase ADMA level and therefore cause endothelial dysfunction. In our study, serum ADMA concentration was higher in patients with chronic periodontitis than in periodontally healthy subjects, and there was a decrease in ADMA concentration from baseline with initial periodontal treatment. These results may be related to the fact that periodontitis, which is known to cause oxidative stress, may have also increased ADMA levels.

NO is secreted by the endothelium, and it has protective effects on vascular structure and function. Inhibition of smooth muscle proliferation, prevention of leukocyte adhesion, and platelet aggregation are among these effects. Damage to the endothelium causes a decrease in NO levels, thus leading to impairment in vascular function. In the absence of NO, proliferation is observed in the vascular smooth muscles and the elasticity of the vessel wall is reduced; hence, loss of flow-dependent vasodilatation occurs (29). In endothelial cells, NO is produced by endothelial NOS. ADMA inhibits NOS in humans and causes a decrease in NO levels, resulting in endothelial dysfunction (30). The increased level of eNOS with periodontal treatment in our study may have occurred due to decreased ADMA concentration.

Hcy is a sulfur-containing amino acid and is methylated to methionine as a metabolite. Elevated plasma Hcy levels are associated with oxidative damage to the vascular endothelium, vascular smooth muscle proliferation, and lipid peroxidation, and may result in peripheral arterial diseases and atherothrombosis (31). Possible mechanisms include the production of pro-inflammatory cytokines such as interleukins and TNF-alpha from inflammatory periodontal tissues. These mediators may initiate an inflammatory cascade with the potential to disrupt Hcy homeostasis and thereby increase plasma Hcy concentrations (32). In a case-control study, plasma Hcy levels were found to be significantly higher in patients with periodontitis than in periodontally

healthy subjects (33). In our study, high serum Hcy concentration in the test group compared with the control group can be explained by the presence of periodontitis, which leads to a systemic inflammatory process.

MCP-1 is a chemokine involved in cell migration throughout the inflammation process (34) and secreted from endothelial cells and vascular smooth muscle cells to attract circulating monocytes to the inflammation site (35). It is known that MCP-1 is associated with oral infections, owing to its monocyte chemotactic abilities (36). Previous studies have shown that MCP-1 expression is high in gingival biopsies from diseased periodontal areas (37) and that MCP-1 levels are high in gingival crevicular fluids of patients with periodontitis (38). In our study, serum MCP-1 concentration was found to be significantly higher in patients with chronic periodontitis than periodontally healthy patients, and a significant decrease was observed in the test group after treatment compared with baseline values, indicating that MCP-1 can be used as a marker for CVD in patients with periodontitis.

Unknown inflammatory conditions might have an effect on the observed cardiovascular risk biomarker levels. The lack of information on the individuals' cardiac or comprehensive medical profile represents a limitation of the study.

## Conclusion

Increased of periodontal inflammation may indicate raised levels of systemic cardiovascular risk biomarkers in systemically healthy subjects and initial periodontal treatment may but not totally reverse this condition. Proper periodontal management should be suggested to improve the systemic health conditions of patients. Further studies can be performed with a group consisted of CVDs to confirm the importance of periodontal treatment.

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## Ethics

**Ethics Committee Approval:** The study was approved by the Ethics Committee of Kırıkkale University Kırıkkale, Turkey. (date: 06.07.2015, no: 19/07)

**Informed Consent:** Patients were entered into the study only after verbal consent was obtained from each subject.

**Peer-review:** Externally and internally peer-reviewed.

#### Authorship Contributions

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

**Conflict of Interest:** The authors declare that they have no conflict of interest.

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# Patient-specific Root-analogue Immediate Titanium Premolar Dental Implants: Prospective Evaluation of Fifteen Patients with One-year Follow-up

*Kişiyeye Özel Kök Analogu İmmediat Titanyum Premolar Dental İmplantlar: On beş Hastanın Bir Yıllık Takiplerle İleriye Dönük Değerlendirilmesi*

✉ Ahmet Emin Demirbaş<sup>1</sup>, ✉ Emine Fulya Akkoyun<sup>1</sup>, ✉ Hasan Önder Gümüş<sup>2</sup>, ✉ Banu Arzu Alkan<sup>3</sup>, ✉ Alper Alkan<sup>4</sup>

<sup>1</sup>Erciyes University Faculty of Dentistry, Department of Oral and Maxillofacial Surgery, Kayseri, Turkey

<sup>2</sup>Erciyes University Faculty of Dentistry, Department of Prosthodontics, Kayseri, Turkey

<sup>3</sup>Bezmialem Vakıf University Faculty of Dentistry, Department of Periodontology, İstanbul, Turkey

<sup>4</sup>Bezmialem Vakıf University Faculty of Dentistry, Department of Oral and Maxillofacial Surgery, İstanbul, Turkey



## Keywords

CAD/CAM, dental implant, immediate, patient-specific, titanium

## Anahtar Kelimeler

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## Address for Correspondence/Yazışma Adresi:

Emine Fulya Akkoyun DDS, PhD,  
Erciyes University Faculty of Dentistry,  
Department of Oral and Maxillofacial Surgery,  
Kayseri, Turkey  
Phone : +90 352 207 66 00  
E-mail : efulyaakkoyun@hotmail.com

ORCID ID: orcid.org/0000-0003-4154-2503

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## Abstract

**Objective:** This study aimed to evaluate the clinical use and outcomes of the patient-specific root-analogue immediate titanium premolar dental implants (PRIs) that are manufactured by using computer numerical control (CNC) machine.

**Materials and Methods:** Three-dimensional models of the 15 non-restorable premolar teeth were constructed from cone-beam computed tomography datasets and transferred to a specific modelling software to design the PRIs. PRIs were manufactured from titanium by using a CNC machine and placed immediately after tooth extraction. Implants were evaluated clinically and radiologically one-year after implant placement.

**Results:** Fifteen patients (five males, 10 females), aged between 18-53 years (average 29.9) were included in the study. The success rate was 80% after one-year follow up. There was no peri-implant radiolucency around survival implants. The 1-year mean marginal bone loss was 1.1 mm ( $\pm 0.4$ ). Clinically healthy gingival margins were observed without any signs of periodontitis or implant mobility. The mean Periotest® value was  $-4.7 \pm 0.3$ .

**Conclusion:** Relatively high success rate was observed for upper premolar PRIs (92.3%) compared to lower premolar PRIs (50%). PRI concept is a new promising treatment modality. Further studies with long-term follow-up are necessary.

## Öz

**Amaç:** Bu çalışmanın amacı, dental bilgisayarlı sayısal kontrol (CNC) cihazıyla üretilen kişiyeye özel kök analogu immediat titanyum premolar dental implantların (KÖİ) klinik kullanımını ve sonuçlarını değerlendirmektir.

**Gereç ve Yöntemler:** On beş adet restore edilemeyen premolar dişin konik ışınli bilgisayarlı tomografi kesitleri kullanılarak 3 boyutlu modelleri oluşturuldu ve KÖİ tasarımı için özel bir modelleme yazılımına aktarıldı. CNC cihazı kullanılarak titanyumdan KÖİ'ler üretildi ve diş çekiminden hemen sonra yerleştirildi. İmplantlar,

yerleştirilmelerinden 1 yıl sonra klinik ve radyolojik olarak değerlendirildi.

**Bulgular:** On sekiz - yirmi üç (ortalama 29,9) yaşları arasında 15 (beş erkek, 10 kadın) hasta çalışmaya dahil edildi. Bir yıllık takip sonrasında sağkalım oranı %80 idi. Sağkalan implantlar etrafında peri-implant radyolusensi yoktu. Bir yıllık ortalama marjinal kemik kaybı 1,1 mm ( $\pm 0,4$ ) idi. Periodontitis bulgusu ya da implant mobilitesi olmaksızın klinik olarak sağlıklı gingival marjinler gözlemlendi. Ortalama Periotest® değeri  $-4,7 \pm 0,3$  idi.

**Sonuç:** Üst çenedeki implantlarda alt çenedekilere göre daha yüksek sağkalım oranı gözlemlenmiştir. KÖİ konsepti yeni ve umut vadeden bir tedavi yöntemidir. Daha uzun takip süreli ileri çalışmalara ihtiyaç vardır.

## Introduction

Prosthetic rehabilitation of edentulous patients with dental implants is a widely used treatment modality with long-term high success rates (1). Recently, with the development of immediate implant replacement, number of surgical procedures are reduced and overall treatment time is shortened when compared to delayed implant placement (2,3). After immediate implantation, guided bone regeneration is usually necessary due to incongruence between the implant and the extraction socket. Besides, this incongruence may cause the lack of primary stability. Hodosh et al. (4) introduced the patient-specific root-analogue dental implant (PRI) concept in 1969 from the idea of fabricating a more congruent implant to the extraction socket. PRIs are identical copies of the teeth to be extracted. Researchers have taken more interest in PRI concept recently with the advancements in Computer aided design (CAD)/computer aided manufacturing (CAM) technologies.

After Hodosh et al. (4) reported failure of autopolymerizing and heat-processed polymethacrylate PRIs, Lundgren et al. (5) used titanium root analogue implants in an experimental study in beagle dogs and a survival rate of 88% was reported. The major factor for the success of these implants was attributed to close-fitting between the implant and the socket. Therefore, Kohal et al. (6) enlarged the coronal part of the root analogue implants to compensate the width of the lost periodontium for better congruence but, the buccal alveolar bone fracture was occurred while placing the implants. However, direct bone-to-implant contact observed in all evaluated implants. In a prospective clinical study by Kohal et al. (7), 31 custom-made titanium implants were evaluated. During an average observation period of 9.1 months, 15 implants were lost before prosthodontic reconstruction and two were lost after crown insertion. This implant system

was not recommended for clinical application, due to this high failure rate over a short time period (7).

Recently, several clinical studies that titanium and zirconia PRIs were manufactured with different CAD/CAM techniques were reported. Direct metal laser sintering (DLMS), an additive manufacturing technology, was used to manufacture PRIs and high success rates were reported (8-10). DLMS technology is widely used in routine dental practice to fabricate the metallic frameworks of removable partial dentures, porcelain-fused-to-metal restorations and implant frameworks. However, these restorations are mostly fabricated from chrome-cobalt alloy powder. Titanium and its alloys are not used as much as chrome-cobalt due to its expense and limited indications. Unlike the DLMS machines, dental computer numerical control (CNC) machines are relatively cheaper, so they can be found in many dental clinics. Subtractive manufacturing of zirconia PRIs with dental CNC machines were also presented (11-16). A hybrid root analogue implant system was introduced recently in a pilot study. Moin et al. (17) fused together a titanium milled root analogue implant and a ceramic milled abutment portion to create one-piece implant.

PRIs with different design and manufacturing techniques and different modifications were evaluated in many experimental and clinical studies. However, there is a few CNC machined titanium PRI studies in the literature. The aim of the study is to show the feasibility of titanium premolar CNC-machined PRIs and to evaluate the clinical use and outcomes of the implants.

## Materials and Methods

### Patient Selection

This prospective study followed the Declaration of Helsinki on Medical Protocol and Ethics; and it was approved by the Local Ethics Committee of Erciyes University (2014/193). Between July 2015 and December 2016, all patients referred to Erciyes

University Faculty of Dentistry, Department of Oral and Maxillofacial Surgery were considered for inclusion in this study.

Patients who needed premolar tooth extraction due to root caries, vertical/horizontal root fracture, endodontic lesion, and unsuccessful root canal treatment were examined clinically and radiographically. Fractured and/or non-restorable teeth with uncompromised periodontal ligaments were included in the study. Exclusion criteria were uncontrolled systemic disease, bruxism, poor oral hygiene, and active periodontal disease. Chronic apical periodontitis and fenestration/dehiscence defects were not exclusion criteria. In case of chronic apical periodontitis, the area of infection was removed and fenestration/dehiscence defects were restored with alloplastic bone grafts after PRIs were placed. The study protocol was explained to each patient, and a signed informed consent was obtained.

### Cone Beam Computed Tomography Scan and Implant Design

Computed tomography (CT) datasets of the teeth were acquired using a CBCT (Cone Beam Computed Tomography) scanner (NewTom 5G, QR, Verona, Italy). CT datasets with voxel size of 0.25x0.25x0.25 mm were transferred in the DICOM format to a specific 3D reconstruction software (Mimics<sup>R</sup>, Materialise, Leuven, Belgium) and virtual 3D models of tooth, surrounding bone and opposing jaw were constructed for each patient. The tooth models were smoothed for obtaining a regular surface. The virtual models were exported as stereolithographic (STL) files and transferred to 3-matic<sup>R</sup> Modeling Software (Materialise, Leuven, Belgium). PRIs were designed by using this software. Macro-retentions on interdental surfaces of root were added. Reduction on the buccal and lingual faces of roots (0.1-0.2 mm) was made to avoid fractures on thin alveolar bone walls. The abutments in the shape of a prepared tooth with a taper of 5 degrees and chamfer margins were designed. Finally, all designed parts were merged to create a PRI. PRIs were smoothed and exported as STL files with three different sizes (original size, 5% increased and 5% decreased) to avoid potential distortions or errors related to the 3D projection steps. All these three STL copies were used to manufacture the PRIs using a dental CNC machine (Figure 1).

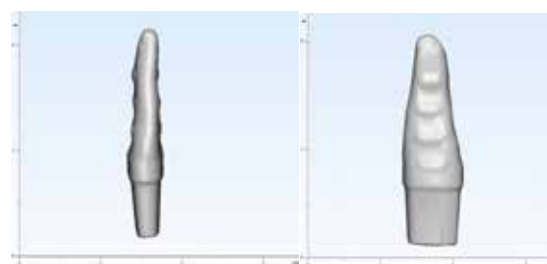
### Implant Manufacturing

The PRIs were milled from Ti-6Al-4V alloy blanks (Cupra Ti-5 Titanblank, Whitepeaks Dental Solutions GmbH&Co. KG, Essen, Germany) by using a five-axis CNC machine (Yenadent DC40 CAM, Yenadent Ltd, Istanbul, Turkey). Three PRIs (original, 5% increased and 5% decreased sizes) were fabricated for each case. The extraosseous part of each implant was polished. The intraosseous part of the implant was roughened by sandblasting with alumina and acid-etching with a mixture of orthophosphoric acid and nitric acid (15-20% diluted with distilled water) at 65 °C. Then PRIs were washed for 10 min. in distilled water at 45 °C in an ultrasonic bath. Finally, the implants were packaged and sterilized in a steam sterilizer (Getinge HS44, Getinge Infection Control AB, Switzerland) at 134 °C for 45 min.

The surface topography of PRIs was evaluated with scanning electron microscope and energy-dispersive X-ray spectroscopy analysis. The average surface roughness ( $R_a$ ) was measured with a profilometer (SurfTest SJ-301, Mitutoyo Corp, Kanagawa, Japan) and between 1.5-2  $\mu$ m which is accepted as ideal for osseointegration (18).

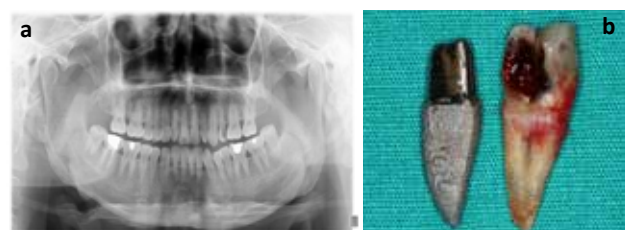
### Surgical Procedure and Postoperative Evaluation

All patients received nonsurgical periodontal therapy and oral hygiene education before implant placement. To reduce the risk of post-extraction bacteremia, 0.12% chlorhexidine gluconate mouth wash (Klorhex<sup>®</sup>, DrogSan, Ankara, Turkey) was administered 30 min before surgery. Under local anesthesia by infiltrating articaine 4% containing 1:100,000 adrenaline (Ultracain DS forte, Sanofi Aventis, Istanbul, Turkey) an intrasulcular incision was made and a minimally invasive flap was released to expose alveolar bone margins. Teeth were carefully extracted by applying predominantly vertical forces avoiding any damage to the socket and soft tissue



**Figure 1.** Stereolithographic file of the premolar dental implants, lateral and frontal view

(Figure 2). Then the extraction sockets were carefully debrided and irrigated with saline solution. The PRIs (first original size, but in case of incongruence, undersized-95% or oversized-105% PRIs) were placed in the sockets under finger pressure and gently tapped into the sockets with a hammer and a mallet (Figure 3). Primary stability was checked by percussion and palpation. At the end of the surgical procedure, interrupted sutures (Propilen, Doğsan, İstanbul, Turkey) were positioned and primary stability was measured by using Periotest® M (Medizintechnik Gulden, Modautal, Germany). In case of fenestration/dehiscence defects or minimum trauma of alveolar bone, (if primary stability could be obtained) the defects were reconstructed with particulate bone graft (Tutobone, RTI Biologics, Tutogen, Alachua, Fla, ABD) and collagen membrane (Tutopatch, RTI Biologics, Tutogen, Alachua, Fla, ABD). The patients received postoperative analgesics (Arveles<sup>®</sup>, Menarini, L'Aquila, Italy) on demand and antibiotherapy (Augmentin<sup>®</sup> 1 g, GalaxoSmithKline, Beecham, Brentford, UK) for 5 days. Mouth rinses with 0.12% chlorhexidine gluconate (Klorhex<sup>®</sup>, Drogosan, Ankara, Turkey) were also administered for seven days. Detailed instructions about oral hygiene were given. The patients were instructed to chew predominantly on the contralateral side and avoid hard foods.



**Figure 2.** a) Preoperative panoramic radiograph, b) The extracted upper left second premolar tooth and the premolar dental implants before placement



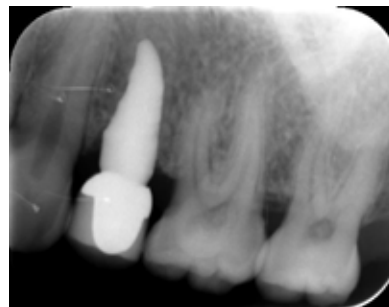
**Figure 3.** Premolar dental implants was placed in the socket and interrupted sutures were positioned

Immediately after implant placement, periapical radiographs were taken to confirm that PRIs were at the right position in the extraction sockets and to measure the distance between the implant apex and the first visible bone contact in millimeters for later measurement of marginal bone loss. Although parallel cone technique and film holders were used for reproducible radiographs, measurements were compared to the real implant length in case of dimensional distortions. Sutures were removed at seventh day after the surgery. The patients were seen weekly during the first month, then monthly until prosthetic rehabilitation. Three months later, metal-ceramic crowns were cemented (Figure 4). After that patients were seen every six months. In case of alveolar bone damage or incongruence of PRIs with the extraction sockets, conventional screw type implants were placed after bone healing.

After one year of functional loading, PRIs were evaluated clinically and radiographically. Presence of bleeding on probing, pocket depth, suppuration, pain, and mobility were investigated. Stability of PRIs were measured with Periotest® M. Periotest values (PTV) lower than 0 were accepted as well osseointegrated. Radiographically, peri-implant radiolucency and excessive bone loss were evaluated on periapical



**Figure 4.** The crown restoration 1-year after premolar dental implants placement



**Figure 5.** Periapical radiograph at 1-year follow-up

radiographs (Figure 5). Marginal bone level was measured and changes at 1 year were registered.

The success of the PRIs was defined, according to the criteria suggested for determination of success with reference to clinical and radiological parameters by Mangano et al. (9). PRIs that were still functional at the end of the study, after 1 year of functional loading, were categorized as survival. Implants presenting pain on function, suppuration, or clinical mobility were removed and categorized as failures (19). To achieve implant success, the following clinical and radiographic success criteria had to be fulfilled: absence of pain on function; absence of suppuration or exudation; absence of clinically detectable implant mobility; PTV <0; absence of continuous peri-implant radiolucency; and absence of prosthetic complications.

### Statistical Analysis

Survival of the implants was computed using the Kaplan-Meier method (SPSS Statistics 17.0, Chicago, IL). An implant survival curve with a 95% confidence interval (CI) was constructed. Data were analyzed descriptively for patients and quoted as mean values  $\pm$  SD.

### Results

Fifteen patients (five males, 10 females), aged between 18-53 years ( $29.9 \pm 10.9$ , mean  $\pm$  SD) were

included in the study (Table 1). A PRI was placed into fresh extraction socket immediately after premolar tooth extraction for each patient. Primary stability was achieved, PTVs were between -1.4 and -6.2 ( $3.9 \pm 1.4$ ). Four PRIs were placed in the mandible and 11 were in the maxilla. Bone grafts were used for dehiscence defects in 3 patients and fenestration defects in two patients and none of those implants were failed. At first week control visit, no complications, such as swelling, inflammation, bleeding and pain, were observed. The mean initial PTV was  $-2.1 \pm 1.8$  for lost implants and  $-4.5 \pm 0.8$  for survival implants. Three of 15 (20%) implants were lost within 24-53 days ( $40 \pm 19$ , mean  $\pm$  SD), before functional loading (Figure 6). Implants

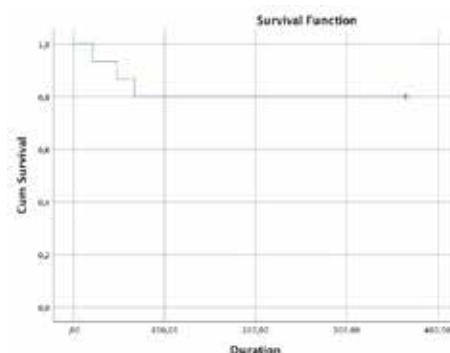


Figure 6. Kaplan-Meier estimate of implant survival

Table 1. Summary of patient information and clinical results of implant placement

Patient	Age (years)	Gender	Extracted tooth number	Implant status	Implant size (%)	Initial PTV	PTV at first year
1	34	F	25	Survival	100	-4.5	-5.4
2	40	F	15	Survival	100	-4.3	-5.4
3	22	F	25	Survival	100	-4.2	-4.4
4	38	M	24	Survival	100	-6.2	-5.6
5	20	M	24	Survival	100	-4	-5.8
6	49	F	15	Survival	100	-4.3	-3.7
7	30	F	15	Survival	95	-5.1	-6.0
8	19	F	25	Fail	95	-3.7	N/A
9	30	F	35	Survival	95	-4.4	-4.0
10	29	M	44	Fail	95	-1.4	N/A
11	23	F	24	Survival	95	-3.5	-4.1
12	21	M	25	Survival	95	-3.4	-3.5
13	23	M	35	Survival	100	-5.4	-5.5
14	53	F	25	Survival	100	-4.4	-2.7
15	18	F	35	Fail	100	-0.5	N/A

PTV: Periotest values, F: Female, M: Male, N/A: Not available, 100: Original size implant, 95: 5% decreased size implant



were loosened and suddenly lost without any pain, infection, noticeable bone resorption or soft tissue inflammation. After failed implants were removed, a soft tissue capsulation surrounding the socket walls was seen.

Three months later, metal-ceramic crowns were cemented. Patients were followed for 15-26 months. There was no peri-implant radiolucency around survival implants. Survival rate was 80% at the end of the observation period. The 1-year mean marginal bone loss was  $1.1 \pm 0.4$  mm (median, 0.5; CI 95%, 0.1-4.4). Clinically healthy gingival margins were observed without any signs of periodontitis or implant mobility. The mean PTV measured at one-year follow-up was  $-4.7 \pm 0.3$ . PTVs for each survival implant were lower than 0 at the end of the study. No prosthetic complications were observed. The prosthetic restorations were stable with good functional and esthetic results.

## Discussion

In recent years, the CAD/CAM technology has been widely used in dentistry with the advantages of improving the patient's comfort, reducing treatment time and amount of visits. New techniques for design and manufacturing of PRI were also developed with the advancements in this technology. In a clinical study, Pirker and Kocher (15) evaluated 18 zirconia PRIs that were placed in premolar/anterior region. In one group ( $n=6$ ), PRIs were roughened by sandblasting only, however all of the PRIs were lost. In the second group ( $n=12$ ), root was modified by adding microretentions, strictly limited to the interdental space, and by reducing the buccal and lingual face by approximately 0.1-0.2 mm, preventing fractures of the thin cortical bone layer at insertion before laser scanning and all of the PRIs in this group were survived. The authors concluded that by introducing significant modifications, primary stability and excellent osseointegration of immediate PRIs can be achieved, while preventing unaesthetic bone resorption. In the present study, these modifications were also applied digitally on implants with the help of a specific software before tooth extraction. In this manner, we are of the opinion that these modifications could be done in a more standardized way for each implant. In addition to these modifications, the abutments in the

shape of a prepared tooth with a taper of 5 degrees and chamfer margins were designed by using the same software. This design provided ease of prosthodontic procedures and resistant and retentive restorations with aesthetic results. In another study by Mangano et al. (9), 15 DLMS titanium premolar PRIs (eight in maxilla, seven in mandible) were evaluated. At the 1-year follow-up, a survival rate of 100% was reported. All implants were stable, with no signs of infection. In this study, there were no microretentions on root surface but, the authors also made a reduction of the diameter (0.1-0.3 mm) of the implant neck next to the thin vestibular cortical bone.

In the present study, survival rate was 80% which is lower than previously reported success rates in other studies. The primary and secondary stabilities were measured by using Periotest® M which is a quantitative test method. PTV ranges between -8 (clinically rigid) and +50 PTVs (very mobile). Lower PTVs indicate more stable implants. The mean initial PTV was  $-2.1 \pm 1.8$  for lost PRIs and  $-4.5 \pm 0.8$  for survival PRIs. These results indicate the importance of primary stability for implant survival.

Even though unchanged peri-implant marginal bone levels after 1 to 2.5 year follow-up were reported in several PRI studies (8,9,11-16), mean marginal bone loss was  $1.1 \pm 0.4$  mm (median, 0.5; CI 95%, 0.1-4.4) at first year in the present study. Nevertheless, all of the survival implants were stable and on function without any signs of peri-implantitis or implant mobility. Healthy gingival margins were observed with good esthetic results.

According to our previous clinical experience, molar or incisor/canine teeth were not included in the study. Titanium is a very hard material which makes it difficult to be milled with CNC-machines. The bifurcation of the molar implants was not milled accurate enough, so the implants were interfering with interradicular septum. Primary stability was achieved by adapting the septum to the implants. However, it resulted in high PTVs and initiation of bone resorption in the furcation area. Hence the most of the titanium molar PRIs were lost within 2-3 weeks. Despite this, the successful upper and lower molar zirconia PRIs were reported in the literature (12-14). Pre-sintered zirconia is a relatively softer material and easy to mill with CNC-machines. The grey color of titanium may

be reflected by the gingiva or it may get exposed in case of gingival recession, so it may not be favorable in the anterior region. Because of all of these, only premolar teeth were included in the study.

Implants were manufactured in three different sizes (original size, 5% increased and 5% decreased) to avoid potential distortions or errors related to the 3D projection steps. Differently from other studies in the literature (8,9,13,15,16), 5% decreased size implants were created instead of %10 increased-sizes. Because, in some cases a smaller implant which is more compatible with the extraction socket may be necessary, not only larger ones. In fact, none of the 5% increased-size CAIs were used in this study.

There are some conditions limiting the application of PRI technique. In the presence of curved and divergent roots, atraumatic tooth extraction and implant placement is difficult. Malposition, large periapical lesions, inadequate alveolar socket height also limits the feasibility. Even if this technique has high success rates, the precise patient selection criteria limit the feasibility of the technique.

## Conclusions

This study differs from other studies in terms of PRI design and manufacturing techniques. CNC machined premolar PRIs were placed and evaluated. Relatively high success rate was observed for upper premolar PRIs (92.3%) compared to lower premolar PRIs (50%). PRI concept is a new promising treatment modality. Further studies with long-term follow-up are necessary.

## Ethics

**Ethics Committee Approval:** This prospective study followed the Declaration of Helsinki on Medical Protocol and Ethics; and it was approved by the Local Ethics Committee of Erciyes University (2014/193).

**Informed Consent:** The study protocol was explained to each patient, and a signed informed consent was obtained.

**Peer-review:** Externally peer-reviewed.

## Authorship Contributions

Surgical and Medical Practices: E.F.A., A.E.D., H.Ö.G., A.A., A.A., Concept: E.F.A., A.E.D., H.Ö.G., A.A., Design: E.F.A., A.E.D., A.A Data Collection or Processing: E.F.A., A.E.D., Analysis or

Interpretation: E.F.A., A.E.D., A.A., B.A.A., Literature Search: E.F.A., A.E.D., A.A., Writing: E.F.A., A.E.D.

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# Evaluation of Double and Triple Antibiotic Paste Removal Efficiency of Various Irrigation Protocols

## Çeşitli İrrigasyon Protokollerinin İkili ve Üçlü Antibiyotik Patlarını Uzaklaştırma Etkinliklerinin Değerlendirilmesi

Evren Sarıyılmaz<sup>1</sup>, Cangül Keskin<sup>2</sup>

<sup>1</sup>Ordu University Faculty of Dentistry, Department of Endodontics, Ordu, Turkey

<sup>2</sup>Öndokuz Mayıs University Faculty of Dentistry, Department of Endodontics, Samsun, Turkey



### Keywords

Double antibiotic paste, triple antibiotic paste, regenerative endodontics, irrigation, removal efficiency

### Anahtar Kelimeler

İkili antibiyotik patı, üçlü antibiyotik patı, rejeneratif endodonti, irrigasyon, uzaklaştırma etkinliği

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### Address for Correspondence/Yazışma Adresi:

Evren Sarıyılmaz MD,  
Ordu University Faculty of Dentistry,  
Department of Endodontics, Ordu, Turkey  
E-mail : evrensariyilmaz@yahoo.com

ORCID ID: orcid.org/0000-0003-1711-7056

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### Abstract

**Objective:** Aim of this study was to compare the double (DAP) and triple (TAP) antibiotic paste removal efficiency of conventional syringe irrigation (CSI), EndoActivator (EA), passive ultrasonic irrigation (PUI) and XP-endo finisher (XPF) from simulated immature root canals.

**Materials and Methods:** A total of 180 extracted mandibular canine roots were used. The apical 2 mm of the roots were removed, and Unicore drills were penetrated through the apical to the coronal direction approximately 1 mm to simulate an immature apex. Canals were prepared up to size 40 by using the Mtwo system. Roots were split longitudinally and then temporarily reassembled. The samples were randomly divided into 2 main groups as DAP and TAP. Five specimens for each main group were kept as negative control group. Then the pastes were placed with lentulo spiral. After 1 week five specimens for each main group were kept as positive control group. Then the remaining specimens in DAP and TAP groups were further assigned to 4 subgroups according to the removal technique (group 1: CSI; group 2: EA; group 3: PUI, group 4: XPF). The root halves were examined under a stereomicroscope at 10x magnification. Kruskal-Wallis H and Mann-Whitney U tests were used for statistical analyses.

**Results:** TAP and DAP were more effectively removed by XPF and PUI compared to CSI and EA ( $p<0.05$ ). XPF and PUI DAP/TAP removal effectiveness was statistically similar ( $p>0.05$ ). Moreover, CSI and EA showed similar effectiveness for both paste groups ( $p>0.05$ ). Regarding the type of antibiotic paste, no significant difference between TAP and DAP was found ( $p>0.05$ ).

**Conclusion:** The tested irrigation systems could not completely remove either TAP or DAP. The use of PUI or the XPF was more effective compared to the use of the EA and CSI.

### Öz

**Amaç:** Bu çalışmanın amacı konvansiyonel şırınga irrigasyonu (KŞİ), EndoActivator (EA), pasif ultrasonik irrigasyon (PUI), ve XP-endo finisher (XPF) ile ikili (DAP) ve üçlü (TAP) antibiyotik patlarının uzaklaştırılabilme etkinliğinin karşılaştırılması amaçlanmaktadır.

**Gereç ve Yöntemler:** Toplam 180 adet çekilmiş mandibular kanin diş kullanılmıştır. Köklerin apikal 2 mm kısmı uzaklaştırılmıştır ve unicore frezler ile apikalden koronal

yöne doğru yaklaşık 1 mm ilerlenerek immature apeks simule edilmiştir. Kanallar Mtwo sistemiyle 40 numara genişliğine kadar prepare edilmiştir. Kökler longitudinal olarak ikiye ayrıldı ve sonrasında geçici olarak yeniden birleştirildi. Örnekler rastgele DAP ve TAP olarak iki ana gruba ayrıldı. Her ana grup için beşer numune negatif kontrol grubu olarak ayrıldı. Sonrasında lentulo ile patlar yerleştirildi. Bir hafta sonra her ana grup için beşer numune pozitif kontrol grubu olarak ayrıldı. Sonrasında DAP ve TAP gruplarındaki kalan numuneler uzaklaştırma yöntemine göre dörder alt gruba ayrıldı (grup 1: KŞİ; grup 2: EA; grup 3: PUI, grup 4: XPF). Kök yarıları 10X büyütmede stereo mikroskop ile incelendi. Kruskal-Wallis H ve Mann-Whitney U testleri istatistiksel analizlerde kullanıldı.

**Bulgular:** XPF ve PUI, KŞİ ve EA'ya kıyasla daha etkin olarak TAP ve DAP uzaklaştırmıştır ( $p < 0,05$ ). XPF ve PUI DAP/TAP uzaklaştırabilme etkinliği istatistiksel olarak benzerdi ( $p > 0,05$ ). Dahası, KŞİ ve EA de her iki antibiyotik patı için benzer etkinlik göstermiştir ( $p > 0,05$ ). Antibiyotik patı tiplerine bakıldığında ise TAP ve DAP arasında anlamlı farklılık bulunamamıştır ( $p > 0,05$ ).

**Sonuç:** Test edilen irrigasyon sistemlerinin hiçbirisi TAP yada DAP'yi tamamen uzaklaştıramamıştır. PUI veya XPF kullanımı EA ve KŞİ ile kıyaslandığında daha etkilidir.

## Introduction

Regenerative endodontics aims to restore necrotic immature teeth to a healthy state and to promote further root development and reinforcement of dentinal walls by the deposition of hard tissue. The success of regenerative endodontic treatment depends on the triad of pulp tissue engineering, which consists of infection control, use of biomaterials, and use of stem cells (1). Infection control is an indispensable step in endodontic regeneration to resolve periodontitis and to prevent persistent pathologic inflammation signals, which interfere with differentiation and maturation of the apical papilla cells and prevent root maturation (1). Disinfection of root canals in regenerative endodontics primarily depends on chemical debridement with minimal or no mechanical instrumentation (2). However, the polymicrobial nature of root canal infection necessitates the use of antibiotic combinations to achieve effective disinfection of root canal systems (3). Triple antibiotic paste (TAP), which consists of ciprofloxacin, metronidazole, and minocycline, has been proven highly effective against endodontic bacteria (4). Moreover, further studies were conducted to determine alternative medicament options for regenerative endodontics due to possible penicillin allergy (3) and/or the discoloration potential of the integrants, especially minocycline (5). Cefaclor (6), amoxicillin (7) and fosfomycin (8) were some recommended alternatives to minocycline in TAP and have been shown to be effective in eliminating bacteria in root canal systems. In addition, the clinical effectiveness of a double antibiotic paste (DAP) made from the combination of metronidazole and ciprofloxacin was also utilized (9).

The concentrations of antibiotics used locally in regenerative endodontics is 5.000 to 10.000 times greater than those in blood circulating levels. Therefore, the host cells as well as stem cells, which are essential for regenerative endodontics, have been under threat from non-selective toxicity of antibiotics (10). Ruparel et al. (11) revealed that TAP was destructive for stem cells. The stem cells in the periradicular region are crucial for endodontic regenerative treatments; Banch and Trope (12) study reported that the continued root formation and revascularization of the pulp occurred following the differentiation of these cells. Therefore, double and/or triple of any kind of antibiotic paste in the root canal must be removed to prevent possible tooth discoloration and detrimental effects on the host cells.

Various techniques are used for TAP removal from root canals; however, none of them can completely remove it from the root canal (10,13). The recently introduced XP-endo finisher file (XPF) (FKG Dentaire SA, La Chaux-de-Fonds, Switzerland) is a unique instrument that promises an enhanced final cleaning, even in the irregularities of root canal systems, due to its enhanced flexibility and expansion capability up to 3 mm in diameter to touch to the canal walls in three-dimensions (14). Previously, studies investigated the intracanal content removal effectiveness of the XPF with various irrigation systems (15-18). However, only a few studies have evaluated the efficacy of the XPF in removing TAP or DAP. One study compared the XPF with conventional syringe irrigation (CSI), laser activated irrigation, and Vibringe in removing DAP from artificial grooves, whereas another recent study compared XPF with passive ultrasonic irrigation (PUI) in the removal of TAP from immature roots (17). According to our literature research, there is no study that compares TAP and DAP removal efficiency of the



XPF with the EndoActivator (EA). Therefore, the aim of this study was to investigate the efficacy of the use of XPF, PUI, EA, and CSI in the removal of TAP and DAP from simulated immature root canals. The null hypotheses were that paste removal was not affected by the technique or antibiotic ingredients.

## Materials and Methods

This study was performed at both Ordu University and Ondokuz Mayıs University. The Ondokuz Mayıs University Clinical Researches Ethical Committee board approved the study protocol (OMU KAEK 2016/403). This study was performed in accordance with the World Medical Association Declaration of Helsinki and written informed consent was obtained from all participants. A total of 180 freshly extracted human mandibular canine teeth were selected. Teeth, which had immature apices, any kind of resorption, previous endodontic treatment, cracks or fractures, were excluded. The presence of single patent root canals was confirmed by using radiographs taken from the mesiodistal and buccolingual aspects. Samples were stored at 100% humidity and 37 °C till the experiments were conducted. Two mm of apical region the roots were removed and size 4 green 1.5 mm diameter Unicore drills (Ultradent Products, Inc., South Jordan, UT, USA) were penetrated approximately one mm through the apical to the coronal direction to simulate an immature apex. The crowns of the teeth were partially removed to standardize working lengths as 16 mm using a sterile diamond burs under water-cooling and then the endodontic access was prepared. Afterwards, a #10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) was used to extirpate the pulp. Mtwo NiTi rotary file system (VDW, Munich, Germany) up to #40 (40.04) file was used to prepare root canals under 1.5% sodium hypochlorite (NaOCl) irrigation. A 30-g side-vented irrigation needle (NaviTip; Ultradent, South Jordan, UT, USA) was used for irrigant delivery. After the chemomechanical root canal preparation, the root canals were irrigated using distilled water (2 mL), 17% ethylenediaminetetraacetic acid (EDTA), (2.5 mL) and finally distilled water (2 mL), respectively. Then, #40 paper points were used to dry the root canals of the specimens.

A test apparatus that described in a previous study by Topçuoğlu et al. (19), was used for this study. The

roots were placed in silicone impression material (Zetaplus; Zhermack, Rovigo, Italy) and then placed in 1.5 mL Eppendorf tubes. Afterwards, waited until the silicone was completely set. Next, the teeth were removed from the test apparatus and longitudinal grooves on the lingual and buccal surfaces of the roots were prepared. Subsequently, the roots were split into halves using a hammer and chisel. Debris from the root halves was removed using a toothbrush under running tap water. A small amount of cyanoacrylate glue (Scotch Super Glue Gel; 3M, St. Paul, MN, USA) was used to temporarily brought the root halves together to have adequate structural durability to the effect of DAP/TAP placement and removal procedures and transportation till the microscopic investigations. Subsequently, the samples were remounted into the impression in the Eppendorf tubes. The samples were divided into two major groups: the DAP and TAP groups.

Ten specimens were randomly chosen as negative controls for the DAP and TAP groups (n=5) and were not subjected to further procedures. In the TAP group, equal portions of metronidazole (Sanofi, İstanbul, Turkey), ciprofloxacin (BioFarma, İstanbul, Turkey), and cefaclor (Basel, İstanbul, Turkey) powders were mixed (1:1:1); whereas metronidazole and ciprofloxacin were mixed in a 1:1 ratio for DAP. Then distilled water was mixed with antibiotic powder mixtures (liquid/powder ratio of 1:3) (20) and then this freshly prepared TAP and DAP was placed into each root canal with a lentulo spiral according to its working length and packed by using size 0 Buchanan hand plugger (SybronEndo, Scafati, Italy). Then the specimen was removed from the tube and the presence of excess antibiotic paste was confirmed at the immature root tip to ensure that the root canals were completely filled with the antibiotic paste. The extruded part of the paste was removed by using moist gauge and the specimens were remounted into the tubes. Cotton pellets were placed on the TAP and DAP, and than some temporary sealing material (Cavit G; 3M ESPE, Seefeld, Germany) was used to seal the access cavities. The apical openings were also sealed with temporary sealing material to prevent the pastes from dissolving due to humidity during the storage period. Afterwards, all the samples were stored at 37 °C at 100% humidity for one month.

At the end of one month, 10 specimens from the TAP and DAP groups were kept as positive controls ( $n=5$ ), and so any removal procedure did not applied to them. A #15 K-file was introduced into the root canal to loosen the antibiotic paste to create a pathway for the syringe needle. The samples in the DAP and TAP groups were divided into four subgroups as described below ( $n=20$ ).

Group 1 (CSI): Five mL of 1.5% NaOCl was used to irrigate the root canals for one minute followed by a one minute irrigation with five mL of 17% EDTA using a 30-g side-vented irrigation needle (NaviTip; Ultradent) that placed one mm short of the WL.

Group 2 (EA): Five mL of 1.5% NaOCl was used to irrigate the root canals and then a polymer tip (25/04) that mounted on a EA device was introduced into the canal two mm short of the WL and it was run at 10.000 cpm (cycles per minute) with vertical movements of an amplitude of two mm for one minute. Next, the canals were irrigated with five mL of 17% EDTA, which was activated using the EA device for one minute. One polymer tip was used for one sample and then discarded.

Group 3 (PUI): Five mL of 1.5% NaOCl was used to irrigate the root canals and then a 15.02 ultrasonic tip [ESI instrument, piezoelectric ultrasonic unit (EMS), Le Sentier, Switzerland], which mounted on a EMS with the power setting at 6, was placed one mm short of the WL and activated for one minute to ultrasonically activate the irrigant. The root canals were irrigated again using five mL of 17% EDTA and then the same procedure that described above was applied for the following 1 minute. Each of the ultrasonic tips was discarded after three usages.

Group 4 (XPF): The XPF was mounted on an endodontic motor (VDW Gold, Munich, Germany) and then it was cooled down using Chloroethyl (Dr. Georg Henning GmbH, Walldorf, Germany). Afterwards the plastic tube, which was covered the XPF, was removed. The XPF was inserted to the root canal that flushed with five mL of 1.5% NaOCl and the XPF was run according to manufacturer's recommendations, which was 800 rpm speed and 1 N.cm torque values, for one minute with vertical strokes of 7-8 mm to the full WL. Afterwards, the canals were irrigated again using five mL of 17% EDTA and the XPF was activated for another one minute. One XPF per sample was used and then discarded.

Finally, each samples was flushed using five mL of distilled water. The total irrigant volume of 15 mL (5 mL of NaOCl, EDTA and distilled water) was used for each sample in all groups. One experienced endodontist performed all these described procedures. The temporary glued root halves were separated into two again and each of these halves was investigated under 10x magnification using a stereomicroscope, and digital images of them were taken. Two experienced endodontists, who were blinded to the groups, were scored the digital images using a classification that previously described by van der Sluis et al. (21):

Score 0: No DAP/TAP residuals were observed in the root canal.

Score 1: DAP/TAP residuals was observed in less than half of the root canal.

Score 2: DAP/TAP residuals was observed in more than half of the root canal.

Score 3: The root canal was completely filled with DAP/TAP residuals.

Shapiro-Wilk test showed that the data were not normally distributed using IBM SPSS Statistics 21.0 software (IBM, Armonk, NY, USA) ( $p<0.05$ ). The Kruskal-Wallis H and Dunn's tests were used to analyse the differences among TAP and DAP removal scores. The level of significance was set at 95%.

## Results

The result of the Kappa test revealed that the interexaminer agreement was 97.1%. The positive control samples verified that no TAP or DAP was removed from the root canals during the transportation and disassembly processes. The scores of the positive control group and the negative control group were significantly different from all tested groups ( $p<0.05$ ). Table 1 and Table 2 detail the distribution of scores of all groups. The Kruskal-Wallis H test showed that there were statistically significant differences in the TAP and DAP removal scores among the tested groups ( $p<0.05$ ). The PUI and XPF removed significantly more TAP and DAP than CSI and the EA ( $p<0.05$ ). There were no statistically significant differences between the XPF and PUI in both the TAP and DAP groups ( $p>0.05$ ). There were no significant differences between CSI and the EA in the TAP and DAP groups ( $p>0.05$ ). Regarding the type of the antibiotic paste, no significant difference between TAP and DAP was found ( $p>0.05$ ).

**Table 1. Distribution and the percentage of the scores of the tested instruments to remove triple antibiotic paste**

Group	n (N)	0	1	2	3
Negative control	5 (10)	10 (100%)	0 (0%)	0 (0%)	0 (0%)
Positive control	5 (10)	0 (0%)	0 (0%)	0 (0%)	10 (100%)
Group 1 (CSI) <sup>a</sup>	20 (40)	0 (0%)	12 (30%)	11 (27.5%)	17 (42.5%)
Group 2 (EA) <sup>a</sup>	20 (40)	0 (0%)	20 (50%)	11 (27.5%)	9 (22.5%)
Group 3 (PUI) <sup>b</sup>	20 (40)	19 (47.5%)	18 (45%)	3 (7.5%)	0 (0%)
Group 4 (XPF) <sup>b</sup>	20 (40)	23 (57.5%)	15 (37.5%)	2 (5%)	0 (0%)

CSI: Conventional syringe irrigation, EA: Endo activator, PUI: Passive ultrasonic irrigation, XPF: XP-endo finisher file, n: Number of specimens, N: Total number of the scored specimens

a,b: Groups that do not share the same superscript letter are significantly different (p<0.05)

**Table 2. Distribution and the percentage of the scores of the tested instruments to remove double antibiotic paste**

Group	n (N)	0	1	2	3
Negative control	5 (10)	10 (100%)	0 (0%)	0 (0%)	0 (0%)
Positive control	5 (10)	0 (0%)	0 (0%)	0 (0%)	10 (100%)
Group 1 (CSI) <sup>a</sup>	20 (40)	0 (0%)	12 (30%)	11 (27.5%)	17 (42.5%)
Group 2 (EA) <sup>a</sup>	20 (40)	2 (5%)	21 (52.5%)	14 (35%)	3 (7.5%)
Group 3 (PUI) <sup>b</sup>	20 (40)	13 (32.5%)	26 (65%)	1 (2.5%)	0 (0%)
Group 4 (XPF) <sup>b</sup>	20 (40)	20 (50%)	16 (40%)	4 (10%)	0 (0%)

CSI: Conventional syringe irrigation, EA: Endo activator, PUI: Passive ultrasonic irrigation, XPF: XP-endo finisher file, n: Number of specimens, N: Total number of the scored specimens

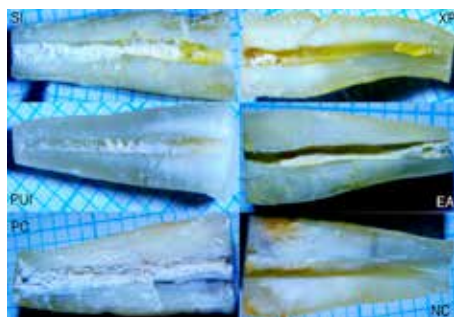
a,b: Groups that do not share the same superscript letter are significantly different (p<0.05)

## Discussion

Antibiotic paste mixtures are used for the disinfection of the root canal system during regenerative endodontic procedures or as an intracanal medicament for further disinfection. However, previous studies led some concerns about their usage due to allergic reactions, bacterial resistance, tooth discoloration, decrease in root dentin microhardness, and their detrimental effects on stem cells (3,5,11,22). Ruparel et al. (11) reported that any antibiotic pastes used for regenerative endodontic procedures present concentration-dependent effects on the survival of stem cells. The authors also emphasized that the survival rate of the stem cells dramatically increased when the selected antibiotic concentration was below 1 mg/mL. Thus, in order to increase the survival rate of the stem cells, any antibiotic paste remnants should also be removed completely at the last session of regenerative endodontic therapy.

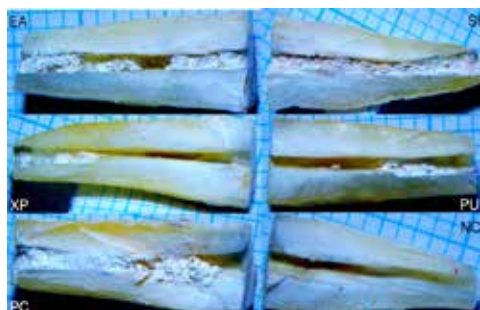
NaOCl also has concentration-dependent detrimental effects on the stem cells of the apical papilla; however, a 1.5% concentration of NaOCl has been shown to present minimal detrimental effects (23). Therefore, a 1.5% NaOCl solution was preferred as an irrigant in this study, and it was combined with 17% EDTA. A previous study showed that combining EDTA and NaOCl could more effectively remove TAP than either NaOCl or EDTA alone, especially from the apical third of the root canal (13).

The novel XPF file was developed in order to fit to the root canal system three dimensionally, and it is suggested for intracanal paste removal by the manufacturer. The present study compared the efficacy of the use of XPF, EA, PUI, and CSI in the removal of TAP or DAP from root canals with simulated immature apices. To the authors' knowledge, there is no study comparing the novel XPF with the EA regarding TAP and DAP removal efficacy. The results of our study



**Figure 1.** Representative images of the double antibiotic paste groups. Persistence of double antibiotic paste residues can be seen in all specimens

SI: Conventional syringe irrigation, PUI: Passive ultrasonic irrigation, XP: XP endo finisher; EA: EndoActivator, PC: Positive control, NC: Negative control



**Figure 2.** Representative images of the triple antibiotic paste groups. Persistence of triple antibiotic paste residues can be seen in all specimens. SI; Conventional syringe irrigation

PUI: Passive ultrasonic irrigation, XP: XP endo finisher; EA: EndoActivator, PC: Positive control, NC: Negative control, SI: Conventional syringe irrigation

revealed that the use of XPF, EA PUI, and CSI failed to completely remove DAP (Figure 1) and TAP (Figure 2).

Previous studies reported that no method could completely remove polyantibiotic pastes from root canal system up to date (10,24,25). The findings of this study were supported the literature. Nevertheless, the use of the XPF and PUI improved TAP and DAP removal compared to needle irrigation and the use of the EA. Therefore, the first null hypothesis that there would be no difference among the various irrigation activation systems in removing TAP or DAP could be rejected.

In the present study, the TAP mixture was modified using cefaclor instead of minocycline. Minocycline, which was one of the substances of TAP, binds to calcium ions via chelation and forms an insoluble complex that has been lead to tooth discoloration (26). To overcome this problem, it was suggested to

remove the minocycline from the combination or substitute minocycline with alternative antibiotics (6,22,23). Cefaclor, which was reported to exhibit sufficient antimicrobial effectiveness within the canal system, has been used as an alternative to minocycline (6,8). The second null hypothesis was accepted, since the presence of TAP or DAP within the root canal system did not influence the removal efficacy of the tested activation systems, which was in accordance with the findings of Arslan et al. (20).

The similarity between the efficacies of the EA and CSI is in accordance with the previous literature that evaluated the calcium hydroxide paste removal efficacy of the EA and CSI (18,19). These results might be attributed to the inability of sonic activation to create a cavitation effect. However, a direct comparison could not be made among studies since Berkhoff et al. (10) reported in their quantitative analysis that it was more difficult to remove TAP than calcium hydroxide.

Previous studies have revealed the superiority of PUI over CSI in the removal of TAP or DAP from canal surfaces or irregularities (17,19). The efficacy of ultrasonically activated irrigation has been determined as a result of sufficient evidence found through a systematic review. In the present study, PUI removed TAP and DAP completely in 47.5% and 32.5% of the cases, respectively. A cavitation effect resulting in a high velocity of irrigant might contribute to the removal of antibiotic pastes from root canal systems. The XPF removed TAP and DAP completely in 57.5% and 50% of the specimens, respectively. The XPF instrument is designed with an ISO apical size of 25 and a 0.00 taper. The efficacy of the XPF in removing intracanal medicaments from canal irregularities has been reported (18), and the results were similar to what was found in the present study. The manufacturer claims that this unique instrument could expand up to 6 mm in diameter when activated in the root canal to adapt to the canal anatomy 3-dimensionally, so it could improve the efficiency of irrigation even in the irregularities of the root canal system.

An immature root model is useful for proving the standardization of the width and length of root canal spaces. However, the limitations of this model include its lack of ability to reflect the complexity of the root canal system that involves grooves or irregularities on root canal walls and the 2-dimensional evaluation of

remaining intracanal polyantibiotic paste remnants from the stereomicroscopic images. Within the limitations of this study, the tested irrigation systems could not completely remove either TAP or DAP from simulated immature roots. The use of the XPF and PUI were more effective when compared to the use of the EA and CSI for the removal of antibiotic root canal pastes.

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### Ethics

**Ethics Committee Approval:** This study was performed at both Ordu University and Ondokuz Mayıs University. The Ondokuz Mayıs University Clinical Researches Ethical Committee board approved the study protocol (OMU KAEK 2016/403).

**Informed Consent:** Written informed consent was obtained from all participants.

**Peer-review:** Externally and internally peer-reviewed.

### Authorship Contributions

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

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

**Financial Disclosure:** The authors deny any conflicts of interest related to this study.

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# The Comparison of Different Surface Preparation Methods in Terms of Shear Bond Strength of Tri-ceram Porcelain-titanium Alloy

*Tri-ceram Porselen ve Titanyum Alaşımı Arasındaki Makaslama Bağlantı Direnci Üzerine Farklı Yüzey Uygulamalarının Etkisi*

✉ Berivan Dünder Yılmaz<sup>1</sup>, ✉ Ayşe Meşe<sup>2</sup>, ✉ Eylem Kaya<sup>2</sup>

<sup>1</sup>Dicle University Faculty of Dentistry, Department of Prosthodontics, Diyarbakır, Turkey

<sup>2</sup>Mersin Dental Hospital, Mersin, Turkey



## Keywords

Shear bond strength, titanium alloy, surface preparation, Tri-ceram porcelain

## Anahtar Kelimeler

Makaslama kopma direnci, titanyum alaşımı, yüzey pürüzlendirme, Tri-ceram porselen

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## Address for Correspondence/Yazışma Adresi:

Berivan Dünder Yılmaz MD,  
Dicle University Faculty of Dentistry,  
Department of Prosthodontics, Diyarbakır,  
Turkey  
E-mail : berivandnr@yahoo.com  
ORCID ID: orcid.org/0000-0002-5471-555X

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## Abstract

**Objective:** Several studies have been made to evaluate effects of the surface preparation on shear bond strength (SBS) of titanium-porcelain complex. However, a completed picture has not been obtained yet. Therefore, such studies appear frequently on recent literature. The purpose of this study is to determine a simple method providing strong SBS and using fewer parameters.

**Materials and Methods:** Sixty titanium samples were equally divided into five groups. Group 1: Control (C). Group 2: Airborne-particle abrasion with 250  $\mu$  Al<sub>2</sub>O<sub>3</sub> (250  $\mu$  AbPA). Group 3: Etching with 10% HCl (10% HCl). Group 4: Etching with Nd:YAG Laser at 6W (Nd6). Group 5: Etching with Nd:YAG laser at 7 W (Nd7).

**Results:** Mean SBS value of C (10.69 MPa) was close to that of 250  $\mu$  AbPA (10.57 MPa). The mean value of 10% HCl (19.37 MPa) was nearly twice higher than C, whereas those of laser groups (8.89 MPa and 8.77 MPa) were smaller than C. There was no overlap between SBS values of samples etched with 10% HCl and those of other samples. Multiple comparisons indicated a significant difference between acid group and others ( $p=0.00$ ). Laser groups were different from control, too. The failure mode of % HCl group was 67% adhesive and 33% mix, while those of other groups were cohesive or mix.

**Conclusion:** Titanium surfaces etched with 10% HCl provides significantly strong SBS values. This method requires only the use of aqueous solution of 10% HCl, and boiling process for 30 minutes. Such a simplicity suggests that etching with 10% HCl provides a very simple surface preparation method which involves in use of fewer parameters.

## Öz

**Amaç:** Yüzey pürüzlendirme işlemlerinin titanyum porselen bağlantısının kopma direnci üzerine etkilerini değerlendiren birçok çalışma yapılmıştır. Buna rağmen henüz, bu alandaki yöntemlerin standardizasyonu sağlanamamıştır. Bu nedenle son yıllarda bu konuyla ilgili çalışmalara sıkça rastlanmaktadır. Bu çalışmanın amacı, yüzey uygulama metodları ve doğru parametreleri kullanarak kopma direnci sonuçlarını değerlendirmektir.

**Gereç ve Yöntemler:** Altmış titanyum örnek 5 gruba eşit sayıda bölündü. Grup 1: Kontrol, grup 2: Al<sub>2</sub>O<sub>3</sub> (250 µm) ile kumlama, grup 3: Asit (%10 HCl) ile pürüzlendirme, grup 4: 6 W lazer (Nd:YAG) uygulama, grup 5: 7 W lazer (Nd:YAG) uygulama.

**Bulgular:** Kontrol grubunun ortalama değerleri (10,69 MPa), kumlama grubuyla (10,57 MPa) yakın bulundu. Asit grubunun ortalama değerleri (19,37 MPa) önemli ölçüde kontrol grubundan fazla bulunurken, lazer grupları değerleri (8,89 MPa ve 8,77 MPa) kontrol grubundan düşük bulundu. Çoklu karşılaştırma testi sonucunda asit grubuyla diğer gruplar arasında anlamlı bir fark bulundu ( $p < 0,05$ ). Altı W'luk lazer uygulaması dışındaki gruplarla kontrol grubu arasında anlamlı bir fark bulunmadı. Asitle pürüzlendirme grubunda %75 adeziv ve %25 miks kopma görülürken diğer gruplarda kohesiv ve miks kopma görüldü. Adeziv kopma tipinin ortalama makaslama bağlanma direnci değeri (20,31 MPa), kohesiv (9,48 MPa) ve miks (10,90 MPa) tipleri değerlerinden yüksek elde edildi. Adeziv kopmanın değerleri istatistiksel olarak diğerlerinden farklı bulundu ( $p < 0,05$ ).

**Sonuç:** Titanyum yüzeyinin %10 HCl asit ile pürüzlendirilmesi güçlü bir makaslama kopma direnci göstermektedir. Bununla, titanyum yüzeyinin asitle pürüzlendirilmesinde uygun bir konsantrasyon olduğunu önerilmektedir.

## Introduction

Several studies have been carried out to investigate the effects of surface preparation on shear bond strength (SBS) of metal-porcelain complex. The SBS values for titanium-porcelain complex have been evaluated by several methods such as acid etching (1-17), alumina airborne-particle abrasion (7-14,17-30), laser irradiation (6,15,16,22,27,30), fluoride etchant application (8,9,12,13), nanotechnology (10), machining/milling/thermic treatments (8,9,13,23,26,31) and cooperative use of simple surface preparation methods (2-4,9). The SBS has also been investigated for the influence of various parameters such as acid types and concentrations (2-4,9), treatment with and without vacuum (3), laser types (6,16,30), radiation power of laser irradiation (15,27;30), size of Al<sub>2</sub>O<sub>3</sub> particles (22,23,25,26,28), resin cements/bonding agents (3,16,17,21,25,26,32-35), surface coating (20,24,36,37), interfacial oxidation (38-40), ceramic types (5,7,15,41,42). Duration and temperature for surface preparation (2,5,8,9,13,15), firing temperature (20,43), storage in water and water loading (7,9,11,16,26,27,41,44), thermocycling (8,13,16,18,25, 26), distance in airborne abrasion (13), area fraction of adherent porcelain (19) and crosshead speed of universal testing machine (13,14,22,24). In addition, several review papers have been published in this field (42,45-47)

Different materials, different methods, different experimental conditions, various parameter settings, and combination of several methods for the surface preparation have yielded different values of the SBS. Depending on experimental conditions and chosen parameters, even contradictory results have been obtained in some studies (1,6,13,14,16,17,22,27-29). In the other words, a completed picture has not been

obtained yet. Therefore, the studies on the SBS of titanium-ceramic complex are still interest of clinical research, and such studies appear frequently on the recent literature (5,10,11,13,14,18,28-30,35,42). For these reasons, a new study done by using the simple surface preparation methods and specific parameters may contribute to complementary studies in this field.

The aim of this *in vitro* study was to reveal simple surface preparation methods providing strong SBS for Tri-ceram porcelain-titanium complex. The hypothesis of the study was to obtain confirmative results contributing to the studies on the strong SBS. For these purposes, the SBS values between titanium Tri-ceram porcelain complex were obtained by simple surface preparation methods such as acid etching, alumina airborne-particle abrasion and laser etching. Some particular parameters for each method were also chosen. The effects of these methods on the SBS between a titanium and porcelain were compared.

## Materials and Methods

### Sample Preparation and Measurements

Titanium bars (ASTM F67-00; Titanium Industries, Inc, Rockaway, NJ) were sectioned with a lathe (computer numerical control auto lathe type SA-12 S/N 0910; Star Micronics Co, Ltd, Shizuoka, Japan) into 60 specimens, with 2.2 mm length and 5.7 mm in diameter. All specimens were machine cut from long metal rods to the same specified dimensions. No specific surface treatment was performed for the machined surface group, which served as the control group (C). The samples were equally divided into five groups for surface preparation (12 samples for each group).

The airborne-particle-abraded surface specimens (12 samples) were abraded with alumina particles

(250  $\mu\text{m}$ ) with a dental airborne-particle-abrasion unit (Micro-blaster; Daedong Industrial Co, Ltd, Daegu, Korea). The air pressure was set at 2 bar, and the distance between the nozzle tip and the specimen surface was maintained at 15 mm, during the airborne-particle abrasion, for 20 seconds (10 scans in 20 seconds, at the rate of 1 scan every 2 seconds).

The acid-etched surface specimens were subjected to chemical surface treatment by submerging the specimens in a 10%-by weight aqueous solution of HCl (DC Chemical Co, Ltd, Seoul, Korea) in a heat-resistant glass container and boiling for 30 minutes, taking care to avoid contact between specimens.

The laser-etched surface specimens were treated using a custom-made pulsed Nd:YAG laser (Jenoptic Laser Optik Systeme GmbH, Jena, Germany). The titanium surfaces of the specimens were irradiated by the linear movement of a glass fiber of the Nd:YAG laser at a power setting of 7 W and 6 W, representing energy and frequency levels of 120 mJ with 50-Hz frequency

The groups were as follows:

Group 1 (C): Control (no treatment).

Group 2 (250 m AbPA): Airborne-particle abrasion with  $\text{Al}_2\text{O}_3$  particles (250 m).

Group 3 (%10 HCl) : Surfaces etched with %10 HCl

Group 4 (Nd6): Surfaces etched with Laser (Nd:YAG laser) at 6 W irradiation power.

Group 5 (Nd7): Surfaces etched with Laser (Nd:YAG laser) at 7 W irradiation power.

Before application of porcelain, the samples were replaced in ultrasonic cleaning apparatus at 80 °C for 10 minutes. Then, they were washed with distilled water. Low-fusing porcelain (Tri-ceram; Esprident GmbH, Ispringen, Germany) was used in this investigation. Firing temperature and times were in accordance with the manufacturer's specifications and directions. Heat pretreatment of the specimens was performed immediately after cleaning procedures in a dental porcelain furnace (Austromat 3001; Dekema GmbH, Freilassing, Germany). Opaque porcelain was mixed as a powder and liquid until it reached a creamy consistency, then applied in 2 uniform coats with a brush on each treated porcelain-bearing surface. After the opaque porcelain firing cycle (795 °C), the dentin porcelain was subsequently formed on the opaque layer, using a specially designed silicone mold, and fired at 500 °C to 755 °C with a heat rate of 55 °C/

min under a vacuum of 72 cm/Hg. The firing shrinkage was compensated for with a second body porcelain application, until an approximately 4-mm thick porcelain layer was obtained. A glazing procedure was not performed. Porcelain application for all of the specimens was performed by a single dental technician. The Tri-ceram were then adhered to the surface treated samples using Clearfil SE Protect (Kuraray).

The following treatments were applied to the prepared samples:

1- The samples were mechanically loaded (20.000 cycles; 50 N load; distilled water at 37 °C).

2- The samples were thermocycled (3.000 cycles; 5-55 °C, dwell time: 30 sec).

To evaluate the bond strength of the interface between the metal and ceramic, the shear bond test, which has been described by other investigators, was performed. For the shear bond test, a special stainless steel device was fabricated. This device enabled the specimen to be held firmly during the shear bond test. The device containing the metal ceramic specimen was placed in a tensile testing machine (micro 500, type U4000, Maywood Instruments. Limited Basingstoke Hants. England). The power loading point was 5 mm far from metal porcelain connection, while the speed of loading was 5 mm/minute. The load was applied until fracture of the metal-porcelain interface occurred, and the maximum load at fracture was expressed in megapascals (MPa). After fracture, scanning electron microscope (SEM) (JSM-6700F; JEOL Ltd, Tokyo, Japan) observation was once more performed to evaluate the nature of the fractured surfaces. Three photomicrographs with x2000 magnification were made of different regions of the treated surface and the fractured surface of each specimen.

#### Statistical Analysis

Results were presented as the mean + standard deviations (SD). One-way ANOVA followed by Games-Howell post hoc test ( $\alpha=0.05$ ) was used for comparisons.  $P<0.05$  was considered as statistically significant.

#### Results

Mean SBS values and SD of all groups were given in Table 1. The mean values of C are close to that of alumina airborne-particle abrasion. The mean value of acid group is almost twice higher than control,

whereas those of laser groups are smaller than control. There is no overlap between maximum SBS values for 250  $\mu$  AAbPA and minimum SBS values for acid etching. There is large overlap among the SBS of other groups.

Multiple comparisons of groups are given in Table 2. It is seen that the SBS of 10% HCl etching group is highly significantly different than those of other groups ( $p=0.00$ ). There is no significant difference between 250 m AbPA and control, and also between 250 m AbPA and Nd6 ( $p>0.05$ ). 250 m AbPA is different from Nd7 ( $p=0.05$ ). In addition, C is different from both laser groups ( $p<0.05$ ).

The numbers of adhesive, cohesive and mix failure modes, determined by SEM, were 8, 21 and 31 respectively. Adhesive failure mode was only observed in 8 of 12 samples etched with acid, but cohesive mode was found in all groups other than 10% HCl. Mixed failure mode was exist in all groups.

#### SEM Images Obtained From Surface Preparations

SEM images of C, 250  $\mu$  AbPA, 10% HCL and Nd7 are shown in Figures 1,2,3 and 4, respectively The

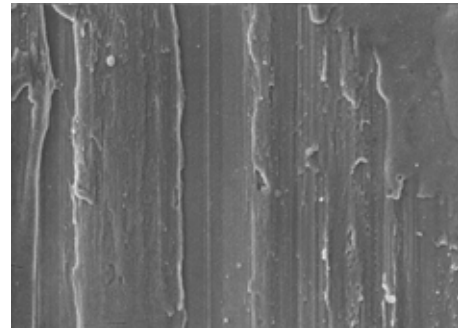
**Table 1. The mean values of shear bond strength together with standard deviation for investigated groups**

Groups	Number	Mean SBS values $\pm$ SD (MPa)	min-max
Control (C)	12	10.69+1.40	7.20-12.10
250 $\mu$ AbPA	12	10.57+1.47	8.40-12.90
10% HCl	12	19.37+2.99	14.40-25.40
Nd6	12	8.89+1.11	7.30-10.90
Nd7	12	8.88+2.34	6.60-13.80

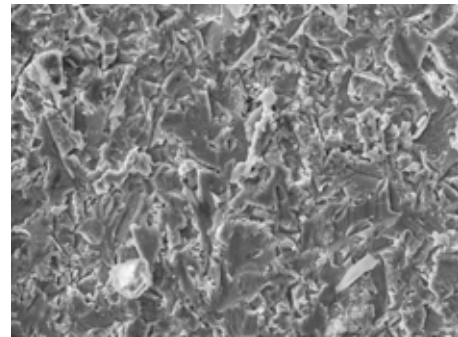
SBS: Shear bond strength, SD: Standard deviation, MPa: Megapascal, min: Minimum, max: Maximum

**Table 2. Statistical significances between groups**

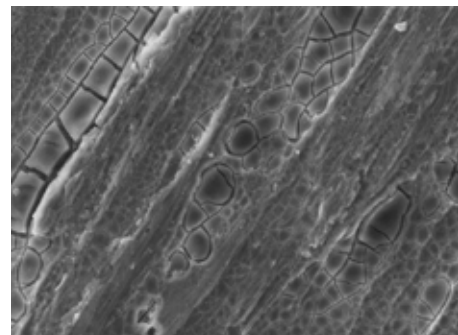
Comparison between groups		Significance (p)
10% HCl	C	0.000
	250 $\mu$ AAbPA	0.000
	Nd6	0.000
	Nd7	0.000
250 mA AbPA	C	0.998
	Nd6	0.075
	Nd7	0.050
C	Nd6	0.049
	Nd7	0.032
Nd6	Nd7	0.990



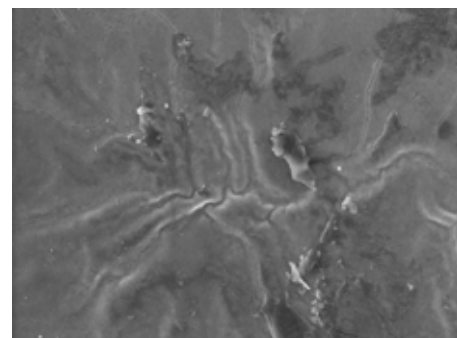
**Figure 1.** Scanning electron microscope image of control group



**Figure 2.** Scanning electron microscope image of airborne-particle ablated with alumina particle group



**Figure 3.** Scanning electron microscope image of acid etched group



**Figure 4.** Scanning electron microscope image of laser group at 7 Watt

image for Nd6 was not given for the sake of simplicity. Smooth surface was observed for both C and Nd6. The roughest surface was observed in 250  $\mu$  AbPA. In addition to these, laser irradiated samples at Nd7 had a smoother surface. Nevertheless some detonation points and black areas were found on SEM images of the Nd7.

## Discussion

The success of the porcelain-fused-alloy restoration depends widely on the strong bonding between porcelain and the titanium. In the current work, the titanium surface treatment with 10% HCl has provided strong SBS values, and acid etching was found to be more effective than alumina airborne-particle abrasion, laser etching and C. In fact, acid etching has been indicated as mostly quite effective method for increasing SBS in titanium-porcelain system (1-4,12,16,18). Therefore, the present results obtained with 10% HCl are in consistent with the previous studies related to surface acid treatment. However, in the previous studies, the surface acid etching has been reinforced by other treatment methods (2,4,16). Also titanium surfaces have been treated by using highly concentrated acids (e.g. 48%  $H_2SO_4$ ) different than HCl (3,12,16). On the contrary, even 1 N (about 3%) HCl is known to erodes the titanium surface effectively and to provide increasing SBS values (18).

Airborne-particle abrasion has been noted to weaken the metal ceramic bonding in some studies (1,6,24). Alternative methods to the airborne-particle abrasion have also presented (1,3,6,10,12,27). However, airborne-particle abrasion with certain size of  $Al_2O_3$  (e.g. 110 and 250 m  $Al_2O_3$ ) is known to increase bonding in the metal ceramic system (6,13,18,29). It was noted that SB increased as particle size increased (29). Despite this, the combined use of alumina airborne-particle abrasion with other surface preparation materials and methods has mostly been used to obtain strong SBS (2,8,9,14,17,19,21,23,26). The diversity of SBS values including the current value for 250  $\mu$  AbPA should be related to parameter settings and experimental conditions. In fact, the strong SBS in the airborne-particle abrasion is dependent on many factors such as particle size of  $Al_2O_3$ , bonding agent, water storage, etching times, laser welding, metal conditioners, vacuum firing, cooperative use of simple surface preparation methods, thermic treatment,

order of cooperative treatment, pressure and angle used (3,7, 13,14,17,18,21,22,24,26,28,31).

The Nd:YAG laser etching has been found found to be effective improving bond strength of titanium-ceramic system in some studies (6,15,27), whereas lower SBS was obtained by laser irradiation in other studies (16). High variability was observed in adhesion values obtained by laser etching (42). In fact, the efficiency of laser irradiation is dependent on various parameters such as irradiation power, laser type etc. (15,30). For example, Er:YAG and Nd:YAG lasers applied with certain power have yielded the stronger SBS (27,30). However, Nd:YAG laser was found to be more successful than Er:YAG and Ho:YAG lasers for bonding low fusion porcelain to metal alloy (30). In addition to these, laser applications or other surface preparation methods may be more successful when applied to ceramics or other type of metal alloys. The SBS obtained with laser etching or without laser have been found to be strong for the ceramic-Ni-Cr or ceramic- Co-Cr alloys (48-51).

Adhesion is the tendency of dissimilar surfaces to stick to one another. There are many types of forces that can occur when surfaces come in close contact (48,49). Rather than inter molecular forces between dislike molecules, mechanical and chemical forces provide binding of metal to ceramic where failure mode is adhesive. In mechanical binding, a strong bond is formed between the substrate and the adhesive (48,49). Also, Chemical binding is usually the strongest form of adhesion (48,49). Since adhesive failure is dominant on the surfaces treated with 10% HCl, the current strong mean SBS obtained for titanium-ceramic system should be related to ontrrol mechanical or chemical binding.

Smooth surface appearance obtained by SEM for laser irradiation groups are in agreement with the small SBS and cohesive failure mode of these groups. The SEM appearance with relatively rough surface in acid etching group is in consistent with strong adhesive bonding that is likely produced through mechanical or chemical interactions. The roughness in the alumina airborne-particle abrasion may be related to long-distance adhesive interactions since cohesive failure mode is dominant for this case. In addition to these, the detonation points and black areas on SEM images of laser group etched at 7 W power express that increasing energy creates burning areas. The

connection between titanium and porcelain becomes weaker in this area. Therefore, the use of a laser at 6 W energy should be more convenient.

As mentioned earlier, variability of the SBS values obtained from titanium-ceramic complex is dependent on surface preparation methods, experimental conditions and parameter settings. Multitude of parameters and conditions requires elimination of some of them which are not providing simple method and strong SBS. In our case, etching titanium surface by using acid requires only the use of aqueous solution of 10% HCL, and boiling process for 30 minutes. Therefore, it is a very simple method providing significantly strong SBS. Thus the aim or hypothesis of our study was fulfilled.

## Conclusion

Etching titanium surface with 10% HCL has provided a strong bonding for titanium-ceramic complex. This suggests that etching with 10% HCL provides very simple method for surface preparation of titanium.

## Ethics

**Ethics Committee Approval:** The study were approved by the Dicle University of Local Ethics Committee (protocol number: 2018/7).

**Informed Consent:** The authors confirm that this article content has no conflict of interest.

**Peer-review:** Internally peer-reviewed.

## Authorship Contributions

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

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# Effect of Different Surface Finishing Methods and Colorant Solutions on Translucency of Monolithic CAD/CAM Ceramics

*Farklı Yüzey Bitirme İşlemlerinin ve Renklendirici Solüsyonların Monolitik CAD/CAM Seramiklerin Translüsensisi Üzerine Etkisi*

Yeliz Hayran, Işıl Sarıkaya

Tokat Gaziosmanpaşa University Faculty of Dentistry, Department of Prosthodontics, Tokat, Turkey



## Keywords

Ceramics, color, dental porcelain, opalescence

## Anahtar Kelimeler

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## Address for Correspondence/Yazışma Adresi:

Yeliz Hayran MD,  
Tokat Gaziosmanpaşa University Faculty of  
Dentistry, Department of Prosthodontics,  
Tokat, Turkey  
Phone : +90 356 212 42 22  
E-mail : yeliz.hayran@gop.edu.tr

ORCID ID: orcid.org/0000-0002-8664-9083

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## Abstract

**Objective:** An ideal aesthetic restoration should be morphologically and optically compatible with the natural teeth and, at the same time, biologically acceptable. The translucency of the restoration is the most important factor in achieving a natural tooth appearance. The aim of this study was to compare the translucency parameters (TPs) of two types of monolithic computer-aided design/computer-aided manufacturing (CAD/CAM) ceramics, Vita Suprinity HT (VS) and IPS e.max CAD HT (IPS), after utilizing three different surface finishing methods and being exposed to cola, coffee, and black tea solutions.

**Materials and Methods:** A total of 24 groups consisting of 10 samples each were created from VS and IPS e.max CAD ceramics. Glaze, Shofu polishers, and Sof-lex polishing discs were used to finish the surface of the ceramics. Cola, coffee, and black tea were used as coloring solutions. For the control groups, samples were kept in distilled water. The TP was determined for optical properties. Data were statistically analyzed using three-way analysis of variance (ANOVA). The Bonferroni test was used for multiple comparisons.

**Results:** Material, method, group and interaction effects were found statistically significant ( $p < 0.001$ ). There was a statistically significant difference in TP value between the materials in terms of applied surface treatments ( $p < 0.05$ ). The TP value for each group was different for the exposed solutions after surface finishing ( $p < 0.05$ ).

**Conclusion:** Translucency was affected by type of the monolithic CAD/CAM ceramic material used. The translucency of the VS ceramic was found to be higher than the IPS ceramic. Colorant solutions decreased the translucency of all ceramics. The lowest TP value for the IPS group was observed in the samples exposed to cola, while for the VS group it was observed in the samples exposed to black tea. The IPS ceramic finished with Sof-lex and the VS ceramic finished with Shofu exhibited higher translucency values.

## Öz

**Amaç:** İdeal bir estetik restorasyon, doğal dişlerle morfolojik ve optik olarak uyumlu aynı zamanda biyolojik olarak kabul edilebilir olmalıdır. Restorasyonun translüsensisi doğal diş görünümü için en önemli faktördür. Bu çalışmanın amacı, Vita Suprinity HT (VS) ve IPS e.max bilgisayar destekli tasarım (CAD) HT (IPS) olmak

üzere iki tip monolitik CAD bilgisayar destekli üretim (CAM) seramiğine üç farklı yüzey sonlandırma işleminin uygulanmasının ardından kola, kahve ve siyah çaya maruz bırakılarak seramiklerin translüsensi parametresini (TP) karşılaştırmaktır.

**Gereç ve Yöntemler:** VS ve IPS e.max CAD seramiklerinden her birinde 10 örnekten oluşan toplam 24 grup oluşturuldu. Seramiklerin yüzeyini bitirmek için glaze, Shofu polisajları ve Sof-lex polisaj diskleri kullanıldı. Renklendirme çözeltisi olarak kola, kahve ve siyah çay seçildi. Kontrol grupları için, örnekler damıtılmış suda tutuldu. Optik özellikler için TP belirlendi. Veriler, üç yönlü varyans analizi kullanılarak istatistiksel olarak analiz edildi. Çoklu karşılaştırma için Bonferroni testi kullanıldı.

**Bulgular:** Materyal, yöntem, grup ve etkileşim etkileri istatistiksel olarak anlamlı bulundu ( $p<0,001$ ). Uygulanan yüzey işlemleri açısından materyaller arasında TP değerinde istatistiksel olarak anlamlı fark vardı ( $p<0,05$ ). Her bir grup için TP değeri, yüzey bitirme işlemi sonrası maruz bırakılan çözeltiler açısından farklıydı ( $p<0,05$ ).

**Sonuç:** Translüsensi, monolitik CAD/CAM seramik tiplerinden etkilenmiştir. VS seramiğin translüsensisi IPS seramikten daha yüksek bulunmuştur. Renklendirici solüsyonlara maruz bırakılan seramik gruplarının TP'si azaldı. IPS grubu için en düşük TP değeri kolayca maruz kalan örneklerde gözlenirken, VS grubu için siyah çaya maruz kalan örneklerde gözlenmiştir. Sof-lex ile bitirilen IPS seramik ve Shofu ile tamamlanmış VS seramik yüksek translüsensi değerleri sergiledi.

## Introduction

The search for the ideal anterior ceramic material has accelerated, with the increase in the aesthetic expectations of patients in recent years. An ideal aesthetic restoration should be morphologically and optically compatible with the natural teeth and biologically acceptable. All ceramic restorations offer an aesthetic appearance close to the natural optical properties of the tooth structure (1,2). However, it is necessary to provide harmony with the neighboring teeth for a natural appearance, but this desired natural appearance is not always achievable due to difficulties in matching the natural tooth color with the existing tooth porcelains (3,4). Hue, chroma, value, and translucency/opacity are color elements that affect the aesthetics of dental restorations (5,6). The translucency of the restoration is the most important factor in achieving a natural tooth appearance (7-10). Since human enamels have a natural translucency, aesthetic materials must reproduce the translucency of natural teeth (11,12). Especially for younger individuals, aesthetic ceramics must have high translucency in order to mimic the natural tooth structure (13). The translucency parameter (TP) and contrast ratio (CR) are used to measure translucency of dental materials (14). A high TP indicates that the material is less opaque (15). Also, the ceramic materials of fixed dental prostheses must provide long-term color stability to avoid repeated restoration.

The surface finishing affects many aspects of the final restoration, such as staining, the degree of color change, plaque buildup, and the wear resistance of ceramics (16,17). Food and other residues will stick to the surface, if a restoration presents a rough

surface. It has been shown that significant color change occurs on rough surfaces (18). The surface cracks that develop during the preparation of the restoration are reduced by the polishing process (19). Therefore a dental restoration should have a highly polished surface due to this aesthetic, mechanical, and biological considerations.

When choosing an all-ceramic system, factors, such as the color of the ceramic material and neighboring teeth and the forces foreseen in that area, should be considered (20,21). For example, the fragile structure of conventional glass ceramics limits the use of these ceramics in the posterior area (22). The use of zirconia as a core material has improved the mechanical properties of all ceramic restorations, but zirconia copings need to be a coated with a ceramic application to attain proper aesthetics (23,24). With the widespread use of dental computer-aided design/computer-aided manufacturing (CAD/CAM) systems, various ceramics offering both durable and optimal aesthetics have been introduced into the dental market. One of these is monolithic glass-ceramics, which was developed to provide aesthetics without the need for coating ceramics (25). Elimination of the connection between the core and the coating ceramic can make the restoration more structurally sound and longer-lasting (26,27). Lithium disilicate ceramic restoration is one of the most popular monolithic ceramic systems for anterior and posterior single crowns and partial veneered restorations because of its favorable physical properties (28). However, the durability of this ceramic in the posterior region may not be optimal (29-31). Recently, zirconia-reinforced lithium silicate glass-ceramics were introduced for inlays, onlays, implant abutments, and partial and full

crowns manufactured using the CAD/CAM system. Zirconia particles are included to strengthen the ceramic build (32).

To the best of the authors' knowledge, no information is available on the clinical and laboratory translucency change of monolithic CAD/CAM ceramics subjected to different surface finishing methods and immersed in coloring solutions, such as cola, coffee, and black tea. Therefore, the aim of this study was to compare the TP of two types of monolithic CAD/CAM ceramics, the Vita Suprinity HT (VS) and the IPS e.max CAD HT (IPS), after three different surface finishing methods and exposure to cola, coffee, and black tea solutions. The null hypothesis was that the type of surface finishing and all colorant solutions would not affect the translucency of the monolithic CAD/CAM ceramics.

## Materials and Methods

### Preparation of Samples

Two monolithic CAD/CAM ceramics, including a zirconia-reinforced lithium silicate ceramic (VS, Vitazahnfabrik) and a lithium disilicate ceramic (IPS e.max CAD, Ivoclar, Vivadent AG) were evaluated (Table 1).

Ceramic blocks were cut at 150 rpm using a precision cutting machine (Micracut 201, Bursa, Turkey) with a diamond disc to render a sample thickness of 1.2 mm. Both ceramic materials were divided into three main groups and control group (n=40) for the three different surface finishing methods: glaze, sandpaper discs (Sof-lex), and silicone-carbide rubber points (Shofu). After the surface treatments, each group was divided into four subgroups according to the exposed solutions: distilled water (control), cola, coffee, and black tea solutions. A total of 240 specimens were prepared, 10 in each group (n=10). After the blocks were cut,

all samples were subjected to the crystallization cycle according to the manufacturer's instructions. Then, both surfaces of the samples were sanded with 600, 800, and 1.200 grained silicon carbide abrasives (English Abrasives, London, UK) under water cooling at 100 rev/min before surface treatments were applied. Each ceramic material received one coat of glaze according to the manufacturer's instructions (Vita akzent plus glaze, Vita, Germany and IPS Empress Universal glaze, Ivoclar, Vivadent). For the specimens to be surface treated with Sof-lex, Sof-lex polishing discs (Sof-lex polishing discs, 3M ESPE, St. Paul, ABD) with a diameter of 12.7 mm were used in accordance with the manufacturer's recommendation. Polishing was first carried out at 10.000 rpm with thick and medium discs, and then followed with thin and super-thin discs. For the specimens to be surface treated with Shofu, first, a white stone (Dura-white Stone, Shofu, Japan), and, then, three silicone-carbide rubber points (Ceramiste Standard, Ultra and Ultra II, Shofu, Japan) were used, respectively, for smoothing the surface. Polishing operations were performed by the same applicator using a low-speed rotary hand tool (Kavo Ewl 4990; KaVo Dental GmbH, Germany). Sample thicknesses were checked with a digital caliper (Absolute Digimatic, Mitutoyo, Japan). After polishing, all samples were sterilized for 10 s using distilled water in an ultrasonic cleaner (Pro-Sonic 600; Sultan Healthcare, NJ, USA), and then air-dried. The groups were formed by random selection of the ten samples for each group. The groups are shown in Table 2. I1a, I2a, I3a, V1a, V2a, and V3a served as the control groups.

### Preparation of Colorant Solutions

Each of colorant solutions were prepared for consumption 5 cups per day. Instant coffee sticks

**Table 1. Tested ceramics**

Material	Chemical composition	Code	Manufacturer
IPS e.max CAD (A2-HT)	Lithium disilicate glass-ceramic (58-80% SiO <sub>2</sub> , 11-19% Li <sub>2</sub> O, 0-13% K <sub>2</sub> O, 0-5% MgO, 0-8% ZrO <sub>2</sub> , 0-8% ZnO, 0-11% P <sub>2</sub> O <sub>5</sub> , 0-5% Al <sub>2</sub> O <sub>3</sub> , 0-8% colouring oxides)*	IPS	Ivoclar Vivadent AG
Vita suprinity (2M2-HT)	Zirconia reinforced lithium silicate glass-ceramic (56-64% SiO <sub>2</sub> , 15-21% Li <sub>2</sub> O, 1-4% K <sub>2</sub> O, 3-8% P <sub>2</sub> O <sub>5</sub> , 1-4% Al <sub>2</sub> O <sub>3</sub> , 8-12% ZrO <sub>2</sub> , 0-4% CeO <sub>2</sub> , 0-6% pigments)*	VS	Vita Zahnfabrick
*As indicated by manufacturers			

**Table 2. Groups in the study**

Materials	Surface finishing and colorant solution groups			
IPS (I)	Glaze + distilled water I1a	Glaze + cola I1b	Glaze + tea I1c	Glaze + coffee I1d
	Sof-lex + distilled water I2a	Sof-lex + cola I2b	Sof-lex + tea I2c	Sof-lex + coffee I2d
	Shofu + distilled water I3a	Shofu + cola I3b	Shofu + tea I3c	Shofu + coffee I3d
VS (V)	Glaze + distilled water V1a	Glaze + cola V1b	Glaze + tea V1c	Glaze + coffee V1d
	Sof-lex + distilled water V2a	Sof-lex + cola V2b	Sof-lex + tea V2c	Sof-lex + coffee V2d
	Shofu + distilled water V3a	Shofu + cola V3b	Shofu + tea V3c	Shofu + coffee V3d

(Nescafe Classic, Nestle, Bursa, Turkey) and tea bags (Earl Grey, Doğuş, Ordu, Turkey) were used in the study and prepared according to the manufacturer's suggested concentrations. The 18.5 g x5 coffee sticks were added to 1 L of boiled distilled water. To prepare the tea solution, 10 g x5 black tea bags were added to 1 L of boiled water and allowed to steep for 3 min. A one L cola (Coca-Cola, Coca-Cola Co, İstanbul, Turkey) solution was used in the same manner.

#### Coloring Methods

Distilled water was used for the control group in the study. The samples were stored in cola, coffee, and black tea, distilled water solutions in the incubator at  $37 \pm 1$  °C. As 144 h corresponds to about six months of coffee consumption (33), all sample groups were exposed to the relevant solutions for 144 h to ensure standardization. After removal from the colorant solutions, the samples were washed with distilled water for five min, and then dried.

#### Color Measurements

Color measurements were performed before and after exposure to the solutions after surface treatments were applied. Color measurements were made with a clinical spectrophotometer (Vita Easy Shade Advance, Vita Zahnfabrik, Germany) using a black and white background under D65 lighting conditions. Translucency values of the samples were determined by the TP according to the CIE L \* a \* b \* system. For the translucency measurement, the formula  $TP = [(L * B - L * W)^2 + (a * B - a * W)^2 + (b * B - b * W)^2]^{1/2}$  was used. Data's were statistically analyzed using three-way analysis of variance (ANOVA). The Bonferroni test was used for multiple comparisons.

The SPSS (Statistical Package for the Social Sciens) for Windows computer program was used for statistical analyses. Material, method, group and interaction effects were considered to be significant ( $p < 0.001$ ).

#### Results

The TP values obtained for each group are summarized in Table 3. When a material has a TP of 100, TP is considered transparent; a TP of 0 indicates that the material is opaque. TP shows the color difference ( $DE^*$ ) on a black and white background of a material of the same thickness and is calculated with the color difference formula (14). A color difference equal to zero ( $DE^*=0$ ) describes a non-translucent material with excellent masking properties (34).

Material, method, group and interaction effects were found statistically significant ( $p < 0.001$ ). There was a statistically significant difference in Total TP value between the materials in terms of applied surface treatments ( $p < 0.05$ ). The ranking of the TP values of materials for control group after the surface finishing process from highest to lowest was Sof-lex > Glaze = Shofu for IPS and Shofu > Sof-lex > Glaze for VS. After surface finishing, the Total TP value of the VS material ( $17.18 \pm 2.63$ ) was found to be higher than that of the IPS material ( $15.55 \pm 1.93$ ). The ranking of the Total TP values after the surface finishing process from highest to lowest was Sof-lex > Glaze > Shofu for IPS and Shofu > Glaze > Sof-lex for VS. The TP value for each group was different in terms of exposed solutions after surface finishing ( $p < 0.05$ ). The highest TP value was demonstrated by the VS control group treated with Shofu (V3a) ( $TP: 21.36 \pm 0.07$ ). The lowest translucency

**Table 3. Mean and standard deviation of translucency parameter values and differences between the groups**

Material	Method (n=10)	Group								Total	
		Control		Cola		Tea		Coffee			
IPS	Glaze	16.08±0.39	(A,x,a)	11.89±0.15	(B,x,a)	16.63±0.2	(C,x,a)	15.01±0.23	(D,x,a)	15.41±1.97	(x,a)
	Sof-lex	18.41±0.2	(A,x,b)	12.58±0.11	(B,x,b)	15.33±0.14	(C,x,b)	16.05±0.12	(D,x,b)	16.08±2.13	(x,b)
	Shofu	1.08±0.1	(A,x,a)	12.75±0.05	(B,x,c)	14.78±0.06	(C,x,c)	14.67±0.1	(D,x,c)	15.14±1.58	(x,c)
	Total	16.6±1.15	(A,x)	12.41±0.39	(B,x)	15.58±0.8	(C,x)	15.24±0.62	(D,x)	15.55±1.93	(x)
VS	Glaze	18.54±0.08	(A,y,a)	18.19±0.1	(B,y,a)	19.62±0.1	(C,y,a)	15.88±0.08	(D,y,a)	17.12±2.26	(y,a)
	Sof-lex	20.07±0.11	(A,y,b)	16.73±0.06	(B,y,b)	11.76±0.07	(C,y,b)	14.82±0.06	(D,y,b)	16.13±2.78	(y,b)
	Shofu	21.36±0.07	(A,y,c)	17.65±0.11	(B,y,c)	14.98±0.1	(C,y,c)	16.86±0.07	(D,y,c)	18.3±2.41	(y,c)
	Total	19.99±1.18	(A,y)	17.52±0.62	(B,y)	15.45±3.28	(C,y)	15.85±0.85	(D,y)	17.18±2.63	(y)
Total	Glaze	17.31±1.29	(A,a)	15.04±3.23	(B,a)	18.13±1.54	(C,a)	15.45±0.48	(D,a)	16.27±2.27	(a)
	Sof-lex	19.24±0.86	(A,b)	14.65±2.13	(B,b)	13.54±1.83	(C,b)	15.44±0.64	(D,a)	16.11±2.47	(b)
	Shofu	18.72±2.71	(A,c)	15.2±2.51	(B,c)	14.88±0.13	(C,c)	15.76±1.13	(D,b)	16.72±2.57	(c)
	Total	18.42±1.95	(A)	14.96±2.63	(B)	15.52±2.37	(C)	15.55±0.8	(C)	16.36±2.45	

(ABCD): Intergroup comparison for interaction and main effects, (xy): Comparison of materials for interaction and main effects, (abc): Comparison between methods for interaction and main effects, VS: Vita suprimity, IPS: IPS e.max cad

Three-way ANOVA was used. The Bonferroni test was used for multiple comparisons. Material, method, group, and interaction effects were significant ( $p<0.001$ ).

value was found in the VS group treated with Sof-Lex and exposed to black tea (V2c) (TP: 11.76±0.07). The translucency value of each material was generally statistically significant in terms of exposed solutions after the applied surface treatment ( $p<0.05$ ). The greatest decrease in TP values was observed in the IPS groups incubated in the cola solution. There was a statistically significant difference in TP values after exposure to colorant solutions for both materials ( $p<0.05$ ). The lowest TP value for the IPS group was observed in the samples exposed to cola, while, for the VS group, it was observed in the samples exposed to black tea.

## Discussion

Based on the results of our work, the null hypotheses were rejected as the translucency of the monolithic CAD/CAM ceramics was affected by surface finishing and colorant solutions. Significant differences were shown among the ceramic groups for TP value.

Translucency is a very important factor in the selection of metal-free materials. Translucency is usually determined by CR or TP. Color determination in dentistry is done visually or instrumentally using spectrophotometers and colorimeters (35). Color measurements made with instruments indicate the potential to eliminate subjective errors in color evaluation and more importantly, are more sensitive when compared to the naked eye, without measuring the slight differences between the colors of colored objects on flat surfaces (36). During color measurement, both the true color of the surface and the lighting condition where the surface is measured affect the color that is measured (33). Therefore standard illuminant D65 was used in the study.

Color stability is as important as translucency in the long-term clinical success of ceramic restorations. Since researchers focus on the mechanical properties of ceramics, little is known about the color changes which occur over time in ceramic restorations. The absorption of colorants from external sources could



cause staining in dental materials (37). Several studies have shown that nicotine (38), coffee and tea (39) were coloring solutions for polymeric materials. However, there is little research showing the effects of these liquids on ceramic surfaces. Surface roughness and surface finishing method have been shown to affect the paint ability of dental materials (37). Surface roughness has a direct effect on the sensitivity of the material to the extrinsic stain. However, finishing and polishing procedures, as well as the composition of the dental material, can affect the surface quality of the material; premature coloration is thought to be due to this (40). The purpose of this study was to observe the translucency change in monolithic glass ceramics exposed to cola, coffee, and black tea for six months. The effect of various surface finishing methods on the translucency of ceramics was also investigated.

Dental restorations should have a highly polished surface prior to use. If there is a rough surface on a restoration or prosthesis, food and other residues will stick to the rough surface. The ceramic surface is traditionally subjected to a surface treatment known as glaze. The glaze process closes the open pores on the surface after firing, providing better optical properties and more surface smoothness (41). At the same time, a smooth surface is formed to reduce biofilm accumulation (41). However, direct finishing and polishing methods on the restoration surface are widely used intraorally. Abrasive rubbers, aluminum oxide discs or diamond polishing paste combined with felt and silicone rubber discs can be used for this process. This procedure produces smooth surfaces, shortens the working time, and can be used to restore brightness after glaze processing, giving the restoration a more natural appearance (42). However, studies are lacking on the best finishing and polishing methods for VS and IPS ceramics to obtain a uniform ceramic surface. The Shofu abrasive tire system consists of ceramic polishing lacquer for pre-polishing, ultra for polishing, and ultra II for a high gloss finish. Sof-lex discs are aluminum oxide discs. Polishing using small to large discs is performed. In our study, the groups which were finished with Sof-lex for IPS ceramics and were finished with Shofu for VS ceramics showed the highest TP values. The difference in the TP values could be the result of the different dimensions of the ceramic material crystals. At the same time, the distribution of crystals and the

hardness of the material could affect the TP.

According to our results, statistically different TP values were determined for the different groups. The TP values of the ceramics ranged from 11.76 (V3b) to 21.36 (V1c). It was found that the TP value for the controls of the total VS material after surface treatment was higher than that of the controls of the total IPS material. Few studies have reported the TP values of the monolithic ceramics used in this study. In the present study, it was found that the TP value of the zirconia-reinforced glass-ceramic was higher than that of the lithium disilicate ceramic, similar to the findings of Awad et al. (43) and Sen and Us (44) studies. The researchers attributed this transparency difference to the fact that the lithium disilicate crystals in the lithium disilicate were four to eight times larger than those in the zirconium-reinforced glass-ceramics (43,44). It has been reported that smaller crystals in the zirconia-reinforced lithium silicate glassy matrix of the ceramic exhibit better TP values due to the high glass content (43). In light of this information, we think that differences in TP values are responsible for the crystal content, grain size and microstructural differences in the materials.

To the best of the authors' knowledge, there is no study evaluating the TP of the specific ceramics used in our study after being kept in colorant solutions. The results of Ramakrishnaiah et al. (45) study showed an increase in the surface wettability and surface roughness of VS and IPS ceramics in direct proportion to the hydrofluoric acid etching time. In this study, generally the translucency value decreased in the groups treated with cola, coffee, and black tea for both VS and IPS groups according to the control groups. The lowest TP value for the IPS group was observed in the samples exposed to cola, while for the VS group it was observed in the samples exposed to tea. Cola, tea and coffee are acidic liquids (46). It is thought that this decrease in translucency may be because of the increased wettability of the ceramics as a result of the increase in the roughness of the ceramics due to the acidic effects of cola, coffee, and black tea. al-Hiyasat et al. (47) reported that cola abrades various ceramics and that this erosion can lead to a tribochemical corrosion mechanism. Crispin and Caputo (18) have shown that significant color change occurs on rough surfaces. The decrease in TP values after treatment with cola, coffee, and black tea indicates that these

solutions cause color change on the VS and IPS ceramics. It is reported that theaflavins in tea leaves cause color change. Likewise, caffeine and caffeic acid cause a color change in the materials (48).

### Study Limitation

One limitation of this study is that the investigation was carried out under *in vitro* conditions. Further *in vivo* studies are required to simulate clinical conditions for TP of VS and IPS ceramics.

### Conclusions

Within the limitations of this study, the following conclusions were drawn. The translucency was affected according to the monolithic CAD/CAM ceramic type. The translucency of the zirconia-reinforced glass-ceramic was found to be higher than that of the lithium disilicate glass-ceramic. Colorant solutions decreased the translucency of both VS and IPS ceramics. The lowest TP value for the IPS group was observed in the samples exposed to cola, while, for the VS group, it was observed in the samples exposed to black tea. According to the results of this study, IPS ceramics finished with Sof-Lex and VS ceramics finished with Shofu exhibited higher translucency values.

### Ethics

**Ethics Committee Approval:** The ethics committee approval was not necessary since the study was *in vitro*.

**Informed Consent:** This study is a laboratory study. Therefore, patient consent is not required.

**Peer-review:** Externally peer-reviewed

### Authorship Contributions

Concept: Y.H., I.S., Design: Y.H., I.S., Data Collection or Processing: Y.H., I.S., Analysis or Interpretation: Y.H., I.S., Literature Search: Y.H., Writing: Y.H.

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# The Nutritional Characteristics of Greenhouse Workers Exposed to Intensive Pesticides and Determining the Levels of Xanthine Oxidase, Nitric Oxide and Arylesterase in Their Blood

## Yoğun Pestisit Maruz Kalan Sera İşçilerinin Kanlarındaki Ksantin Oksidaz, Nitrik Oksit ve Arilesteraz Seviyelerinin ve Beslenme Alışkanlıklarının Belirlenmesi

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<sup>1</sup>Adnan Menderes University Faculty of Health Science, Department of Nutrition and Dietetics, Aydın, Turkey

<sup>2</sup>Süleyman Demirel University Faculty of Pharmacy, Department of Pharmaceutical Microbiology, Isparta, Turkey

<sup>3</sup>Health Sciences University Faculty of Pharmacy, Department of Biochemistry İstanbul, Turkey

<sup>4</sup>Süleyman Demirel University Faculty of Engineering, Department of Food Engineering, Isparta, Turkey



### Keywords

Arylesterase, greenhouse workers, nitric oxide, nutritional habits, pesticide, xanthine oxidase

### Anahtar Kelimeler

Arilesteraz, sera işçileri, nitrik oksit, beslenme alışkanlıkları, pestisit, ksantin oksidaz

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### Address for Correspondence/Yazışma Adresi:

Serdal Ögüt PhD,  
Adnan Menderes University Faculty of  
Health Science, Department of Nutrition and  
Dietetics, Aydın, Turkey  
Phone : +90 256 213 87 55  
E-mail : serdalogut@yahoo.com  
ORCID ID: orcid.org/0000-0001-8863-7249

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### Abstract

**Objective:** Agricultural spraying in greenhouses may cause workers be exposed to the adverse effects of pesticides more than outdoor land workers. High dose application and spraying of pesticides without prevention may increase risks of harmful effect remarkably. In this study, influence of greenhouse workers from pesticides was investigated.

**Materials and Methods:** For this study 74 people were chosen, including 44 workers who have been exposed to pesticides and 30 people who have never been directly exposed pesticides. To compare continuous variables, the Student's t-test was used where appropriate.

**Results:** According to nutritional habits 52.5% of greenhouse workers consumed their production of tomatoes and also other fruits and vegetables without washing. In addition, 59% of greenhouse workers have consumed water and 54.5% of them have consumed some food while spraying pesticides. The statistics showed that levels of xanthine oxidase (XO) and nitric oxide (NO) in pesticides-exposed workers were significantly higher in comparison to non-exposed group and the level of ARE was significantly lower in pesticides-exposed workers when compared with non-exposed group, respectively (p<0.05).

**Conclusion:** The results of study revealed that levels of XO and NO in study group were significantly (p>0.05) higher than control group and the level of arylesterase was significantly (p<0.05) lower than control group, respectively.

### Öz

**Amaç:** Sera işçileri açık alan tarım işçilerine kıyasla pestisitlerin zararlı etkilerine daha fazla maruz kalabilirler. Gerekli koruma önlemleri alınmadan yapılan pestisit uygulamaları, uygulayıcılarda dikkate değer şekilde sağlık problemlerini artırmaktadır. Bu çalışmada sera işçilerinin pestisitlerden etkilenme durumu araştırılmıştır.

**Gereç ve Yöntemler:** Bu çalışmada, en az 3 senedir seralarda zirai ilaçlama yapan işçilerin kanlarında ksantin oksidaz (XO), nitrik oksit (NO) ve arilesteraz enzim

seviyeleri araştırılmıştır. Buna ek bu işçilerin beslenme alışkanlıkları da değerlendirilmiştir. Bu amaçla 74 kişi çalışmaya dahil edilmiştir. Bu kişilerden 44'ü seralarda çalışan tarım işçilerinden oluşturulan araştırma grubu, 30'u ise hayatlarının hiçbir döneminde doğrudan pestisit maruz kalmamış kişilerden oluşturulan kontrol grubudur.

**Bulgular:** Yapılan analizler sonucu tarım işçilerinden oluşan araştırma grubunun XO, NO değerlerinin kontrol grubuna göre anlamlı arttığı, arilesteraz enzim seviyelerinin ise anlamlı azaldığı belirlenmiştir ( $p<0,05$ ). Tarım işçilerinin beslenme alışkanlıkları değerlendirildiğinde ise; işçilerin %52,5'inin tükettikleri meyve ve sebzeleri yıkamadan tükettikleri belirlenmiştir. Buna ek olarak işçilerin pestisit uygulaması yaparken %59'unun su tükettiği, %54,5'inin ise bazı besinler tükettiği belirlenmiştir.

**Sonuç:** Çalışma sonuçları değerlendirildiğinde, deney grubunun XO ve NO seviyeleri kontrol grubundan anlamlı ( $p<0,05$ ) yüksek, arilesteraz seviyeleri ise anlamlı ( $p<0,05$ ) düşük bulunmuştur.

## Introduction

Organophosphate pesticides (OPs), synthetic pyrethroid and carbamates are the most commonly used insecticides worldwide for the pest control of crops. With the green revolution and industrialization, they have become household items of agriculturists (1,2). Pesticides are mainly used in the agricultural sector in Turkey, too. While OPs are commonly used in Turkey, the use of organochlorines is not allowed because of their high toxicity, compose of endosulfan and dicofol. Considering the research, consumption of pesticide has increased 45% between 1979 and 2012 in this country. And forty percent of annual pesticide used takes place in the Mediterranean and Aegean regions (3,4).

Exposed to pesticides cause harmful effect on agriculture and agricultural spraying workers. The most affected parameters in oxidant and antioxidant systems within agriculture and greenhouse workers are the levels of xanthine oxidase (XO), nitric oxide (NO), catalase and superoxide dismutase and arylesterase (ARE). ARE, is related to increased susceptibility to low density lipoprotein oxidation and development of atherosclerosis (5-12).

Nutritional habits and diet style have great importance in every age and every occupational group. Intensive nutrition becomes even more important in workers exposed to pesticides (9).

In this study the workers who are so heavily exposed to pesticides while spraying in greenhouses and the XO, ARE and NO levels in their blood were investigated, and nutritional characteristics were determined.

## Materials and Methods

This experimental study was conducted between 2010 and 2011 in Isparta, Turkey. Forty-four greenhouse workers who have worked at greenhouse

(applying insecticides on tomato and carnation agriculturally) at least 3 years were taken as models for this research. The study was approved by the Research Ethics Committee of Süleyman Demirel University (20.02.2008 date, 01-17 number). All participants gave their verbal and written consents. In this study 74 individuals (44 subjects, 30 control group) were selected. The results of a power analysis, with power 84.9% and a significance level (alpha) of 0.050 suggested that a selection of 74 patients was sufficient for the sample. The control group consisted of persons who were not directly exposed to pesticide. Demographic characteristics (gender, age, BMI etc.) were calculated with descriptive statistics (Student's t-test). The characteristics of the control group and the study group were chosen to be similar ( $p>0,05$ ) (Table 1).

**Detection of XO, NO, ARE Level:** The blood samples of each group were collected and the levels of XO, NO and ARE in blood samples were analyzed. Blood samples of the study group were obtained 30 minutes after the pesticide application into the ethylene diamine tetraacetic acid tubes. Blood samples were transferred to the laboratory in appropriate cold boxes.

Blood samples centrifuged (Nüve brand NF-400 model centrifuge) at 3000 g for 15 minutes. First of all erythrocytes were filtered by taking into the eppendorf tubes after the end of centrifuge. Then erythrocytes were washed by serum physiological for 3 times and stored at -80 °C in deepfreeze until operation day. Analogously, plasma samples which were taken into eppendorf tubes stored at -80 °C in deep freeze until operation day. The blood samples of control group who have never been exposed to pesticides were prepared analogously and stored. Then, all blood samples were diluted 10 times with cold deionizes water. All spectrophotometric analyses were carried out by Perkin Elmer marked UV/Vis model lamb 20 spectrophotometer.

**Table 1. Socio-demographic characteristics of study and control groups**

	Study n=44		Control n=30	
<b>Age</b>	<b>39.4±8.7</b>		<b>38.4±7.5</b>	
BMI (kg/m <sup>2</sup> )	24.3±3.2		25.6±3.6	
Gender	Male	Female	Male	Female
n (%)	36 (81.8)	8 (18.2)	36 (81.8)	8 (18.2)
Smoking	Yes	No	Yes	No
(Ten or more smokers a day)	24	20	16	14
Alcohol	Yes	No	Yes	No
(Consuming alcohol at least once a week)	8	36	7	23
BMI: Body mass index				

**Table 2. Nutrition questionnaire in green house workers**

Questions	Options	n	%
How many times do you eat meal for a day?	1 meal	0	0.0
	2 meals	6	13.8
	3 meals	30	68.1
	>3 meals	8	18.1
Do you skip breakfast?	Yes	12	27.2
	No	32	72.8
Do you skip lunch?	Yes	8	18.1
	No	36	71.9
Do you skip dinner?	Yes	2	4.5
	No	42	95.5
Do you eat snacks?	Yes	4	9
	No	40	91
Do you eat fruits without peeling or washing during application of pesticides to apples/cherries or after application of pesticides?	Yes	22	50
	No	22	50
Do you eat tomatoes before application of pesticides without washing?	Yes	23	52.2
	No	21	47.8
Do you eat other food/fruits during application of pesticides?	Yes	24	54.5
	No	20	45.5
Do you drink water during application of pesticides?	Yes	26	59
	No	18	41
How many times do you eat meat per week?	No	4	9
	1-3 times	31	70.4
	4-6 times	9	20.6
	6 times	0	0.0
How many times do you eat vegetables per week?	No	0	0.0
	1-3 times	32	72.7
	4-6 times	8	18.1
	>6 times	4	9.2



Table 2. continued

How many times do you eat fruits per week?	No	0	0.0
	1-3 times	7	15.9
	4-6 times	15	34.1
	>6 times	22	50
How many times do you drink milk per week?	No	18	40.9
	1-3 times	18	40.9
	4-6 times	8	18.2
	>6 times	0	0.0
How many times do you eat milk products such as yogurt, cheese, and butter vs per week?	No	0	0.0
	1-3 times	6	13.7
	4-6 times	20	45.4
	>6 times	18	40.9
How do you eat fruits? (%)	After wash	15	34.1
	After peel	7	15.9
	Both wash and peel	2	4.5
	Without wash or peel	20	45.5

Assessment of XO activity is based on the principle of producing uric acid from xanthine in XO environment by accepting the presence of XO in the sample (13-15).

NO assesment was performed by the Moshage et al. (15) method. Measurements were done in 545 nm and the results were evaluated as  $\mu\text{mol/L}$  (16,17).

ARE measurement have been realized by the help of ARE kits spectrophotometrically which were developed by Aslan et al. (18).

**Questionnaire:** In order to learn nutrition qualities of agriculture workers, nutrition questionnaires were applied (Table 2). The questionnaires were prepared by the study group. The data in this research was collected by questionnaire form which was prepared by possibility nutritional habits about changing some parameters in blood related with pesticides. Especially habits of washing fruits and vegetables and consuming of food while working were asked to workers. The data in this research was collected by questionnaire form which was prepared by surveying all the related literature by the researcher. Data were evaluated using correlation, crosstab, percentage and frequency distributions on the computer program SPSS statistical package program (17.0)

Table 3. The levels xanthine oxidase, arylesterase and nitric oxide in blood samples of each groups

	Study group (n=44)	Control group (n=30)	p
XO (U/mL)	2.01 $\pm$ 0.26	0.83 $\pm$ 0.36	0.0372
NO ( $\mu\text{mol/L}$ )	8.25 $\pm$ 1.69	5.87 $\pm$ 2.13	0.0458
ARE (U/L)	7.25 $\pm$ 1.22	10.25 $\pm$ 1.63	0.0354

XO: Xanthine oxidase, ARE: Arylesterase, NO: Nitric oxide

### Statistical Analysis

All statistical analyses were performed using commercially available software (SPSS version 17.0; SPSS, Inc, Chicago, Illinois). The datas were evaluated by the Kolmogorov-Smirnov test because they did not show a normal distribution. In evaluation of nutrition questionnaires frequencies were used. The data are provided as median (min-max). To compare continuous variables, the Student t-test was used. Statistical significance was set at  $p < 0.05$ .

### Results

Socio-demographic characteristics of each group were shown in Table 1. The results of greenhouse workers' nutritional habits were shown in Table 2. The levels of XO, ARE and NO in blood samples of each groups were shown in Table 3. The statistics showed

that levels of XO and NO in pesticides-exposed workers were significantly higher compared to non-exposed group and the level of ARE was significantly lower in pesticides-exposed workers when compared with non-exposed group, respectively ( $p<0.05$ ).

The results of study showed that workers eat fruits without washing (45.5%). Another important result in this research is that workers are feeding during application of pesticides (45.5%). Workers skip breakfast compared to other meals (27.2%).

## Discussion

Recently, Isparta has become one of the most important provinces known for its productions of greenhouse vegetable cultivation and greenhouse carnation besides over production of apples and cherries (19). Therefore, greenhouse workers have adverse effects as a result of spraying pesticides in these regions. The most harmful toxicological impacts of these pesticides are over balance of oxidant-antioxidant, and several studies also support this (4-9,20-22). Greenhouse workers and other workers in this area are more susceptible to harmful effects of pesticides as a result of spraying. Because pesticides are not diffused during spraying and after spraying they fold over there a long time. So, greenhouse workers are exposed to harmful effects of pesticides.

In this study, the blood of greenhouse workers was taken just after spraying. According to the results, increase of XO and NO levels, and significantly decrease of ARE level which is one of the antioxidant defense system enzymes are detected, respectively.

To examine role of oxidative/nitrosative stress in human, measurement of cellular lipid peroxidation, total antioxidant capacity, concentration of total thiol group, and determination of total NO in blood are recommended (23,24).

El-Gohary et al. (23) have been examined kind of pyrethroid on rats NO production and its effect. And as a result, effects of pesticides on NO production have been determined.

Ayub et al. (24) determined that malathion which is OPs has effected NO production on rats. At present study activity of NO has been found about  $8.25\pm1.69$  in workers and control groups  $5.87\pm2.13$ . Activity of NO shows a significant increase in workers group than control group ( $p<0.05$ ).

Terzi et al. (25) have examined rotenone's effects which is plant origin pesticide on NO levels of Wistar

albino rats. They have given rotenone three different dose to three different group and they haven't given any rotenone to last group. The results of study showed that there have been no differences between control group and other group in XO activity (25).

XO catalyzes the conversion of hypoxanthine to xanthine and that of xanthine to uric acid, which are the last steps in purine catabolism; product of these processes is a toxic superoxide radical. This reaction is, therefore, considered to be an important potential source of oxygen free radicals *in vivo* (26,27).

At present study while XO activity was identified average in worker group as  $2.01\pm0.26$ , in control group this rate was identified average as  $0.83\pm0.36$ . XO activity increased considerably in worker group as compared to control group. Investigations applied in this study region showed that the most intense usage of Ops came true in this region and each in turn synthetic pyrethroid group and insecticides and carbamate group insecticides followed it. Besides these results, it was identified in this research that workers were not protected or they take a bit measures during application of drug. A great deal of study applied in this region and country support it (28-30). So agrarian drug enforcers and producers considered not enough warning of authorities. Therefore environment and health problems occur of course (31,32).

Human serum ARE [(ARE, EC 3.1.1.2, also known as paraoxonase1 (PON1)] originates from liver with two subtypes. PON1 activity in serum was associated with plasma lipid peroxidation and cardiovascular diseases (33). PON-1 and ARE (ARY) are considered two separate enzymes in spite of their marked biological similarities. Plasma PON-1 catalyzes the hydrolysis of the bioactive organophosphate metabolite paraoxon to nontoxic products as p-nitrophenol and diethyl-phosphoric acid. At the same time, ARE is another aromatic esterase that inactivates the same organophosphate pesticide but unlike PON-1 is not inhibited by cholinesterase inhibitors as phosostigmine (eserine) (34). In this research, ARE levels were determined low levels of workers. The decline in blood levels may be due to chronic pesticide exposure. Workers eat fruits without washing (if fruits are washed, pesticides remove largely).

In this paper while evaluating XO, NO and ARE level of pesticides exposure works at the same time some questions about nutritional habitual were

asked. Because not only application of pesticides but also wrong habits while working can cause exposure of pesticides. And according to the results of questionnaire, greenhouse workers made some mistakes about their nutrition. Especially, consuming food/fruits during application of pesticides is the worst mistake. Since workers eat any food, pesticides can get into the body (via the digestive tract). And eating vegetables or fruits without washing is another significant mistake.

As given in Table 2, workers are fed three meals a day largely (68.1%). This is a condition required and recommended. Workers generally do not skip meals. They do not have breakfast because workers start to work early in the morning. Workers skip the dinner meal minimal. The reason for this, all family members want to get together at dinner time. Another remarkable point in Table 3 is that workers feed during application of pesticides. This is an extremely dangerous situation because workers may be exposed to pesticides through skin and respiratory tract.

One point that is salient in nutrition surveys is that half of agricultural workers eat foods without cleaning and peeling, when they eat fruit. Also, agricultural workers eat tomatoes in greenhouse during their production without cleaning. Up taking of pesticide through digestive system by agricultural workers may derive from tomatoes that are eaten during and afterwards pest control, therefore parameters in this research may be impressed from it. Research carried out in this region show us that agricultural workers eat fruits during pest control and don't take measures efficiently (35,36). Also agricultural workers eat, drink during pest control, therefore quantity of pesticide uptake may go up; it may give rise to several health problems. Also levels of XO, NO and ARE may be impressed negatively.

## Conclusion

All in all pesticide usage is urgent in modern agriculture. But while using pesticide against diseases in crops, human health should be considered also. And negative impressions of agricultural pesticides on humans are very big social issue. This research is aimed to determine whether the pesticides are indeed negative impressions of oxidant- antioxidant balance of agricultural workers. And this oxidant- antioxidant imbalance can be increased hazard of atherosclerosis,

abiotrophy and cancer in agricultural workers. Avoiding and reducing exposures to pesticides will reduce the harmful effects from pesticides. Some taking sample measurement can be provided protection. In order to avoid exposures, safety systems can be used- such as closed systems and enclosed cabs, and wearing appropriate personal protective equipment. And also washing exposed areas often may be another solution for this problem. Researchers must be sensitive above this issue and to try to find new solutions. As we can see from workers nutritional habits, they don't wash their fruits and vegetables and they drink water while working. If they don't do these habitual actions while working they may reduce harmful effect of pesticides, too. We believe that these problems can be reduced with the application of necessary precautions and pest control with stated doses.

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## Ethics

**Ethics Committee Approval:** The study was approved by the Research Ethics Committee of Süleyman Demirel University (date: 20.02.2008, no: 01-17).

**Informed Consent:** All participants gave their verbal and written consents.

## Authorship Contributions

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

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# Relationship Between the Number of Cesarean Deliveries and Maternal-fetal Complications

## *Sezeryan Sayısının Anne ve Çocuk Komplikasyonları ile İlişkisi*

Özgür Deniz Turan, Ezgi Özdemir, Tolga Atakul, Emre Zafer

Aydın Adnan Menderes University Faculty of Medicine, Department of Obstetrics and Gynecology, Aydın, Turkey



### Keywords

Cesarean, fetal, maternal, complications

### Anahtar Kelimeler

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### Address for Correspondence/Yazışma Adresi:

Özgür Deniz Turan MD,  
Aydın Adnan Menderes University Faculty  
of Medicine, Department of Obstetrics and  
Gynecology, Aydın, Turkey  
Phone : +90 532 448 16 43  
E-mail : ozgurdenizturhan@gmail.com

ORCID ID: orcid.org/0000-0003-2230-8404

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### Abstract

**Objective:** Cesarean operation is very common and it is being performed increasingly all over the world. In this study, the effect of the number of cesarean deliveries on maternal and fetal health was evaluated.

**Materials and Methods:** We included patients who underwent cesarean section in our clinic between October 2014 and October 2017, who had no additional disease, between 38 and 40 gestational weeks, and ages between 18 and 40 years. Postnatal records were retrospectively reviewed. Patients who had pregnancy complications such as pregnancy hypertension, gestational diabetes, oligohydramnios, polyhydramnios, or who had a dysmorphic appearing baby or with any genetic syndrome were excluded. Patients were divided into three groups according to the number of cesareans they had undergone. Group 1 consisted of patients with history of two cesarean deliveries, group 2 consisted of patients with 3 cesarean deliveries and group three consisted of patients with 4 or more cesarean deliveries. Groups were then compared to each other for their demographic data and maternal - fetal outcomes.

**Results:** Mean age of the patients studied was 32.4 years [standard deviation (SD): 4.4], mean gestational week was 38 weeks and 2 days (SD: 0.55), and mean hospitalization duration was: 4.4 days (SD: 1.0). There was no significant correlation between the number of cesarean section and the parameters of birth weight, duration of hospital stay, and appearance, pulse, grimace, activity, and respiration scores.

**Conclusion:** The history for the number of previous cesarean deliveries for a pregnancy may not seem to be correlated with increased maternal or fetal complications up to four previous cesarean deliveries. There is a need for future studies with even larger patients to support our findings.

### Öz

**Amaç:** Sezeryan operasyonu tüm dünyada çok yaygın olarak ve giderek artan oranlarda yapılmaktadır. Sunulan bu çalışmada sezeryan sayısının maternal ve fetal sağlığı üzerine etkilerinin incelenmesidir.

**Gereç ve Yöntemler:** Çalışmaya 1 Ekim 2014 - 1 Ekim 2017 tarihleri arasında kliniğimizde sezeryan operasyonu geçirmiş ve ek hastalığı olmayan, gebelik haftası 38-40 hafta arasında olan, gebeliği 18-40 yaşlarında olan kişiler dahil edilmiştir. Bu kişilerin doğum sonrası kayıtları retrospektif olarak incelenmiştir. Gebelik

tansiyonu, gestasyonel diyabet, oligohidramniyoz, polihidroamniyoz gibi gebelik komplikasyonu olan ya da bebeğe ait anomali ya da herhangi bir genetik sendrom tanısı konmuş olan hastalar çalışma dışında bırakıldı. Hastalar sezeryan sayılarına göre üç gruba ayrıldı. Sezeryan sayısı 2 ise grup 1, üç ise grup 2, dört ise grup 3 olarak tanımlandı. Her bir grup demografik veriler, maternal ve fetal sonuçlar açısından karşılaştırıldı.

**Bulgular:** Çalışmaya alınan hastaların yaş ortalaması 32,4 [standart deviasyon (SD): 4,4], ortalama gebelik haftası: 38 hafta iki gün (SD: 0,55), ortalama hastanede kalma süreleri: 4,4 (SD: 1,0). gündü Sezeryan sayısı ile doğum ağırlığı, hastanede kalış süresi, 1. ve 5. dakika görünüm, nabız, yüz buruşturma, etkinlik ve solunum skorları arasında anlamlı korelasyon gözlenmedi.

**Sonuç:** Dördüncü sezeryan sayısına kadar geçirilmiş sezeryan öyküsü; gebelikte maternal ve fetal komplikasyonlarla korelasyon göstermeyebilir. Bulgularımızı desteklemek için gelecekte daha büyük hastalarla yapılacak olan çalışmalara ihtiyaç vardır.

## Introduction

The cesarean section is the most commonly performed obstetric operation in the world. The rates of cesarean operation in recent years have increased in many countries as well as in Turkey. It has been reported that cesarean rates have increased by 10-15% over the last 30 years throughout the world (1). In Turkey, while 8% cesarean rate was reported in 1993, it has risen to 37% in 2008. Despite the increased cesarean rates, the rate of tubal sterilization incidence among married women in our country has been reported to be only 2.7% (2).

It is well known that cesarean section operation has a significantly increased maternal mortality and morbidity risk compared to normal vaginal birth (3,4). Postpartum maternal complications of cesarean sections may include wound infection, endometritis, bladder injury complications related to blood transfusions, hemorrhage, disseminated intravascular coagulation and urinary tract infections. Important possible fetal-neonatal complications are neonatal asphyxia, respiratory distress syndrome, transient tachypnea neonatal sepsis and neonatal morbidity (5).

Increased number of cesarean rates has been shown to be correlated with decreased fertility, increased early pregnancy loss, higher ectopic pregnancy rates, and low birth weight (6,7).

Therefore, we aimed to investigate the relationship between the history of previous number of cesarean deliveries and maternal-fetal outcomes.

## Materials and Methods

The study was approved by the Ethics Committee for Research of the Adnan Menderes University (19/02/2018-E.10086), and informed written consent was obtained from all subjects.

Ninety-two pregnant women between the ages of 18-40, who had a cesarean section in our clinic between October 2014 and October 2017 with no additional disease, between the ages of 38-40 weeks of gestation were retrospectively screened and included in the study. All cesarean operations had been performed by low segment transverse incision under regional spinal anesthesia. Patients who had had pregnancy complications such as gestational hypertension, gestational diabetes mellitus, oligohydramnios, polyhydramnios, intrauterine growth retardation, multiple pregnancy, or who had a dismorphic baby or baby with genetic syndrome were excluded from the study. Cases were divided into three groups according to the personal history for previous cesarean numbers. Group 1: those who had two cesarean operations, group 2: those who had three cesarean operations, and group 3: women who had four cesarean delivery histories. Demographic data, maternal and fetal outcomes of each group were then compared to each other.

## Statistical Analysis

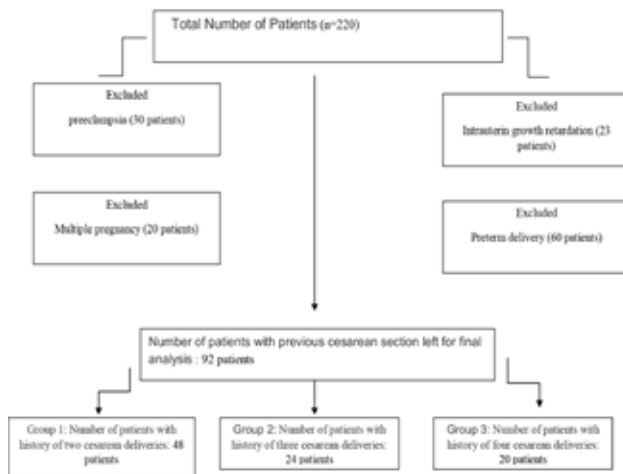
The program SPSS 12 was used for statistical analyses (SPSS Inc., Chicago, IL, USA). The t-test was used to compare normal distribution of variables, ANOVA test was used to compare more than two groups with one dependent variable. A value of  $p < 0.05$  was considered as statistically significant.

## Results

In our study we retrospectively screened 220 patients records who delivered with caesarean section. Pregnants with complication were excluded and a total of 92 patients were included in the study. The study design is shown in the Figure 1. The mean age for the studied population was 32.4 years [standard deviation (SD): 4.4], mean gestational week was 38 weeks and 2 days, mean hospitalization time:

4.4 days (SD: 1.0). Demographic data was given in Table 1.

There was no significant difference with respect to maternal age among groups ( $p=0.806$ ). Smoking rates



**Figure 1.** Study Design

were not significantly different either ( $p=0.958$ ). The rate of higher education was 78.3% in the first group, 21.7% in the second group and 0% in the third group ( $p=0.09$ ). When the fetal status was evaluated, no significant differences were found among the groups in terms of gestational age, birth weight, first and fifth minute appearance, pulse, grimace, activity, and respiration (APGAR) scores ( $p>0.05$ ).

There was no significant correlation between birth weight and number of cesarean section, duration of hospital stay, and 1<sup>st</sup> and 5<sup>th</sup> minute APGAR scores (Table 2 and 3).

## Discussion

Cesarian rates are increasing in Turkey as well as in the majority of other countries. In this study, there was no significant difference between the groups in terms of demographic data.

As the number of previous cesarean deliveries increases, the duration of operation prolongs due

**Table 1. Demographic data and clinical findings in patients**

	Group 1 (n=48)	Group 2 (n=24)	Group 3 (n=20)	p
Maternal age [year, mean (SD)]	32.1 (4.76)	32.7 (3.28)	32.0 (4.31)	0.797
Gestational week at birth [mean (SD)]	38.2 (0.43)	38.2 (0.55)	38.0 (0.44)	0.195
Body mass index [kg/m <sup>2</sup> , mean (SD)]	28.2 (3.61)	28.39 (5.64)	28.0 (5.04)	0.972
Post-operative hemoglobin level [g/dL, mean (SD)]	10.6 (1.15)	10.0 (1.24)	10.3 (1.46)	0.187
Hospitalization time [day, mean (SD)]	4.3 (0.93)	4.5 (1.28)	4.5 (1.19)	0.765
APGAR 1. minute [mean (SD)]	7.8 ( 0.80)	7.9 (0.85)	8.1 (0.71)	0.634
APGAR 5. minute [mean (SD)]	9.1 (0.55)	9.1 ( 0.53)	9.1 (0.58)	0.987

SD: Standard deviation, APGAR: Appearance, pulse, grimace, activity, and respiration

**Table 2. Correlation between the history of previous cesarean delivery number and fetal outcomes**

	Fetal Weight [gr, mean (SD)]	p	APGAR at first minute [mean (SD)]	p	APGAR at fifth minute [mean (SD)]	p
Number of cesarean delivery	3260.5 (459.57)	0.220	7.9 (0.79)	0.270	9.1 (0.57)	0.903

SD: Standard deviation, APGAR: Appearance, pulse, grimace, activity, and respiration

**Table 3. Correlation between the history of previous cesarean delivery number and maternal outcomes**

	Number of cesarean delivery	p
Post-operative hemoglobin level [g/dL, mean (SD)]	10.4 (1.27)	0.454
Hospitalization time [day, mean (SD)]	4.4 (1.06)	0.501

SD: Standard deviation



to increased intra-abdominal adhesions, and post-operative hemoglobin levels may decrease as reported in some studies (8). In this study, the history for previous cesarean delivery number was not found to be significantly correlated with hemoglobin levels ( $p>0.005$ ). The reason for this may be related to our relatively shorter than expected operation time even in the 4th cesarean procedures. The other reason may be our relatively lower complication rates.

In our study, we studied the possible correlation between the number of previous cesarean deliveries and maternal-fetal complications in cases with two or more cesarean section histories. In this study, increased cesarean rates and risk of maternal morbidity were not found to be related. Similar to our study, Rashid and Rashid (9) reported that increasing the number of previous caesarean sections in terms of maternal morbidity was not a risk. However, contrary to our results, increased maternal mortality and morbidity rates with increasing cesarean ratios have also been reported (10-13). In addition, it has been shown that the number of previous cesarean deliveries is directly proportional to the risk of morbidly adhesive diseases of placenta. The incidence of accreta continued to rise with increasing previous cesarean deliveries, up to 6.74% for women with previous cesarean deliveries compared with no previous CD, with an odds ratio of 29.8 (11). Compared with previous normal spontaneous vaginal delivery, previous cesarian delivery was a significant risk factor for placenta previa in many studies (14-21). The highest number of previous cesarean delivery in our study was four. It was reported in a study from Turkey that was conducted on 2460 patients, critical complications developed when there were 4 or more previous cesarean delivery histories (10). The reason for low rate of maternal complications in our study may be due to the lack of cases with more than 4 previous cesarean deliveries.

In this study, there was no difference between the neonatal APGAR scores with respect to the number of previous cesarean sections. Similarly to our findings, in the study of Rashid and Rashid. (9) there was no difference in the APGAR scores whom had 5 to 9 previous cesarean operations when compared to 3 or 4 previous cesarean operations (9). Contrary to these results, it was observed that the APGAR scores were

significantly deteriorated in the group of cesarean section in Yaman Tunc et al. (22) study.

Retrospective design, the small number of patients can be regarded as the limitation of our study. A prospective study design that would compare the maternal - fetal outcomes for the number of previous cesarean deliveries and also of patients with previous vaginal deliveries would be interesting. the small number of patients

## Conclusion

In conclusion, the history of previous cesarean delivery number until four previous cesarean may not increase baseline maternal or fetal complication risk. However, there is a need for prospective controlled studies involving more patients to support our finding.

## Ethics

**Ethics Committee Approval:** The study was approved by the Ethics Committee for Research of the Adnan Menderes University (19/02/2018-E.10086).

**Informed Consent:** Informed written consent was obtained from all subjects.

**Peer-review:** Internally peer-reviewed.

## Authorship Contributions

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

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

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# Nosocomial Infections and Cases of *Sphingomonas Paucimobilis*

## Nosokomiyal Enfeksiyonlar ve *Sphingomonas Paucimobilis*: Olguların Sunumu

✉ Fatma Sirmatel<sup>1</sup>, ✉ Elham Behzadi<sup>2</sup>, ✉ Oğuz Karabay<sup>1</sup>, ✉ Aynur Özensoy<sup>3</sup>

<sup>1</sup>Abant İzzet Baysal University Faculty of Medicine, Department of Infectious Diseases and Clinical Microbiology, Bolu, Turkey

<sup>2</sup>Iranian Academy of Medical Sciences, Department of Infectious Diseases and Clinical Microbiology, Tehran, Iran

<sup>3</sup>Abant İzzet Baysal University Faculty of Medicine, Department of Anesthesiology and Reanimation Bolu, Turkey



### Keywords

*S. paucimobilis*, immunosuppressive patients, nosocomial infections

### Anahtar Kelimeler

*S. paucimobilis*, immunsupresif hastalar, nosokomiyal enfeksiyonlar

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### Address for Correspondence/Yazışma Adresi:

Fatma Sirmatel MD,  
Abant İzzet Baysal University Faculty of  
Medicine, Department of Infectious Diseases  
and Clinical Microbiology, Bolu, Turkey  
E-mail : sirmatel@yahoo.com

ORCID ID: orcid.org/0000-0003-0442-5981

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### Abstract

*Sphingomonas paucimobilis* (*S. paucimobilis*), especially in patients with immune suppressive and very rare in healthy people, attracted attention recently, causing nosocomial infections, is a gram-negative, aerobic bacillus. This bacterial nosocomial infection in our hospital as a first six-month period, six times was isolated from five patients. These five patients were acute peritonitis, vaginal cuff, surgical field, soft tissue and bloodstream infection. The identification of microbiological methods known to *S. paucimobilis* was examined by the antibiotic sensitivity disk diffusion test. Antibiotic sensitivity of the isolated bacteria were the same. From the environment and environment cultures *S. paucimobilis* was isolated once from the use water as a hospital infection effect. It was realized that the chlorination of hospital waters was inadequate during that period. Our hospital was thought to have a small epidemic. In conclusion, *S. paucimobilis* has recently been investigated in the context of literature with the belief that a new nosocomial infection will be affected.

### Öz

*Sphingomonas paucimobilis* (*S. paucimobilis*) özellikle immünsüpresif hastalarda ve sağlıklı insanlarda oldukça nadir görülen, son zamanlarda dikkati çeken, nozokomiyal enfeksiyonlara neden olan, gram-negatif, aerobik bir basildir. Bu bakteri hastanemizde ilk altı aylık bir süreç içerisinde nozokomiyal enfeksiyon etkeni olarak, beş hastadan altı kez izole edildi. Toplam beş hasta; akut peritonit, vaginal cuff, cerrahi alan, yumuşak doku ve kan yolu enfeksiyonu idi. *S. paucimobilis*'in identifikasyonu bilinen mikrobiyolojik yöntemlerle, antibiyotik duyarlılığı ise disk difüzyon testi ile incelendi. İzole edilen bakterilerin antibiyotik duyarlılığı aynı idi. Yapılan çevre ve ortam kültürlerinden *S. paucimobilis* hastane enfeksiyon etkeni olarak bir kez kullanım suyundan izole edildi. O süreç içerisinde hastane sularının klorlamanın yetersiz olduğu fark edildi. Hastanemizde küçük bir epidemi olduğu düşünüldü. Sonuçta *S. paucimobilis* son zamanlarda yeni bir nozokomiyal enfeksiyon etkeni olacağı düşünülerek literatür eşliğinde olgularla irdelendi.

## Introduction

*Sphingomonas paucimobilis* (*S. paucimobilis*), a non-fermentative gram-negative bacillus, has recently begun to be reported as a causative pathogen in rare cases of nosocomial infections (1-4). This bacterium has been shown to be an opportunistic pathogen in environmental samples just like other opportunistic non-fermentative bacteria, especially in immunosuppressive, diabetic and cirrhotic cases (5-14). This micro-organism is a bacterium with poor pathogenicity (12-18), which has the property of being found in low carbon environment (oligotrophic), water, nature and soil. Especially in hospitalized patients, *S. paucimobilis* has been reported as a catheter-associated bacteremia, sepsis, meningitis, peritonitis, genito-urinary, skin and wound infections (6-10). This bacterium has been reported to be community and hospital infections in Turkey and other countries, which are sometimes transmitted by water and food (9,16-22).

In this presentation, five nosocomial infections due to *S. paucimobilis* seen in the hospital within a period of six months were discussed in the context of the literature.

## Case Report

In a university hospital in 2008, *S. paucimobilis* was isolated by conventional methods from six clinical specimens of five patients over a period of six

months. In the hospital microbiology laboratory, the demographic and clinical information of the patients in whom the agent was isolated and the culture samples were belonged to infectious diseases were evaluated by the consul general practitioner.

Identification of the patients: The clinical data were collected from all data records by interviewing the patients with *S. paucimobilis*, the laboratory supervisor, the consultant physician and the clinician in cooperation. Diagnosis, treatment and follow-up of patients were made according to centers for disease control criteria for nosocomial infection (1,2). Environment and patient-use materials were cultured for possible contamination.

Antibiotic sensitivity: The antibiotic sensitivity test of seven *S. paucimobilis* isolates isolated from a total of five patients and one drinking water was carried out by Mueller Hinton agar with disc diffusion method.

Within a total period of 6 months, *S. paucimobilis* was isolated six times from five patients identified as nosocomial infection due to *S. paucimobilis* and once from the use water. The demographic data of the cases are shown in Table 1. Antibiotograms of all isolates were prepared by disk diffusion method; cefazolin, cefepime, cefotaxime and ciprofloxacin are resistant; ampicillin x sulbactam, gentamycin, imipenem, piperacillin x tazobactam, co-trimoxazole, cefaperazone x sulbactam and levofloxacin were found to be sensitive. Antibiotic sensitivity tests of strains that could not be genotypically screened

**Table 1. Isolated nosocomial *Sphingomonas paucimobilis* infections and demographic data of patients**

Age/genus	Where isolated	Clinical findings	Comorbid sick	Therapy	Results
54/male	Blood culture and peritoneal fluid	Fever/vomiting/nausea/abdominal pain	Chronic renal failure (Ambulatory peritoneal dialysis since 5 years)	Ampicilline plus sulbactam	Recovery
55/women	Vaginal cuff	Abdominal pain and vaginal discharge	Endocervical cancer and chemotherapy	Imipenem plus gentamysine	Recovery
67/male	Wound culture from surgical incision	Postoperative surgical incision infection	Cholangio carcinoma	Sefaperazon plus sulbactam	Recovery
63/women	Wound culture from decubitus	Nosocomial soft tissue infection	Multiple myelomatosis	Levofloksasin	Recovery
56/male	Blood culture	Blood stream infection	Hodgking lymphoma and chemotherapy	Piperacillin plus tazobactam	Death

as a molecular were the same. Insulation from any place other than the use water could not be made in the environment survey. When we retrospectively analyzed the 5 cases of our study, we found that all of the cases of ciprofloxacin and the underlying malignancy of the second immunodeficiency epidemic were detected.

Examination of the cases and the underlying risk factors: The first case was a 54-year-old male patient had been on regular peritoneal dialysis to treat chronic renal failure for 3 years. The patient was admitted to the emergency department with fever, nausea and vomiting, and hospitalised for acute pyelonephritis pre-diagnosis and taken for empirical treatment with ciprofloxacin. *E. coli* was produced in the urine culture of the patient and the urine culture was negative on the 5<sup>th</sup> day of treatment. On the 8<sup>th</sup> day of hospitalization, fever, abdominal pain and turbidity were seen in the peritoneal dialysis fluid when the patient's treatment was monitored to be completed at the 10<sup>th</sup> day. *S. paucimobilis* was isolated in the blood and peritoneal fluid cultures. The patient was treated with ampicillin x sulbactam.

Second case; A 55-year-old female patient with endocervical cancer diagnosis and postoperative chemotherapy treatment. The patient had been in hospital for a month. *S. paucimobilis* was isolated from the vaginal cuff culture when the patient complained of fever, abdominal pain and purulent vaginal discharge. The underlying risk factors were diabetes, malignancy and long-term antibiotic use (ciprofloxacin and cefazolin). The patient was treated with a combination of imipenem and gentamycin.

Third case; A 67-year-old male patient who was operated on for cholangiocarcinoma. On the 34<sup>th</sup> day of the hospitalization, *S. paucimobilis* was isolated from the purine stream developed at the site of abdominal incision, in culture. There was an underlying malignancy story. Previously, the treatment of ciprofloxacin and ceftriaxone was replaced with cefoperazone x sulbactam. After ten days of treatment the patient was discharged with the recovery of the incision site.

Fourth case; A 63-year-old female patient who had been diagnosed with multiple myeloma since long-term follow-up. The patient was on the 45<sup>th</sup> day of his admission. *S. paucimobilis* was isolated from cultures of the patient's dorsal rupture of decubitus ulcers

in the back and hips. The patient was treated with levofloxacin and wound care. No regrowth was found in the control cultures of the wounded patients.

In the third and fourth cases, information was obtained that they used ciprofloxacin in advance of the urinary system infection.

Fifth case; A 56-year-old male patient with a Hodking Lymphoma that has been seen since long time. The case was followed up for 12 days in the hospital with the cause of febrile neutropenia after 4<sup>th</sup> cure chemotherapy and taken for carbapenem and ciprofloxacin treatment. *S. paucimobilis* was isolated in a blood culture taken at the height of the fever (38.9 °C) when the patient's discharge was considered. Although the patient was treated with piperacillin x tazobactam, she died of severe respiratory failure on 20<sup>th</sup> day of admission. All cases have been taken with the informed consent form.

## Discussion

*S. paucimobilis* previously known as *Pseudomonas paucimobilis* non-fermentatif gram-negative bacilli, which is a slow moving eyelashes one polar (3,4,19). Bacteria are found in nature, in soil, in water, in the environment outside, and rarely cause nosocomial infections (5,9,12,15-17). In nosocomial infections, where endogenous florans predominate, *S. paucimobilis* is often contaminated by the contamination of sterile solutions (5,6,9). Pathogenicity of the bacteria, although weak, especially when it is stated that patients be treated with immunosuppressive and hematological history is with a 5.5% mortality (12,17). Glupczynski et al. (19) first reported peritonitis in the literature and then Hsueh et al. (17) have isolated a total of eleven *S. paucimobilis* from six patients, influencing nosocomial infection in the large study they performed in 1995-96. In the literature, it has been reported that *S. paucimobilis* is associated with ventilator-associated pneumonia, intravascular catheter infection, wound infection, urinary tract infection, biliary tract infection, and all patients have been treated (14,17,18). In some publications, it has been reported that the patient's blood culture is long-standing and the removal of the catheter allows eradication of the bacterium (3,4,6,8,13).

Our first case was a case with peritoneal dialysis and acute peritonitis was shown in the hospital while

it was seen as urinary system infection. We thought that the patient would have infected *S. paucimobilis* during peritoneal dialysis. Since the peritoneal exchange fluids used are sterile and ready, the agent may be contaminated from the outside. In the case of peritoneal dialysis performed in the hospital, *S. paucimobilis* was detected as an effective pathogen in the developing nosocomial peritonitis and treated with appropriate antibiotic. In this case, it was thought that it could be transmitted from outside environment through the hands and environment culture, but the source was not found. The isolation of the same bacterium from other patients over the next six months of this episode has caught our attention to a possible nosocomial epidemic. Isolation from a waiting cell with utility water in the constructed environment is done.

In our country and abroad, occasionally isolation of peritoneal fluids is occurred (6,8,17,18). Dervisoglu et al. (6) have isolated *S. paucimobilis* in the peritoneal fluid of a patient undergoing chronic ambulatory peritoneal dialysis and have shown that this bacteria remains in the patient's peritoneal fluid for 17 days despite appropriate antibiotic therapy. Some authors have reported that bacterial peritoneal fluid isolation is terminated after changing the patient's catheter in their presentations (3,6). When case-based analysis was performed, *S. paucimobilis* was isolated from both blood and peritoneal fluid (8,17-19). Maragakis et al. (5) detected *S. paucimobilis* bacteremia in a total of six patients and thought that they were contaminated with intravenous fentanyl, but could not isolate bacteria from closed solutions. Likewise, *S. paucimobilis* infections, which are transmitted by water in coronary intensive care patients and hematologic-oncologic outbreaks, have been reported in studies reported by our country (9,10,13). Willke et al. (9) showed *S. paucimobilis* distilled water used in the oxygen flowmeter, to the coronary intensive care patients by molecular examination method. We thought that we could be contaminated by water that was used from outside. But we have not done molecular studies. All isolated strains showed the same sensitivity of antibiotics.

*S. paucimobilis* was isolated from the vaginal cuff culture taken during hospitalization in the second case presented. This phenomenon has not been reported in English sources yet. Having the underlying malignancy of the patient, performing interventional

procedures, and antibiotic use predispose to nosocomial infection. Likewise, the third, fourth and fifth cases are immunosuppressive patients and have multiple risk factors for *S. paucimobilis* infection. The third and fourth cases were thought to be contagious during the dressing and wound care of the bacterium, and during the intravenous application of the fifth case. In the studies conducted, in the development of *S. paucimobilis* infections, as preparatory factors; alcohol use, diabetes and ciprofloxacin have been reported as risk factors (3,11,12,17). Toh et al. (12) reported a total of 55 nosocomial *S. paucimobilis* infections in a four-year study and reported diabetes and alcoholism as underlying factors. The authors reported that 63 of *S. paucimobilis* isolates from 61 patients in this study, half of them reported that they were community-acquired infections and the other half of them commonly had lower respiratory tract infections in hospital-acquired infections (12).

While the second, third and fourth cases were cured with treatment, our fifth case died despite appropriate treatment. Despite the presence of a weak pathogen, the *S. paucimobilis* causative pathogen can be seen as having high morbidity and mortality in immunosuppressive cases. In many studies, when *S. paucimobilis* was not treated as a pathogen, it was associated with high mortality in immunosuppressive patients, with 40-58% of ciprofloxacin resistance (4,7,11,15). In retrospective studies conducted in our country, Turhanoglu and Bilman (20) have isolated this bacterium in 83 materials, including the most sputum, in a ten-year review according to laboratory data. Bayram et al. (21) defined 11 of the 24 cases as nosocomial infection. *S. paucimobilis* infections with poor pathogenicity but on different clinical tables are more mortal, especially in immunosuppressive patients (3,5,12,17,19).

Five cases presented are immunosuppressive patients. The use of broad spectrum antibiotics in the literature of these patients is also a risk factor. In the examination of the cases, there are preparatory factors for the development of *S. paucimobilis* infections, such as ciprofloxacin using history and being immunosuppressive patients. For the first case, it was understood that the risk factors identified in their follow-up were inadequate chlorination and pending distilled waters. When this rare bacterium was isolated from other patients, environmental cultures were taken as a possible risk factor for

**Table 2. Nosocomial *Sphingomonas paucimobilis* cases followed up to date (total 45 cases)**

Autors name	Country	Underlying causes	Number	Clinic findings	Where isolyted	Result	Sources
Yozgat Y.	Turkiye	Rheumatic fever	1	Septic shock	Blood	Recovery	Indian J Med Microbiol. 2014
Gil Diaz A.	Canary islands	Diabetes mellitus	1	Hepatitis	Blood	Recovery	Ir J Med Sci. 2014
Pascale R.	Italy	Chronic osteomyelitis	1	Surgical incision infection	Purulent material	Recovery	
Bayram N.	Turkiye	4 febril neutropenie, one burn, one imperfore anus,	11	Bacteriemia, catheter infections	Blood	Recovery	
Lanoix JP.	France	Lynphoma	1	Bacteriemia, catheter infections	Blood	Recovery	Med Mal Infect. 2012
Thompson SM.	England	Surgical procedure	1	Surgical incision infection	Patien used material	Recovery	Ann R Coll Surg Engl. 2011
Kriet MM.	France	Postpartum panophtalmitis	1	Ophthalmitis	Purulent tears	Recovery	Bull Soc Belge Ophthalmol. 2011
Özdemir M.	Turkiye	Down syndrome	1	Pnemoniae	Blood	Recovery	Int J Med Sci. 2011
Mutlu M.	Turkiye	Neonatal	11	Bacteriemia, catheter infection	Hospital using water	Recovery	Indian Pediatr. 2011
Meric M.	Turkiye	Cardiac operation	2	Pneumoniae	Hospital using water	Recovery	J Infect. 2009
Bulut C.	Turkiye	Ventriculoperitoneal şunt	1	Bacteriemia, catheter infection	Blood	Recovery	Mikrobiyol Bul. 2008
Kilic A.	Turkiye	Lynphoma, leucemia, neuroblastoma	4	Bacteriemia	Blood and hospital using water	Recovery	Jpn J Infect Dis. 2007
Lee JU.	Kore	Peritoneal dialysis	1	Bacteriemia, catheter infection	Blood	Recovery	Kidney Research and Clinical Practice. 2012
Perola O.	Finland	Leukemia	1	Bacteriemia	Blood and hospital using water	Recovery	J Hosp Infect. 2002
Hsueh PR.	Taiwan	Cholangio carcimamatosi, breast cancer, wound, cranial taruma, adenocarcinomatosis	6	Cholangitis, urinary tract infection, wound infection, pneumoniae	Blood, bile, wound, urine	Recovery	Clin Infect Dis. 1998
Krishna S.	India	Renal transplant	1	Urinary tract infection	Urine	Recovery	Int. J. Med. Public Health. 2011

contamination and the same bacteria were isolated from a pending water bomb only. As an infection control team, it was noticed that the hospital waters had not been adequately chlorinated at that time and the necessary precautions were taken, thus preventing a possible major epidemic. In these cases

it is thought that the possible infectious use water can be passed through the hospital staff or through the use water. In the second six months of follow-up we did not identify *S. paucimobilis* and other infection. Seeing gram-negative cocobacil and yellow-colored colonies in routine culture has helped us to



define *S. paucimobilis*. Four of the presented cases were completely healed after appropriate antibiotic treatment. Despite the appropriate antibiotic treatment, the fifth case was lost with developing respiratory failure and septic shock. In our hospital, *S. paucimobilis* was isolated six times in total. The fact that our cases are seen in a period of six months and there is no subsequent isolation confirms that this bacterium may rarely affect hospital infection. The antibiotic susceptibility of the cases varies according to region and country and in "Table 2" we tried to compile similar nosocomial *S. paucimobilis* infections as reported in our country and abroad.

As a result, *S. paucimobilis*, a rare pathogen, can be recognized by a particularly careful laboratory examination and treated with antibiotics that are sensitive.

A total of 45 *S. paucimobilis* related nosocomial infections are reported in the Turkish and English literature review and in PubMed screening until 2015. Especially in opportunistic infections that develop in immunosuppressive patients, environmental contamination should be considered. In this presentation, it has been revealed that it is necessary to be aware of the isolation of *S. paucimobilis* in the microbiology laboratory. Because the nosocomial epidemics that will be seen with the negative environmental conditions in the hospital, we need to be careful in *S. paucimobilis* isolations that we believe will become an important nosocomial pathogen in the future.

#### Ethics

**Informed Consent:** All cases have been taken with the informed consent form.

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Surgical and Medical Practices: F.S., Concept: F.S., E.B., Design: F.S., E.B., Data Collection or Processing: F.S., O.K., E.B., Literature Search: A.O., O.K., Writing: F.S., P.B.

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# A Rare Cause of Paresthesia: Hypophosphatemia

## Nadir Bir Parestezi Nedeni: Hipofosfatemi

✉ Zeynep Özözen Ayas<sup>1</sup>, ✉ Ruhsen Öncel Öcal<sup>2</sup>, ✉ Ayhan Bölük<sup>1</sup>

<sup>1</sup>Sakarya University Training and Research Hospital, Clinic of Neurology, İstanbul, Turkey

<sup>2</sup>Başkent University Hospital, Clinic of Neurology, Ankara, Turkey



### Abstract

Phosphate is a structural molecule for cells and also is used as coenzyme or as a second messenger. Renal or gastrointestinal loss of phosphate, diabetes mellitus, chronic alcoholism, hyperparathyroidism, sepsis, increased glucocorticoid, diuretics and antacids may cause hypophosphatemia. Muscle weakness, paresthesia, confusion, convulsion, tremor and coma are neurological symptoms of hypophosphatemia. Main clinical signs occur due to deterioration of oxygen distribution and reduced intracellular adenosine triphosphate. In the treatment of hypophosphatemia identification of underlying causes is important. In this article, a 26-year-old young male patient with paresthesia that is caused by hypophosphatemia due to D vitamin deficiency is reported. Clinicians must be on the alert about phosphate imbalance which is seen more rare than other electrolytes when investigation of patients with paresis and/or paresthesia.

### Keywords

Hipofosfatemi, parestezi, D vitamin eksikliği

### Anahtar Kelimeler

Hypophosphatemia, paresthesia, D vitamin deficiency

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### Address for Correspondence/Yazışma Adresi:

Zeynep Özözen Ayas MD,  
Sakarya University Training and Research  
Hospital, Clinic of Neurology, İstanbul, Turkey  
E-mail : zozozen@hotmail.com

ORCID ID: orcid.org/0000 0002 9302 5543

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### Öz

Fosfor hücreler için yapısal bir molekül olup ayrıca koenzim ya da ikincil haberci olarak kullanılmaktadır. Fosfatın renal ve gastrointestinal kayıpları, diyabetis mellitus, hiperparatiroidizm, kronik alkolizm, sepsis, glukokortikoid fazlalığı, diüretikler ve antiasitler hipofosfatemiye neden olabilir. Kas güçsüzlüğü, parestezi, konfüzyon, nöbet, tremor ve koma hipofosfateminin nörolojik bulgularındandır. Temel klinik bulgular dokulara oksijen dağılımındaki bozulmaya ve hücre içi adenosin trifosfatın azalmasına bağlı olarak gelişir. Hipofosfateminin tedavisinde altta yatan nedenin belirlenmesi önemlidir. Bu yazıda D vitamini eksikliğine bağlı hipofosfateminin neden olduğu parestezili 26 yaşındaki genç erkek hasta sunulmuştur. Klinisyenler parezi ve/veya parestezili hastaları incelerken diğer elektrolitlere göre daha nadir görülen fosfor bozuklukları hakkında dikkatli olmalıdır.

### Introduction

Phosphate is an electrolyte playing a role in the regulation of calcium levels, arrangement of carbohydrate and lipid metabolism, and maintaining acid-base balance. Hypophosphatemia may develop as a result of renal loss, chronic alcoholism, diabetes, burns, gastrointestinal loss, diuretics, and antiacids. Muscle weakness, paresthesia, confusion,

seizure, tremor, and coma are neurological symptoms of hypophosphatemia. Identification of the underlying cause is an important step for determining the etiology of hypophosphatemia. The treatment includes oral or intravenous replacement of phosphate. In this paper a 26-year-old male patient who was admitted to emergency with paresthesia due to vitamin D deficiency induced hypophosphatemia.

### Case Report

A 26-year-old man who presented with numbness in all four extremities was evaluated. The symptoms began at the distal of extremities and added upwards and with increasing intensity in the last 2 weeks. He additionally had myalgia. He had no history of smoking or alcohol use, nor he had any systemic disease. He also had no familial history of any disease. On neurological examination he was conscious, and had orientation, cooperation. His pupils were isocoric; his light reflexes were bilaterally normal; his eye movements were unrestricted in every direction; he had no nystagmus or facial asymmetry; and he had intact lower cranial nerves and motor function. However, he had diffuse patchy paresthesia on sensory testing; his deep tendon reflexes were hypoactive; and his plantar responses were bilaterally flexor. In laboratory tests, serum glucose, hemoglobin, biochemical parameters (urea, creatinine, sodium, potassium, calcium, chloride, magnesium, ALT, AST and thyroid function tests, and vitamin B12 and folic acid levels) were within normal ranges.

The phosphate level was 1.3 mg/dL (normal range 2.5-4.5 mg/dL). A search for the cause of hypophosphatemia found normal parathormone and glucocorticoid hormone levels and a low vitamin D level (5.7 ng/dL; normal range 20-120 ng/dL). Vitamin D deficiency induced hypophosphatemia was considered as the primary diagnosis. The patient's neurological examination completely returned to normal within 72 hours of phosphate replacement. A search for the cause of vitamin D deficiency revealed no signs of symptoms of nutritional deficiency, intestinal malabsorption (celiac disease, crohn disease, cystic fibrosis, gastric or intestinal perforation), hepatic or renal disorders, abnormal hepatic or renal function tests, or any malabsorption syndrome affecting vitamin B12 or folic acid levels. He also lacked any history of the use of medications preventing vitamin D absorption,

including corticosteroids, orlistat, cholestiramine, or phenytoin. There was also no history of sunlight avoidance causing deficient sunlight exposure. The patients was also administered treatment against vitamin D deficiency.

### Discussion

Phosphate is a structural molecule for human cells which is also used as a coenzyme or second messenger. Human body normally contains 600-700 mg phosphate, and approximately 1000 mg phosphate is consumed daily. Absorption of phosphate is the function of proximal tubule of the intestines. Phosphate is predominantly excreted in urine but some amount also in stool. Phosphate balance is regulated by vitamin D, parathormone, acid-base balance, and glucocorticoids (1). Healthy individuals have a phosphate level of 2.5-4.5 mg/dL.

The main mechanisms leading to hypophosphatemia (serum phosphate level <2.5 mg/dL) include phosphate shift from the extracellular compartment to the intracellular compartment, reduced phosphate intake, reduced phosphate absorption from the intestines, and increased urinary phosphate excretion (2). Hypophosphatemia may develop with glucose, fructose, and amino acid infusion or intravenous hyperalimentation. Hypophosphatemia is seen at a rate of 1-5% in hospitalization. Sepsis, diabetic ketoacidosis, liver failure, and post-renal transplant period are particularly risk for hypophosphatemia, and patients with these conditions should be more closely monitored. Our patient was, however, a young adult without any systemic disorder or a marked risk factor for hypophosphatemia.

Renal losses are among the most common causes of hypophosphatemia. Chronic alcoholism, malabsorption, burns, hypothermia, diabetes, renal losses, vitamin D deficiency, prolonged vomiting, glucocorticoid excess, hyperparathyroidism, antiacid, thiazide diuretic, and phosphate binding agent use are some major causes of hypophosphatemia.

Hypophosphatemia is rarely symptomatic unless serum phosphate level is reduced below 2 mg/dL. Affected patients are typically asymptomatic, with signs and symptoms occurring with deep hypophosphatemia. Main clinical signs and symptoms emerge as a result of disturbed tissue oxygenization and reduced intracellular adenosine triphosphate

concentration (3-5). The patient's phosphate was 1.3 mg/dL which caused the symptoms.

The signs and symptoms may involve all organs and systems. The gastrointestinal effects (loss of appetite, nausea, vomiting, gastric atony), neurological symptoms (weakness, paresthesia, ataxia, tremor, seizure, coma), musculoskeletal signs and symptoms (myalgia, fractures, bone aches, osteomalasia), cardiac signs and symptoms (reduced myocardial contractility), and hematological disturbances (hemolytic anemia, thrombocyte dysfunction) may all be found. Mohseni et al. (6) defined acute hemiparesis in a patient with hypophosphatemia. The patient's phosphate level was 1.3 mg/dL and displayed the symptoms of numbness, myalgia, and sign of diffuse paresthesia, and symmetrically reduced deep tendon reflexes. No sign related to other systems was noted.

A complete evaluation of phosphate level requires the measurement of parathormone, vitamin D, and glucocorticoid hormone levels. In cases with reduced phosphate intake vitamin D deficiency may cause hypophosphatemia by reducing gastrointestinal absorption. Our patient had normal parathormone and glucocorticoid hormone levels but reduced vitamin D level.

We considered that hypophosphatemia was caused by vitamin D deficiency. Appropriate phosphate replacement corrected phosphate level, and the correction of the underlying vitamin D deficiency prevented further renal phosphate loss.

Hypophosphatemia treatment should both aim at phosphate replacement and the identification of the underlying cause (7). We also started vitamin D along with phosphate replacement. In this paper we discussed the clinical and laboratory features of a young male patient with hypophosphatemia who presented to our clinic with diffuse paresthesia, discussion with literature. We aimed to emphasize that calling to mind phosphate

imbalance as a rare cause of paresia and paresthesia along with more common electrolyte disorders such as sodium, potassium, calcium, and magnesium deficiency.

### Ethics

**Informed Consent:** The written informed consent was obtained from the patient for publication.

**Peer-review:** Internally peer-reviewed.

### Authorship Contributions

Surgical and Medical Practices: Z.Ö.A., R.Ö., A.B., Concept: Z.Ö.A., R.Ö., A.B., Design: Z.Ö.A., R.Ö., A.B., Data Collection or Processing: Z.Ö.A., R.Ö., A.B., Analysis or Interpretation: Z.Ö.A., R.Ö., A.B., Literature Search: Z.Ö.A., R.Ö., A.B., Writing: Z.Ö.A., R.Ö., A.B.

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# Management of Diffuse Abdominal Cellulitis After Cesarean Section

## Sezaryen Sonrası Gelişmiş Yaygın Karın Selülit Olgusu Yönetimi

● Sümeyra Nergiz Avcioğlu, ● Emre Zafer, ● Sündüz Özlem Altinkaya, ● Selda Demircan Sezer, ● Hasan Yüksel

Aydın Adnan Menderes University Faculty of Medicine, Department of Obstetrics and Gynecology, Aydın, Turkey



### Abstract

In present case report, diffuse abdominal cellulitis is discussed under light of data in literature. Thirty-nine years old patients referred to our clinic, with the cesarean wound dehiscence and diffuse abdominal erythema, pain, increased temperature at 6th day of cesarean section. Initial diagnosis was diffuse abdominal cellulitis after cesarean section. In spite of antibiotic treatment and drainage, clinic of patient did not regress. So, a transverse incision at left lumbar area near to midline and below umbilicus was performed and phannenstiel incision was extended upwards to right side under general anesthesia. Drainage and debridement was performed. Between two incisions, skin and subcutaneous tissues were left as a bridge and tissue above the fascia was under arch of bridge. Necrotic tissues was excised. The incisions at abdominal area, were expected to heal secondarily. When the clinic and symptoms of patient was improved, at postoperative 36<sup>th</sup> day, she was discharged with daily wound dressing and control. In conclusion, serious superficial and subcutaneous tissue infections developed after cesarean section should be taken into intensive care and treatment protocols. Besides, these cases should be under observation due to increased risk of sepsis.

### Keywords

Cesarean section, cellulitis, debridement

### Anahtar Kelimeler

Sezaryen, selülit, debridman

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### Address for Correspondence/Yazışma Adresi:

Sümeyra Nergiz Avcioğlu MD,  
Aydın Adnan Menderes University Faculty  
of Medicine, Department of Obstetrics and  
Gynecology, Aydın, Turkey  
Phone : +90 256 211 28 71  
E-mail : sumeyranergiz80@gmail.com  
ORCID ID: orcid.org/

### Öz

Bu olgu sunumunda sezaryen sonrası gelişen ciddi karın duvarı selülit olgusu literatür bilgileri eşliğinde tartışılmıştır. Otuz dokuz yaşında hasta sezaryen sonrası postoperatif 6. günde yara yeri açılması ve karın cildinde yaygın eritem, ağrı ve ısı artışı şikayeti ile refere edildi. Sezaryen sonrası gelişen karın duvarı selülit düşünüldü. Antibiyotik tedavisi ve drenaja rağmen kliniği düzelmeyen hastaya, genel anestezi altında umbilikus sol lumbar bölgenin orta hattı geçen yakın yerinden transvers kesi uygulandı ve pfannenstiel kesisi sağa yukarıya doğru uzatıldı. Drenaj ve debridman yapıldı. İki kesi arasında köprü olarak deri ve deri altı enfekte yapılar bırakıldı, fasyanın üstü köprüaltı boşluk olarak kaldı. Nekrotik alanlar cerrahi olarak çıkarıldı. Mevcut açık yaralar sekonder iyileşmeye bırakıldı. Postoperatif 36. günde klinik durumunun düzelmesi üzerine hasta günlük kontrol ve pansumanlara çağrılmak üzere taburcu edildi. Sonuç olarak, sezaryen sonrası gelişen ciddi yüzeysel deri ve deri altı doku enfeksiyon olguları yoğun bir izlem ve tedavi protokolüne alınmalı, bu hastaların sepsise ilerleme riskleri bakımından gözlem altında tutulmalıdır.

## Introduction

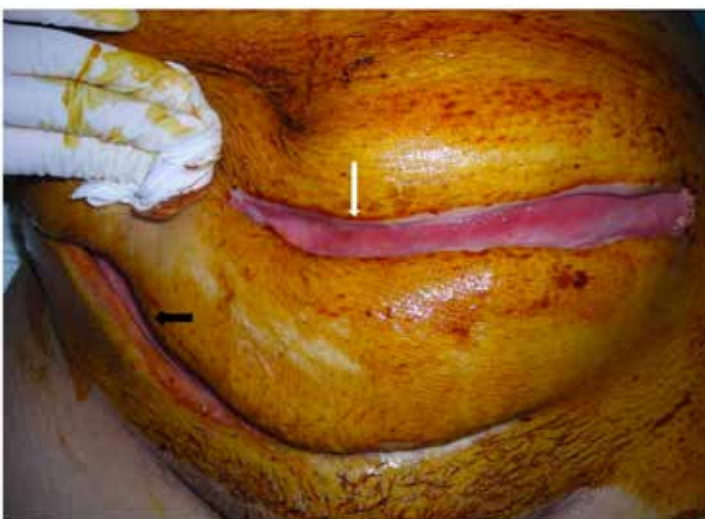
Cellulitis is an infection involving the dermis and the subdermis, having a sudden onset and spreading quickly. Cesarean section is one most frequently performed surgeries in the United States and Turkey. The rate of surgical site infections in clean surgeries like cesarean section is lower than 2% (1). The causative agents are usually *Streptococcus pyogenes* and *Staphylococcus aureus*. Traumatized regions, surgical sites and eczema and chicken pox lesions, where the skin integrity is disrupted, can be the ports of infections; however, in some cases there can be no ports of infections (2). Although cellulitis rarely appears after cesarean section, it causes very severe morbidity when it occurs. In this report, a case of diffuse abdominal cellulitis will be discussed.

## Case Report

A 39-year-old Gravida 3 and Parity 2 with 38-week-gestation underwent cesarean section for non-progressive labor in a health care center. Upon detection of surgical wound dehiscence, diffuse abdominal erythema and increased local temperature during removal of the surgical sutures, the patient was referred to our clinic. When the patient was presented to our clinic, her body temperature was 36.7 °C, her heart beat was 88/min, her respiratory rate was 22/min and her blood pressure was 100/80 mmHg. On physical examination, Pfannenstiel suture line was open and had necrosis and odor. Abundant

purulent discharge was observed in the subdermis, suggestive of a surgical site infection. On inspection, the skin extending to the umbilicus was painful and hyperemic. The skin in the epigastric region 4 cm above the umbilicus was necrotic and gangrenous. Ultrasonography performed in our clinic and in the center where the patient had cesarean section did not show a remarkable feature, but revealed thickness and increased echogenicity and linear fluid, suggestive of abdominal free fluid and edema in the anterior abdominal wall. Hemoglobin was 10.9 g/dL, white cell count was 18.5 ( $\times 10^9/L$ ) and C-reactive protein: 71 mg/L. Surgical site culture was obtained. The patient was diagnosed as superficial skin infection initially and administered cefazolin sodium (sefodin, Bilim ilaç, İstanbul, Turkey) 2 g four times a day intravenously. Drainage tubes were placed in the subdermis and on the fascia in the surgical incision area and in the left paramedian region for evacuation and irrigation of the dermis and subdermis collection extending towards the umbilicus. Two days after initiation of this treatment, it was replaced by ampicillin-sulbactam (Duocid, Pfizer ilaç, İstanbul, Turkey) 1.5 g four times daily through the intravenous route.

On the postoperative eighth day, the wound site was irrigated when the patient was under general anesthesia. When the drainage was insufficient, a transverse incision under the umbilicus 12 cm in length and starting from the left lumbar area was made and the Pfannenstiel incision was lengthened 5 cm both on the right and on the left (Figure 1).



**Figure 1.** Transverse incision extending through the inferior part of the umbilicus and close to the midline of the left lumbar region (white arrow) and pfannenstiel incision extending to the right superior part (black arrow)



Drainage, irrigation, aspiration and debridement were performed. Between the two incisions were dermal and subdermal infected tissues left as a bridge. Necrotic areas were surgically removed. Two drainage tubes were placed in the subdermis; one through the Pfannenstiel incision and the other through the transverse incision in the left lumbar region. Aspiration and irrigation were performed through these tubes three times a day. The wound culture obtained on admission to our clinic showed staphylococcus aureus (sensitive to ampicillin/sulbactam); therefore, the treatment initiated on admission was continued. When the patient's temperature increased, blood, urine and throat cultures were obtained, but they did not show bacterial growth.

On the postoperative 13<sup>th</sup> day, the tissues under the Pfannenstiel incision towards the umbilicus were explored under general anesthesia. The fascia was intact. The drainage tubes placed in the incision sites were removed. Necrotic, purulent and infected tissues in the incision and debridement areas were excised. Obtained specimens were sent to the microbiological laboratory and no bacterial growth was detected. Debrided areas were first irrigated with NaCl solution and then with diluted batticon and rifocine. Debrided areas were left open and the rest was closed with wet tampons. When hemoglobin was found to be 7.6 (g/dL), the patient was given three units of blood and two units of fresh frozen plasma. After these infusions, hemoglobin increased to 11.2 (g/dL). After debridement, we continued to perform wound dressing three times a day. On the postoperative 17<sup>th</sup> day, clinical improvement started. The wounds were left to have secondary healing. Upon complete clinical improvement on the postoperative 36<sup>th</sup> day, the patient was discharged and instructed to come to hospital for daily wound dressing and other controls.

## Discussion

Cellulitis is an acute infection involving the subdermis and subcutaneous fat tissue. It typically presents with tenderness, pain and erythema. The margins of the lesion on the skin were unclear. Local lymphadenopathy (LAP) and lymphangitis were common. Local abscesses and necrosis may appear (1). The case reported here presented with wound

dehiscence and diffuse erythema. She also had necrotic, gangrenous skin 4 cm above the umbilicus in the epigastric region, but did not have LAP.

In most of the obstetric cases, surgical site infections are not life-threatening (1). However, as cellulitis has the risk of spread through lymphatics, it is a serious infection. It can develop both on the basis of traumas (grazes and incisions), surgical wounds and skin lesions and on the intact skin through the hematogenous route. It can rarely develop due to an underlying infection focus (osteomyelitis and abscess). It can appear in the late postoperative period, especially on the postoperative fourth day (2). In the case reported here, it appeared on Pfannenstiel incision on the postoperative sixth day after cesarean section.

The factors predisposing to cellulitis are obesity, cutaneous lesions, venous insufficiency and lymphatic obstruction, immunosuppression, diabetes, chronic diseases, drugs (corticosteroids), malnutrition, age of >60, intravenous administrations of drugs and vascular diseases (3). In the case presented here, there was not a risk factor except for cesarean.

In severe cases of cellulitis, fever, tachycardia, confusion and hypotension may develop together with bacteremia. Polymorphonuclear leukocytosis can be seen. Although petechia and ecchymosis are expected to appear, necrotizing fasciitis should be ruled out when they are diffuse and accompanied by systemic toxicity (4). The case presented here had fever, tachycardia and polymorphonuclear leukocytosis, but did not have hypotension, confusion or ecchymosis. In addition, the fascia was intact initially and throughout the course of the disease.

Indications for hospitalization of cellulitis cases are rapid progression of the disease, asplenia, neutropenia, immunocompromised patients, cirrhosis and cardiac-renal failure. The present case was hospitalized due to wound dehiscence and the rapid progression of the infection. Consistent with the literature (2), wound culture and blood culture were obtained and *Staphylococcus aureus* was isolated from the wound culture.

In patients with cellulitis having an indication for hospitalization, first antibiotics should be given parenterally. When the disease is kept under

control clinically, antibiotics should be given orally (5). Community based cellulitis cases can be given cefazolin (4x 1.0 g intravenously), cefadroxil (1-2 x1.0 g orally), cefalexin, clindamycin or erythromycin. Ampicillin-sulbactam (4x 1-3 g/day intravenously) are the treatment of choice in cellulitis caused by resistant strains. Methicillin resistant *Staphylococcus strains* can be treated with linezolid (zyvoxid 2x 0.6 g intravenously or orally) or glycopeptides (teicoplanin 1x 400 mg/day intravenously and vancomycin 4x 500 mg/day intravenously). Treatment should last 14 days (3,4). The case presented here was first diagnosed as superficial skin infection and instituted cefazolin as recommended by the infectious diseases department; however, upon isolation of *Staphylococcus aureus* on wound culture (sensitive to ampicillin/sulbactam), the treatment was replaced by ampicillin-sulbactam.

Another clinically important point is whether there is a superficial necrotic area in the first lesion. It should be kept in mind that infection can be caused by methicillin resistant *Staphylococcus aureus* in cases having necrotic crust. The exotoxin released by *Staphylococcus aureus* causes severe necrosis in the area where it is secreted. Necrotic skin lesions can create necrotizing pneumonia and deep tissue abscesses. Therefore, a mechanical approach in addition to appropriate antibiotics should be used in patients having necrotic lesions. The infected area above the fascia should be opened and the margins of the wound should be removed and debrided. Wound care and dressing should be performed two or three times daily. This helps formation of granulation tissue and closure of the wound secondarily (3-5). In the case reported here, a large debridement of the wound was carried out under general anesthesia. An incision parallel with the superior part of the wound was made. Our primary aim was to prevent development of an infection involving the fascia. It would have been difficult to evaluate the fascia left under the infection in a vertical incision. In addition, it would not have been possible to prevent spread of the infection to the fascia. However, we left the non-necrotic infected dermal and subdermal tissues. By leaving the dermal and subdermal tissue and thus creating a bridge, we aimed to facilitate secondary healing of the non-necrotic tissues. If we had removed these tissues completely, there would have been a large dermal and subdermal defect and a problem with healing. We

even would have had to create a flap for a large area.

In conclusion, some simple preventive strategies like asepsis in the preoperative incision area, adoption of surgical principles and prophylactic use of systemic antibiotics can reduce the incidence of postsurgical infection (1,6). In addition, cleaning the surgical site with chlorhexidine 24 hours before surgery has been shown to decrease the risk of infection (7,8). Despite all these precautions, infectious morbidity is an inevitable complication of surgery. In cases having diffuse abdominal cellulitis, similar to the one reported here, treatment with antibiotics and mechanical debridement in resistant cases can prevent progression of the clinical picture to complications like necrotizing fasciitis or sepsis.

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#### **Ethics**

**Informed Consent:** Consent form was filled out by all participants.

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#### **Authorship Contributions**

Surgical and Medical Practice: H.Y.Ü., S.N.A., Concept: S.D.S., Design: E.Z., Data Collection or processing: S.N.A., Analysis or Interpretation: H.Y., S.Ö.A., Literature Search: E.Z., Writing: S.N.A.

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# Anaphylaxis After the First Dose of Lansoprazole: A Case Report

## *İlk Doz Lansoprazol Kullanımı Sonrası Gelişen Anafilaksi: Olgu Sunumu*

İskender Ekinci<sup>1</sup>, Yusuf Kayar<sup>2</sup>, Shute Ailia Dae<sup>1</sup>, Ali Dur<sup>3</sup>

<sup>1</sup>Bezmialem Foundation University Faculty of Medicine, Department of Internal Medicine, İstanbul, Turkey

<sup>2</sup>Bezmialem Foundation University Faculty of Medicine, Department of Gastroenterology, İstanbul, Turkey

<sup>3</sup>Bezmialem Foundation University Faculty of Medicine, Department of Emergency Medicine, İstanbul, Turkey



### Abstract

Drug-induced anaphylaxis is a life-threatening condition that is rapid in onset and defined as a serious allergic or hypersensitivity reaction. Proton pump inhibitors are widely used for the treatment of acid-related disorders. These drugs are known as effective, safe and well tolerated. Although these drugs are known to be safe they may lead to life-threatening side effects such as anaphylaxis and interstitial lung disease in addition to minor side effects such as abdominal pain, headache, nausea, vomiting and diarrhea. A small number of anaphylactic reactions to lansoprazole have been described, although it is a commonly prescribed drug. We describe a case of anaphylaxis induced by oral intake of lansoprazole.

### Keywords

Anaphylaxis, lansoprazole, proton pump inhibitors, drug allergy

### Anahtar Kelimeler

Anafilaksi, lansoprazol, proton pompa inhibitörleri, ilaç allerjisi

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### Address for Correspondence/Yazışma Adresi:

İskender Ekinci MD,

Bezmialem Foundation University Faculty of Medicine, Department of Internal Medicine, İstanbul, Turkey

Phone : +90 539 556 25 72

E-mail : driskenderekinci@gmail.com

ORCID ID: orcid.org/0000-0001-7390-9301

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### Öz

İlaçla indüklenen anafilaksi ciddi allerjik veya hipersensitif reaksiyon olarak tanımlanan, hızlı başlangıçlı olabilen ve hayatı tehdit eden bir durumdur. Proton pompa inhibitörleri mide asit salgınımıyla ilişkili birçok hastalıkta gastrik asit sekresyonunu azaltıcı etkileri nedeniyle sıklıkla kullanılan ilaçlardır. Bu ilaçlar etkin, güvenli ve iyi tolere edilen ilaçlar olarak bilinmekle beraber karın ağrısı, baş ağrısı, bulantı, kusma ve ishal gibi hafif yan etkilerin yanında anafilaksi ve interstisyel akciğer hastalığı gibi hayatı tehdit eden ciddi yan etkilere de neden olabilir. Proton pompa inhibitörlerinden biri olan lansoprazol çok sık reçete edilmesine rağmen, lansoprazol kullanımına bağlı anafilaksi çok az sayıda rapor edilmiştir. Burada oral lansoprazol alımı sonrası oluşan bir anafilaksi olgusu takdim edilmiştir.

### Introduction

Anaphylaxis is a life-threatening disorder caused by the release of immunoglobulin E (IgE) in response to allergen substances. IgE sticks to mast cells in basophils and body tissues. When a person with IgE antibodies to a specific allergen is exposed to that allergen again, these cells become activated and release histamine and tryptase into the bloodstream. The signs and symptoms of anaphylaxis generally begin within minutes to hours after exposure to a trigger, such as a

food, a drug, or an insect sting (1). We describe a case of anaphylaxis induced by oral intake of lansoprazole.

### Case Report

A 24-year-old woman was brought to the emergency room with complaints of generalized itching and flushing, shortness of breath, and weakness. We were informed that the patient had taken 30 mg of oral lansoprazole for the first time 45 min earlier for epigastric heartburn. The patient had no history of allergies or anaphylaxis to any drug. She was monitored, and the following findings were recorded: blood pressure of 70/40 mmHg, pulse rate of 120 beats/min, oxygen saturation of 88%, and respiratory rate of 28 breaths/min. The patient was conscious, cooperative, and oriented but agitated. The physical examination revealed generalized erythema, edema of the periorbital region and uvula, and skin wounds due to scratching. These findings were compatible with anaphylaxis due to the use of lansoprazole. Epinephrine (0.5 mg) was administered intramuscularly, and methylprednisolone (120 mg) was injected intravenously. Oxygen was given at a rate of 5 L/min. The patient was placed in the recumbent position, and intravenous saline infusion was started rapidly to treat the hypotension. Diphenhydramine (45.5) mg was given intravenously for the itching. Laboratory studies, including a complete blood count and biochemical parameters, were all in the normal ranges. The patient's condition stabilized 2 h after the initiation of the treatment, and the erythema on the body disappeared 6 h later. The itching also subsided, and the edema of the uvula and periorbital area was resolved. The patient was discharged in a stable condition after follow up for 24 h in the emergency room and advised to make an appointment with an allergy unit for immunological research.

### Discussion

Proton pump inhibitors (PPIs), which decrease gastric acid secretion, are widely used for the treatment of acid-related disorders, including Barret's esophagus, peptic ulcer disease, Zollinger-Ellison syndrome, gastrinomas, and esophagitis/gastritis (2). PPIs reduce basal and stimulated gastric acid secretion by inhibiting the parietal cell enzyme H<sup>+</sup>-K<sup>+</sup>-ATPase. PPIs are prevalent medications owing to their proven

efficacy, safety, and good tolerability, and they are associated with a low incidence of adverse reactions (3). Common adverse effects of PPIs are headache, nausea, vomiting, abdominal pain, diarrhea, itching, and a skin rash. More serious adverse events include glottis edema, anaphylaxis, microscopic colitis, hip fractures, interstitial lung disease, Kounis syndrome, and acute allergic intestinal nephritis (4-10). Candar et al. (11) reported a case of asystole after intake of lansoprazole and stated that the cause of death of the patient was delayed admission to the emergency room. In all the cases reported in the literature, the response to the treatment was better when the time between the intake of the drug and the initiation of treatment was short.

The literature also reports different patterns of cross-reactions between PPIs. Sobretiva Elfau et al. (12) reported the following patterns:

- 1) An allergy to a single PPI and positive cross-reactivity with all other PPIs;
- 2) An allergy to lansoprazole, positive cross-reactivity with rabeprazole, but tolerability of other PPIs or an allergy to omeprazole, positive cross-reactivity with pantoprazole, and tolerability of other PPIs;
- 3) An allergy to omeprazole but tolerability of pantoprazole and lansoprazole (rabeprazole and esomeprazole unknown). The high rate of cross-reactivity between these drugs is closely related to their chemical structure. The pyridine rings of rabeprazole and lansoprazole have a methoxypropoxy and trifluoroethoxy chain, respectively, whereas the pantoprazole and omeprazole benzimidazole rings have a difluoromethoxy and methoxy chain, respectively (13). In the present case, cross-reactivity tests could not be performed in the hospital, but the patient was directed to an allergy unit to undergo these tests after being discharged.

Although anaphylaxis induced by PPIs is rare, patients who are prescribed PPIs should be informed about their potential side effects. Physicians should keep the possibility of PPI induced anaphylaxis in mind because of the frequent use of these drugs without a prescription.

PPI-induced anaphylaxis needs to be quickly diagnosed and treated, as it may progress to anaphylactic shock and death. To avoid the recurrence of a hypersensitivity reaction, cross reactivity studies

should be performed before starting a new PPI when an allergic reaction occurs in response to any PPI.

#### **Ethics**

**Informed Consent:** We received informed consent from the patient.

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# Retroperitoneal Tunneled Hemodialysis Catheter in Inferior Vena Cava: A Report of Three Cases

## *Inferior Vena Kavaya Retroperitoneal Tünelli Hemodiyaliz Katateri: Üç Hastalık Olgu*

● Okay Karaca<sup>1</sup>, ● Ata Ecevit<sup>2</sup>, ● Mehmet Kalender<sup>2</sup>, ● Mehmet Taşar<sup>3</sup>, ● Turan Ege<sup>4</sup>

<sup>1</sup>Düzce University Faculty of Medicine, Cardiovascular Surgery, Düzce, Turkey

<sup>2</sup>Konya Training and Research Hospital, Department of Cardiovascular Surgery, Konya, Turkey

<sup>3</sup>Ankara University Faculty of Medical, Department of Cardiovascular Surgery, Ankara, Turkey

<sup>4</sup>Trakya University Faculty of Medical, Department of Cardiovascular Surgery, Edirne, Turkey



### Keywords

Chronic renal failure, emergency hemodialysis, tunneled catheter

### Anahtar Kelimeler

Kronik böbrek yetersizliği, acil hemodiyaliz, tünelli katater

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### Address for Correspondence/Yazışma Adresi:

Okay Karaca MD,  
Düzce University Faculty of Medicine,  
Cardiovascular Surgery, Düzce, Turkey  
E-mail : drguven@gmail.com

ORCID ID: orcid.org/

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### Abstract

Finding an appropriate vascular access is difficult in long term hemodialysis patients for emergency hemodialysis. The aim of this study was to present tunneled hemodialysis catheter placement to inferior vena cava through retroperitoneal approach as an alternative method for patients who have emergency hemodialysis and no other choice. We placed tunneled hemodialysis catheter into inferior vena cava through retroperitoneal approach in three patients for emergency hemodialysis, two of them being female and one being male. The mean age of the patients was 51.3 years (min: 36, max: 56) and mean dialysis time was for a period of 4.3 years (min: 2, max: 6). In conclusion, placement of tunneled hemodialysis catheter into the inferior vena cava through retroperitoneal approach can be an alternative vascular access procedure for patients especially who have emergency hemodialysis and no other choice.

### Öz

Uzun süre hemodiyalize giren hastalarda acil hemodiyaliz için yeterli damar giriş yolu bulmak zordur. Biz bu çalışmada acil hemodiyalize girecek olan başka bir seçeneği olmayan hastalarda alternatif bir metod olan retroperitoneal yaklaşımla inferior vena kava'ya tünelli hemodiyaliz katateri yerleştirilmesini göstermeyi amaçladık. İki kadın, bir tanası erkek olan üç hastaya retroperitoneal yaklaşımla vena kava inferiora tünelli hemodiyaliz katateri yerleştirdik. Hastaların ortalama yaşı 51,3 (min: 36, maks: 56), ortalama diyaliz süresi 4,3 yıl (min: 2, maks: 6) idi. Sonuçta retroperitoneal yaklaşım ile vena kava inferiora tünelli hemodiyaliz katateri yerleştirilmesi özellikle acil hemodiyalize girecek ve başka bir seçeneği olmayan hastalarda alternatif bir damar giriş yolu olabilir.

### Introduction

Finding an appropriate vascular access is difficult in long term hemodialysis patients for emergency hemodialysis. Tunneled hemodialysis catheter (THC) is a common and highly effective means of administering temporary venous access for dialysis for periods



longer than three weeks (1). THC inserted through a central vein is a useful way for these patients. Commonly, catheterization of the right jugular vein is the first choice (2). Subclavian and femoral veins are also used. Long term hemodialysis requires multiple interventions to central veins, causing thrombosis. Particularly in this group of patients, alternative interventions for vascular access are required. The aim of this study was to present THC placement to inferior vena cava (VCI) through retroperitoneal approach as an alternative method for emergency hemodialysis.

## Case Report

### Patient Selection

Patients who have bilateral occlusion of the jugular veins or subclavian veins, occlusion of the superior vena cava or chest wall abnormalities may be canalized for translumbar catheter placement (3). Patients who have morbid obesity and with stomas or open abdominal walls may be poor candidates for translumbar approach (4).

We placed THC into VCI through retroperitoneal approach in three patients for emergency hemodialysis, two of them being female and one being male. The mean age of the patients was 51.3 years (min: 36, max: 56) and mean dialysis time was for a period of 4.3 years (min: 2, max: 6). The patients were operated between December 2011 and June 2013. Past medical history of the patients revealed peritonitis due to peritoneal dialysis. All patients had previous bilateral upper extremity radiocephalic and brachio basilic arteriovenous fistulas which had failed. Following the failure of the fistulas, THC was placed in right and left jugular veins in all patients. Before the operation, venous Doppler ultrasonography was performed in all patients for both upper extremities and lower extremities. All patients had bilateral jugular and subclavian vein thrombosis. Two patients had venous thrombosis in one of the lower extremities.

### Operative Technique

All patients were operated under general anesthesia with endotracheal intubation. Right retroperitoneal exposure was used to access VCI. VCI was secured by silastic tapes proximally and distally. Systemic 5000 IU unfractionated heparin was applied intravenously. Purse string suture was placed on the VCI. A 19 Fx32 cm silicone double-lumen THC was inserted through

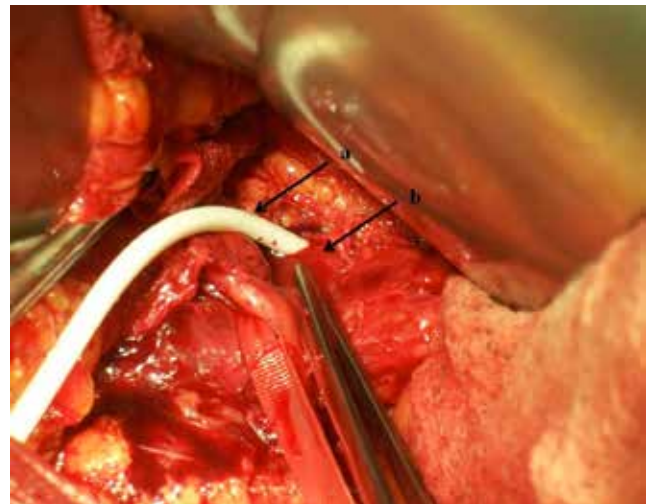
previously prepared tunnel (Figure 1). After insertion of catheter, the purse string suture was tied. A closed suction drain was placed in the retroperitoneal space. The retroperitoneal space was closed in the routine manner. The subcutaneous tissues and skin were closed with an absorbable suture.

### Postoperative Care

All patients were admitted to the postoperative intensive care unit. A single dose of prophylactic antibiotic (cefamezin) was administered. Patients received hemodialysis by the newly inserted catheter in the intensive care, when necessary. All patients were transferred to the surgical ward on the 2<sup>nd</sup> postoperative day.

## Discussion

To create an appropriate vascular access for patients undergoing long term hemodialysis is challenging. In this particular group of patients, alternative vascular access routes can be lifesaving. THCs, as described by Schwab et al. (5) for the first time, are used temporarily while patients have been waiting for fistula maturation, kidney transplantation or they are used for chronic vascular access (2). The dialysis outcomes quality initiative vascular access guideline recommends that no more than 10% of long-term hemodialysis access should be in the type of catheters (2). However, growing number of patients with long term hemodialysis require tunneled or non-tunneled central venous catheters due to increasing rates of thrombosis, vascular stenosis and infection (6-8).



**Figure 1.** a: Tunneled catheter b: inferior vena cava

In most patients, the right internal jugular vein is used more often than the left for central venous catheter. Access through the right internal jugular vein offers direct path to the right atrium, in contrast to the left internal jugular vein (9). The right internal jugular vein has the lowest risk of symptomatic central venous stenosis and thrombosis, and is technically easier to puncture using ultrasound guidance than any other central vein, with the exception of the femoral vein. Furthermore, thrombotic complications of the internal jugular vein catheters are usually asymptomatic because of the abundant network of collateral veins draining the head and neck (10).

Subclavian vein should generally be avoided for hemodialysis unless internal jugular access is unavailable. The subclavian vein has a number of unique disadvantages compared with the internal jugular vein, primarily because it provides venous drainage from the arm. Subclavian access is associated with high rates of stenosis and thrombosis (11). Subclavian vein puncture has the highest risk of pneumothorax and, if the puncture site is too far medial, catheter compression by the subclavius muscle-costoclavicular ligament complex may lead to fatigue and fracture, the so-called pinch-off syndrome (12,13). The subclavian vein should not be used in any patient requiring hemodialysis unless the ipsilateral extremity is unsuitable for synthetic graft or fistula creation.

Femoral vein catheterization is more prone to mechanical and infectious complications when compared to jugular and subclavian veins (14).

Conventional access sites provide satisfactory routes for catheter placement; a variety of options have been developed when they are no longer available for access because of thrombosis from multiple prior catheterizations. Lau et al. (15) described innominate vein catheterization. Moreover, percutaneous translumbar VCI, transsternal, transrenal, transhepatic venous access routes have been described (16-20). In one of our patients, multiple catheterizations, either permanent or temporary, were present in the past medical history together with thrombosis of bilateral jugular veins, subclavian and femoral vein. Numerous autologous arteriovenous fistulae (AVFs) and synthetic AVFs in both upper extremities had ended with failure. In the other two patients, while Doppler ultrasonography did not reveal any thrombosis in only

one of their lower extremities, catheterization failed. Catheterization through this region was present in the medical history. In order to meet the needs for hemodialysis in these three patients, THC was placed immediately into the VCI by the retroperitoneal approach.

The complications related to central venous catheterization may be classified under two groups: complications of device insertion and long-term complications. Complications of device insertion are pneumothorax, perforation, hemothorax and tamponade. Long term complications are infection, thrombosis, malfunction, compression, fracture and embolization (21). Our patients have been undergoing hemodialysis for a mean period of 10 months (min: 8, max: 12) without any complications.

In conclusion, placement of THC into the VCI through retroperitoneal approach can be an alternative vascular access procedure for patients especially who have emergency hemodialysis and no other choice. However, complications and catheter longevity should be taken into consideration.

#### **Ethics**

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#### **Authorship Contributions**

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# Myotonic Dystrophy

## Miyotonik Distrofi

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Celal Bayar University, Department of Physical Medicine and Rehabilitation, Manisa, Turkey



### Abstract

Myotonic dystrophy (MD) is a rare, progressive muscle disease, characterized by autosomal dominant heredity and multisystem involvement. The muscle involvement's being progressive in MD patients leads to negative effects such as dependency of the patients in their daily activities. For this reason, medical treatment and rehabilitation of these patients are very important. We considered 3 patients with MD diagnosis in this series of cases. We made pre-treatment and post-treatment evaluations of 3 patients who were hospitalized in our clinic by giving them a 4 week conventional exercise program and functional electrical stimulation (FES) treatment. The rehabilitation program we applied to the patients contained joint's range of motion exercises, breathing exercises, standing training, gait training, balance coordination exercises, and FES application to ankle and wrist dorsiflexors. We achieved positive changes in the clinical findings that we documented with pre-treatment and post-treatment assessment values.

### Keywords

Myotonic dystrophy, rehabilitation, exercise

### Anahtar Kelimeler

Miyotonik distrofi, rehabilitasyon, egzersiz

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### Address for Correspondence/Yazışma Adresi:

Zeliha Ünlü MD,  
Celal Bayar University, Department of Physical  
Medicine and Rehabilitation, Manisa, Turkey  
Phone : +90 236 236 03 30  
E-mail : zelihaunlu@yahoo.com  
ORCID ID: orcid.org/0000-0003-2270-0414

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### Öz

Miyotonik distrofi (MD) seyrek görülen, otozomal dominant kalıtım ve multisistemik tutulumla seyreden ilerleyici bir kas hastalığıdır. MD hastalarında kas tutulumunun ilerleyici olması hastaların günlük yaşam aktivitelerinde bağımlılık gibi olumsuz etkiler doğurmaktadır. Bu yüzden bu hastaların medikal tedavi ve rehabilitasyonu oldukça önemlidir. Bu olgu serisinde MD tanısı olan 3 olgu gözden geçirildi. Kliniğimizde yatarak tedavi gören 3 MD hastasına 4 hafta geleneksel egzersiz programı ve fonksiyonel elektrik stimülasyonu (FES) tedavisi verildi, hastalar tedavi öncesi ve sonrası değerlendirildi. Hastalara uygulanan rehabilitasyon programı; eklem hareket açıklığı egzersizleri, solunum egzersizleri, ayakta durma eğitimi, yürüme eğitimi, denge koordinasyon egzersizleri, ayak bileği ve el bileği dorsifleksörlerine FES uygulamasını içeriyordu. Hastaların tedavi öncesi ve tedavi sonrası değerlendirme ölççekleriyle dökümente ettiğimiz klinik bulgularında olumlu değişiklikler bulundu.

### Introduction

Myotonic dystrophy (MD) is an autosomal dominant hereditary muscle disease which presenting with progressive muscle weakness. MD is a rare disease with an incidence of 1/8000 (1). This condition is caused by an expanded cytosine-thymine-guanine trinucleotide repeat in the DMPK-gene on chromosome 19q (2,3). MD is not limited to muscle involvement only, it presents with multisystemic symptoms

(4). Along with musculoskeletal system symptoms, such symptoms as cataracts, cardiac pathologies, insulin resistance, alopecia, constipation, interfertility may be present. Progressive muscle weakness, atrophy and myotonia are neuromuscular symptoms of this disease (5). It has 3 sub-groups including myotonic dystrophy-1 (Steinert's disease), MD-2 (proximal myotonic myopathy) and proximal myotonic dystrophy. MD-1 is the most common in adults (6).

The typical involvement pattern in this disease is that it starts with weakness of distal muscles initially and in later stages weakness of proximal muscles is included. Presence of myotonia, as well as muscle weakness, should be considered in these patients. When muscle strength decreases, it also becomes difficult to determine clinical myotonia. Having difficulty in releasing a grasp after making a fist, difficulty in opening eyelids after closing them tightly, occurrence of muscle tightening as a response to hitting muscle with a reflex hammer are clinical symptoms of myotonia.

Although there is no sufficient evidence for the effectiveness of drugs in the treatment of this disease, antidepressants, benzodiazepines, calcium antagonists, taurine (essential amino acids) and prednisone based drugs are among the drugs used (7). Since medical treatment is limited, rehabilitation programs based on exercise are very important in terms of preventing progression of the disease.

Studies conducted with MD patients more often concentrated on the effects of aerobic exercises on progression of the disease and hand skills. In some studies, it was reported that moderate-intensity exercises are not harmful, but their benefit is questionable (8). In this article, it was intended to review MD rehabilitation in the light of recent literature through 3 patients who receive in-patient treatment. The literature concerning MD rehabilitation is limited. It is rather focused on training of subtle skills and aerobic exercise.

### Case Report

In this study, we made pre-treatment and post-treatment evaluations of 3 patients who were hospitalized in our clinic by giving them a 4 week conventional exercise program and functional electrical stimulation (FES) treatment. The rehabilitation program we applied to the patients

contained joint's range of motion (ROM) exercises, breathing exercises, standing training, gait training, balance coordination exercises, and FES application to ankle and wrist dorsiflexors and moderate-intensity lower extremity and upper extremity strengthening exercises for 2 patients. Strengthening exercises were not given to patient 1 because creatine kinase (CK) values were above normal.

**Patient 1 (N.Y.):** Forty-eight-year old female patient diagnosed with MD 20 years ago. She could be mobilized with electric-powered wheelchair. She used unarticulated, solid ankle foot orthosis (AFO). She received outpatient treatment in a physical therapy and rehabilitation center one year ago. She had no family history. In her physical examination findings; she couldn't walk, she couldn't stand. Movements in bed were present; she couldn't take sitting position from lying position.

**PHASE:** It was stage 1. (functional ambulation scale stage 1: physical aid dependent, continuous manual support of a person is required for the patient's ambulation).

Manual muscle strength examination: shoulder flexors-abductors: 4/5, biceps: 3-3+/5, flexor carpi ulnaris - flexor carpi radialis (FCU-FCR): 1/5, finger flexors: 2+/5, hip flexors on the left: 3-/5, on the right: 3+/5, hamstrings: 3+/5, ankle dorsiflexors: 1/5, plantar flexors: 1/5. ROM (joint's ROM) limitations; wrist and ankle active-passive ROM was limited. Deep tendon reflexes (DTR): hypoactive was detected in 4 extremities. There were no pathological reflexes.

**Patient 2 (M.Ç.):** Fifty-eight-year old patient diagnosed with myotonic dystrophy 20 years ago, walk without support was unstable and short distant. Standing, taking sitting position from lying position was present.

**PHASE:** Stage 2 (functional ambulation scale stage 2: physical aid dependent, manual support was to assist balance and coordination). The patient hadn't received a physical therapy program nor had any orthosis or AFO to support walking. It was learned that in the family history, daughter of her uncle, her sister and daughter (3<sup>rd</sup> patient: B.Ç) had the same disease. In her medical history; there was bilateral operated cataracts, a history of arrhythmia and a history of thyroid nodules existed.

In the physical examination; manual muscle strength examination: shoulder flexors-abductors: 4/5, biceps: 4/5, FCU-FCR: 3+/5, finger flexors: 3-/5,

hip flexors: 4/5, hamstrings: 5/5, ankle dorsiflexors on the left: 3+/5, on the right: 3-/5 plantar flexors on the left: 3+/5, on the right: 3-/5. ROM (joint's ROM) limitations; wrist and ankle active ROM was limited, passive was natural. DTR; hypoactive was detected on 4 extremities. There were no pathological reflexes.

**Patient 3 (B.Ç):** The patient, daughter of the patient 2, diagnosed with MD could walk without support and go up and down the stairs.

**PHASE:** It was stage 4-5. (functional ambulation scale stage 4-5: patient can walk on flat ground without support, and may need support in inclined surfaces such as stairs). In the physical examination; manual muscle strength examination; wrist extensor-flexors: 4+/5, ankle dorsiflexors/plantar flexors: 4+/5, other muscles: 5/5. No ROM limitation was detected. Active passive electro-hydrostatic actuator was natural. DTR normoactive and there were no pathologic reflexes.

In this study, we made evaluations of patients with examinations and measurements before and after 4-week rehabilitation program. The conducted evaluation forms are as follows:

1) Daily living activities assessment (DLA), health assessment questionnaire; this is a test where patients' daily activities are examined in 8 categories such as dressing, general self-care, eating, sitting standing etc. Evaluation is made as follows: 0 (I do not have any difficulty), 1 (I have a little difficulty), 2 (I have a lot of difficulty) and 3 (I can't perform it). Higher the score in the survey means worse the health situation (9).

2) Posture analysis; it is a measure where head, chest, shoulder, scapula, spine foot posture and leg length are assessed.

3) Gait analysis [functional ambulation classification (FAC)]; patients' walking levels are categorized in 6 groups. (PHASE Level 0: non-functional ambulation, level 5, independent ambulatory) (10).

4) Manual muscle strength assessment; muscle strength is rated from 0 to 5 (muscle strength 0; no movement, muscle strength 5; normal) (11).

5) Balance - coordination tests; get up and go test, functional reach test and romberg test (12).

6) Hand skills test (bimanual fine motor function); both hand functions are rated on 5 levels (13).

7) Goniometric ROM measurement.

According to the results of the evaluation forms obtained before and after the treatment of the patients, we observed improvement in DLA test, FAC, manual muscle strength assessment and goniometric ROM measurements. We did not detect a difference in hand skills test, posture analysis and balance - coordination tests (except patient 2). In patient 2, there was improvement in 'get up and go test' and functional reach test among balance - coordination tests. Patients' pre-treatment and post-treatment DLA and FAC values are stated in Table 1.

Improved muscle strength values of patient 1 and patient 2, whose muscle strength measurements are stated in the case report section in detail, is shown in Table 2. For Patient 3, left big toe flexors improved from 4/5 to 5/5, other muscle strength measurements were assessed as 5/5.

Those who showed improvement in joint's range of movement measurements are shown in Table 3. In Patient 3, no joint movement limitation was detected pre-treatment or post-treatment. We did not find any difference in patients' other joint range of movement.

## Discussion

Medically, rehabilitationally and socially, a multidisciplinary approach is needed to reduce morbidity and mortality rates in MD patients (14). There is a limited literature concerning implementation of rehabilitation programs in MD and their results. There is not sufficient information about rehabilitation protocol to be applied to these patients. Therefore, the rehabilitation practices applied to these patients are focused on ROM exercises, strengthening exercises, aerobic exercises and relation techniques, which underlie conventional

**Table 1. Pre-treatment and post-treatment evaluation form results**

Patients	HAQ - PreT	HAQ - PostT	FAC - PreT	FAC - PostT
Patient 1	46	41	Stage 0	Stage 1
Patient 2	31	29	Stage 2-3	Stage 3
Patient 3	6	4	Stage 5	Stage 5

PreT: Pre-treatment, PostT: Post-treatment, HAQ: Health Assessment Questionnaire, FAC: Functional Ambulation Classification

rehabilitation approaches. Low-to-moderate-intensity strength and aerobic exercise training, and an active lifestyle are have beneficial evidences in patients with a slowly progressive neuromuscular disease, such as MD. A hand isometric exercise program applied to 35 MD patients for 12 weeks achieved positive outcomes (15). Studies indicate that there is insufficient evidence whether or not strengthening exercises and aerobic exercise rehabilitation programs in muscle diseases (16). Kierkegaard et al. (17) evaluated a comprehensive group exercise programme supported by music (Friskis&Svettis Open Doors Programme) in patients with MD for 14 weeks. They were not revealed evidently beneficial or harmful effects in intention-to-treat programme. The harmful effects are an important manner when the patient has a progressive disease. They were not detected gross improvements in the outcome measures but a majority of participants in the exercise group felt more subjective positive effects and reported increasing

in fitness, strength, flexibility and excessive daytime sleepiness levels.

Due to lack of information and protocol in these patients, we also applied conventional methods to our patients. Considering CK level in this implementation, we avoided overloading during muscle strengthening as in patient 1. We applied moderate-intensity strengthening program to patient 2 and patient 3. At the end of 4-week rehabilitation program, we observed positive developments in our patients as shown in the tables. These observations were documented with measurements. One of the most significant factors for the increased fall frequency in patients with MD is foot drop due to ankle dorsiflexor weakness. Also drop foot is considered a factor decreasing quality of life in these patients. FES might be suggested a safe and effective tool to improve muscle function. We applied FES to stimulate ankle dorsiflexors during one month. Tibialis anterior muscle strength increased all of the cases at the end of the treatment. A pilot study

**Table 2. Pre-treatment and post-treatment, comparative muscle strength values of patient 1 and patient 2**

Manual muscle strength measurement	Patient 1		Patient 2	
	PreT	PostT	PreT	PostT
Hand finger flexion	2+/5	3/5	3/5	3/5
Thumb flexion	2+/5	3/5	3/5	3/5
Ankle plantar flexion (M. gastrocnemius - M. soleus)	1/5	3/5	3/5	4/5
Ankle dorsiflexion	1/5	1/5	3/5	4/5
Ankle inversion (M. tibialis anterior and posterior)	1/5	2/5	3/5	4/5
Ankle aversion (M. peroneus brevis and longus)	1/5	2/5	3/5	4/5
Toe flexion	3/5	3/5	2/5	3/5
PreT: Pre-treatment, PostT: Post-treatment,				

**Table 3. Goniometric measurement of joint's range of movement of patient 1 and patient 2**

	Patient 1				Patient 2			
	Active		Passive		Active		Passive	
EHA goniometric measurement	PreT	PostT	PreT	PostT	PreT	PostT	PreT	PostT
Wrist extension	5	10	50	70	50	55	60	60
Wrist flexion	0	0	0	0	60	60	90	90
Ankle plantar flexion	5	5	45	45	30	35	45	45
Ankle dorsiflexion	0	0	0	0	10	15	20	25
EHA: Electro-hydrostatic actuator, PreT: Pre-treatment, PostT: Post-treatment								



showed that FES lower extremity training (extensor and flexor muscles of the knee as well as ankle dorsiflexor and plantarflexor muscles) has a greater effect on improving muscle strength and endurance when compared with conventional training (18). Recently, improvement of mild tibialis anterior muscle weakness was reported the useful effect of electrical stimulation in 4 patients with MD (19).

Although it is our limitation that we could not provide rehabilitation programs or protocols for these patients due to small number of patients, our observations will constitute preliminary information for wider series of studies to be conducted in the future. MD is a rather neglected disease and new treatment approaches are needed to improve clinical practice in the management of these patients. In this respect, we think our observations will contribute to the literature.

Since MD might be familial, genetic counseling and guidance in these patients are also important. Patient 2 (M.Ç.) and patient 3 (B.Ç.) are noteworthy in this regard. Clinical follow-up of our patients currently continues.

### **Ethics**

**Informed Consent:** Patients were informed and they consented to the study.

**Peer-review:** Internally peer-reviewed.

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