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Review / Derleme

Disagreements in the Approach to the N0 Neck and their Causes in Head and Neck Cancers

Baş Boyun Kanserlerinde, N0 Boyuna Yaklaşımda Fikir Ayrılıkları ve Nedenleri

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Abstract

There are numerous controversial issues in the management of patients with N0 neck. Whether the diagnosis of N0 neck should be made based on the physical examination or imaging methods is contradictory in the first place. The rates of metastasis to the neck and the identification of occult neck metastasis vary according to the location and histopathological characteristics of the primary tumour. A wait-and-see approach may be preferred in the treatment of these patients, whereas an elective neck dissection or elective radiotherapy may be implemented considering all these features. An elective treatment is preferred if the occult metastasis ratio is higher than 15-20%. The neck dissection levels change depending on the primary tumour localisation. In patients with N0 neck, it is required to decide on the most effective prophylactic treatment with low morbidity that can avoid cervical recurrence and distant metastasis.

Keywords
Head and neck cancers, N0, approach

Öz

Baş boyun kanserlerinde, N0 boyunlu hastalara yaklaşımda birçok tartışmalı konu bulunmaktadır. Başta N0 tanısının, muayeneye göre mi, görüntüleme yöntemlerine göre mi konulacağı tartışmalıdır. Primer tümörün yerleşim yerine ve histopatolojik özelliklerine göre boyuna metastaz yapma ve boyunda okült metastaz saptanma oranları değişmektedir. Tüm bu özellikler gözetilerek, bu hastaların tedavisinde, bekle-gör şeklinde bir yaklaşım olabildiği gibi, elektif boyun diseksiyonu veya elektif radyoterapi de uygulanabilmektedir. Boyunda gizli metastaz olasılığı %15-20'nin üzerinde ise elektif tedavi önerilmektedir. Elektif boyun diseksiyonlarında primer tümöre göre diseke edilecek lenf nodu bölgeleri değişmektedir. N0 olan hastalarda, boyun nüksü ve uzak metastazdan koruyan en etkin ve morbiditesi düşük profilaktik tedaviye karar vermek gerekmektedir.

Anahtar Kelimeler
Baş boyun kanserleri, N0, yaklaşım

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Introduction

Patients defined as N0 in head and neck cancers are the patients in whom the pathological lymph nodes are considered to be absent clinically by the examination and the imaging methods. During the decision-making in the N0 neck, the size, the histopathological features, the degree of differentiation, and the location of the tumor have importance (1). Whether there are cancer cells in the lymph nodes and occult metastasis is present or not are significant indeterminate issues in patients defined as N0 (1). The probability of development of clinical cervical metastasis in the future, and when identified, how many of these would benefit from the salvage surgery are also important. The cervical metastases are among the significant causes of death following treatment of head and neck cancers. During the decision-making process for treatment, our primary principle is "Primum non nocere", doing no harm to the patients, first.

In patients with N0 neck, it is required to decide on the most effective prophylactic treatment with low morbidity that can avoid cervical recurrence and distant metastasis, during decision-making.

Imaging in the N0 Neck

First of all, we consider that N0 being related to what is an important issue. Will we consider these patients N0 according to findings on palpation, imaging methods, or the result of the ultrasound- guided fine needle aspiration biopsy? When the results of the computed tomography (CT) only were compared to the magnetic resonance imaging (MRI), they were found to have similar sensitivities (sensitivity: metastasis+, finding+) and specificities (specificity: finding-, disease-) (2). CT, MRI, ultrasonography, positron emission tomography (PET) and palpation are methods used in diagnosing N0 (1). Besides all these, the ultrasound-guided fine needle aspiration biopsy was emphasized to be important in the diagnosis of N0 (1-3). The specificity of positron emission tomography is high (82-85%) in this respect; however, the sensitivity is significantly reduced (50-70%) in N0 necks when compared to the N+ ones. Therefore, in N0 patients, PET was considered as unnecessary concerning neck in N0 patients (4). However, since the stage and the treatment plan might change with PET, it is recommended in T3 and T4 patients. Some suggest using the clinical criteria only, not recommending any imaging method. Suggesting that elective neck dissection can be performed in T2 patients even though no lymph node is identified by imaging, and also, in T1 patients, the detected lymph node does not always imply metastasis, the proponents of this argument do not recommend imaging.

The Therapeutic Approaches

They can be performed as a wait-and-see procedure or a prophylactic/elective treatment [neck dissection (ND), radiotherapy (RT)] (1). The elective treatment can contribute the staging and therapeutic processes (Figure 1) (1).

Wait-and-see

Those who accept this therapeutic approach consider that cancer is not present in the lymph nodes of the patients recognized as N0 clinically. They advocate that salvage surgery can be implemented if cervical metastasis is determined in the future. This approach is accepted if the probability of occult metastasis in under 20%. One of the disadvantages of this approach is the probability of being unable to identify the cervical metastasis early, in the future. When it is identified, the pathological stage might be more advanced than the clinical stage. The salvage treatments have not been very successful. Besides,

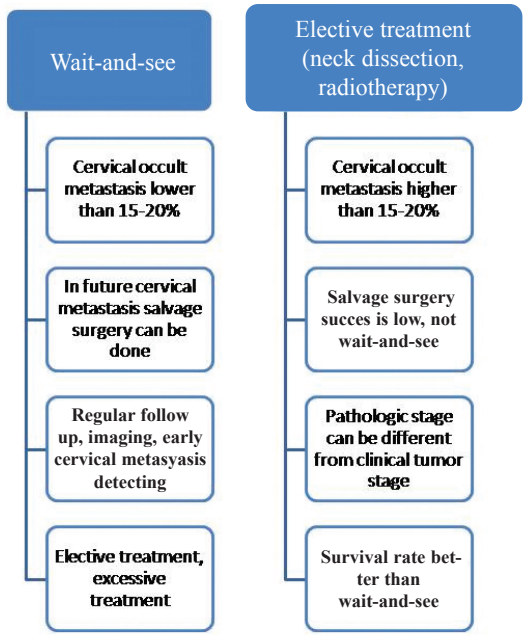

Figure 1. The advantages of treatment options in N0 neck

when cervical metastasis develops, the probability of developing distant metastasis is also elevated. The situations that the wait-and-see approach is not appropriate are as follows: the location of the primary tumor, the likelihood of the occult cervical metastasis being greater than 15-20% related to its stage, the neck being short and thick, the unavailability of medical center resources for frequent imaging, the inability of the patient to attend regular follow-up examinations, and failure to perform immediate salvage surgery (5).

The Elective Treatment

The advocates of this approach consider that even though the patient is N0 clinically, occult metastases might be present in the neck region. Since the success rate of the salvage surgery is small, they defend the necessity of the elective treatment. One of the hesitations of supporters of this treatment is whether the elective surgery would be an excessive treatment if these patients are actually N0. It is recommended that if elective surgery is performed in N0 neck, the method should be similar to the method used in the primary tumor (surgery or RT) for the patient not to undertake the morbidities of both methods. The prophylactic RT is considered to be as effective as surgery. In N0 neck, the surgical treatment is "selective ND" [lateral ND, selective ND (II-IV)]. The type of the selective ND is decided depending on the lymph nodes at risk. The survival rate was determined to be higher in patients who had undergone ND during the neck was in the occult state when compared to those who had undergone surgery following the occurrence of the clinical findings (6,7). While some authors have suggested that the success of the elective RT was similar to that of elective ND (8), others have stated that the cervical recurrence was more frequent following elective RT when compared to ND (9). In a high-grade tumor, elective RT might be preferred if RT is planned following surgery.

N0 According to the Location of the Tumor

A high rate of clinical N-positivity has been determined in cancers of the tongue corpus, the mouth floor, the retromolar trigone, gingiva, nasopharynx, oropharynx, and the supraglottic larynx, even at the time of the diagnosis (1). The identification rate of occult cervical metastasis is high in cancers of the tongue corpus and base, the mouth floor, gingiva, pharyngeal walls, hypopharynx, and the supraglottic larynx, even though the patient is N0 clinically (5).

In oral cavity tumors, the tumor thickness in T1 T2 is quite significant concerning our decision for treatment. It was determined that the elective ND had contributed to the survival positively in tumors with at hickness of 4 mm and above (10). If the depth is over 3-4 mm, the risk of occult metastasis rises to 40% or more in the oral cavity tumors (11).

While the incidence of occult metastasis was 10% in the glottic tumors which were N0 both initially and following treatment, this rate is over 40% in the supraglottic and transglottic tumors (12). The risk of the occult metastasis development is directly proportional to the T stage (13).

Numerous studies have reported rates ranging from 4% to 25% for occult metastasis in lower lip cancers. There are studies suggesting that occult metastasis rates are in parallel with the T stage or stating that the two are not related (14). These ratios are not always related to the tumor size. The skip metastases are important. In N0 patients with lower lip pathologies who undergo suprahyoid ND, recurrence might be observed in zone 3. While the wait-and-see approach is recommended in T1N0 patients in lip tumors, some authors have suggested performing suprahyoid or supraomohyoid NDs in T2-3 N0 and the commissure (15). However, an important issue is that when T1 patients are admitted with cervical recurrence, although rare, their salvage surgeries are quite complicated. In tongue tumors, the rate of occult metastasis is high. While some authors have suggested elective ND even if the patient was T1, some others have stated that in T1, T2 and N0 patients, the success rates of the observation and the elective ND approaches were similar, the ND not providing any therapeutic contribution (16-19). However, in T1, the contralateral ND is not recommended. The probability of contralateral neck metastasis increases when the tumor passes the midline, and the ipsilateral neck is positive.

The perineural invasion, singly, is an important indicator related to the occult metastasis and the regional recurrence (20).

Why Bilateral Neck Dissection in N0?

It is recommended in midline lesions. The supraglottis is considered as a midline structure embryologically; however, when the lesion is laterally located, the ipsilateral ND may be initially performed.

The authors criticizing the routine bilateral implementation have advocated that the disruption of the integrity of the lymph nodes would destroy the barrier against cancer, it the cancer is not present. The prevalence of contralateral metastasis is under 10% in supraglottic cancer, in T1, T2, and unilateral pathologic N0 (5). Therefore, in unilateral pathologic N0 tumor, the contralateral ND might not be performed. However, one of the significant hesitations is which side of the neck should be dissected first. It is suggested that this should be decided depending on the lateralization of the lesion or the presence of the sentinel node. However, as a counter-view, late-term contralateral metastases have been reported in unilateral N0 cases (21).

Although some authors have suggested that contralateral ND should be performed when the suspicious lymph node was reported as N+ in frozen section during the ND, since the entire neck cannot be examined by frozen section, the suspicious lymph not might not be encountered. Elective RT might be administered to the contralateral side, or when the pathology report reveals N+, contralateral ND might be performed. RT should not be preferred particularly in conservative laryngeal surgery.

The Advantages of Selective Neck Dissections

It is an oncologically effective method. The lymph nodes that are at risk are excised. Since the sternocleidomastoid muscle, the accessory nerve, and the jugular vein are preserved, its morbidity is low. Its bilateral implementation can be made simultaneously. The pathological staging together with the prognostic evaluation and requirements for additional treatments can be planned correctly. Provision of additional treatment may occur in N0 cases following the ND. It may be necessary to initiate detailed ND according to the findings and frozen section results during surgery. The preservation of the submandibular gland during ND is one of the controversial issues (22,23). Since it does not involve intraparenchymal lymph nodes, some authors recommend its preservation (22). However, due to the proximity of the gland to the tumors of the mouth floor and tongue base, another opinion has been the non-preservation of the gland (23,24). The preservation of the submandibular gland may lead to difficulties in the exposure of the lymph nodes located behind the gland.

The N0 Neck Results in Salvage Surgery

There are differences in approaching the clinically N0 cases with recurrent laryngeal carcinoma. The wait-and-see approach, additional RT, or selective ND might be performed. It was determined that these therapeutic approaches were not able to create any significant differences in the survival rates (25).

Dissection of Zone IV?

Some of the significant morbidities during the dissection of this zone are the probability of chylous fistula and phrenic nerve injury development. The situations in which the development of zone IV metastasis is possible to occur are the subglottic extension from the larynx and the transglottic tumors (26). In clinically N0 necks, the zone IV metastasis occurs with low rates such as 0-3.5% following the treatment. In numerous studies, the zone IV metastasis was always identified together with zone II or zone III metastases. Therefore, it has been suggested that zone II and zone III might be sent to the frozen-section examination and when the result was reported as (+), the zone IV dissection might be performed (26). The regional recurrences were reported to be frequent in zone IV (27). In oropharyngeal cancers, since skip metastasis is frequently met in zone IV, the dissection of this zone has been recommended (27).

The Dissection of Zone IIb in N0 Laryngeal and Hypopharyngeal Cancers?

In zone IIb dissections performed in the clinically N0 and N+ necks, the metastasis to zone IIb was identified as 1-17%. The most significant morbidity in the dissection of zone IIb is the accessory nerve dysfunction. In N0 laryngeal cancers, the dissection of the zone IIb has not been recommended (28,29). In cancers of the tongue, zone IIb dissection is recommended (30).

The Dissection of Zone V

While the involvement of zone V is 1% in N0 head and neck cancers, the rate increases up to 7% in the cancers of hypopharynx and oropharynx. In parotid tumors, the dissection of zone V is not recommended in N0 cases (31).

The Use of the Sentinel Node in Head and Neck

The sentinel node has been introduced to routine use in the malignant melanomas of the head and neck regions; it has significant contributions concerning the staging process (32). The false-positivity rates are high, and the false-negativity rates are low in

the histopathological examination of the sentinel nodes. During its use in the oral cavity and the oropharynx, the negative predictive value of the sentinel node is 94-95% (33). One of the controversial issues is the examination of the sentinel node by immunohistochemical methods, after staining by haematoxylin-eosin, instead of frozen-section examination. Here, the significant question mark is the probability of skip metastasis.

Conclusion

In head and neck cancers, the appropriate treatment can be decided by taking into consideration the findings of the physical examination and the imaging methods, the location of the primary tumor, the histopathological characteristics of the primary tumor, the T stage and the comorbidities of the patient while approaching the N0 neck.

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Original Article / Özgün Araştırma

Evaluation of the Effect of Different Curing Units and Exposure Times on Pulp Chamber Temperature Using Simulated Pulpal Microcirculation

Yapay Pulpal Mikrosirkülasyonu Kullanılarak Farklı Işınlama Üniteleri ve Işınlama Sürelerinin Pulpa Odası Sıcaklığına Etkisinin Değerlendirilmesi

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Keywords
Curing units, exposure time, pulpal temperature elevation

Anahtar Kelimeler
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Abstract

Objective: To compare the elevations in physiological pulp chamber temperature and blood microcirculation during light curing using four different light sources at two different exposure times.

Materials and Methods: The study was performed on eighty Class-V cavities divided into eight groups of ten experiments each, according to the curing unit and exposure time. Short-term curing was performed in four groups: quartz-tungsten-halogen (QTH)-20 seconds (s) (XL3000, 3M ESPE, St. Paul, MN, USA); Woodpecker (WP)-15s (Lux V Light Cure Unit, WP, Apexion Dental Products); KR-10s (Demi Plus Dental Curing Light, Kerr Dental) and VL-3s (VALO light-emitting diode Curing Light Xtra Power mode, Ultradent Products, South Jordan, UT, USA). The same units were used for long-term curing at double the exposure time (i.e., QHT-40s, WP-30s, KR-20s, VL-3+3s). Composite material (Filtek Ultimate, 3M/ESPE) was placed into the cavities, followed by light curing performed at a distance of 1 mm. Temperature changes in the pulp chamber were recorded. The Kruskal-Wallis test, followed by Mann-Whitney U multiple comparisons test, were used for statistical analyses, with $p<0.001$ considered to be statistically significant.

Results: There were no significant differences among the curing units at the same exposure times. However, increases in pulp chamber temperatures varied significantly depending on the curing time ($p<0.001$). All the curing units induced significantly higher intrapulpal temperature changes at long-term curing times, except the WP groups ($p<0.001$). WP-30s, KR-20s and VL-3+3s exhibited critical temperature increases >5.5 °C.

Conclusion: Longer curing times led to critical temperature increases in the pulp chamber. Shorter curing times can protect the pulp tissue against damage caused by temperature elevations above a certain threshold.

Öz

Amaç: İki farklı ışınlama süresinde dört farklı ışık kaynağı kullanılarak ışıkla sertleştirme sırasında fizyolojik kan mikrosirkülasyonu ile birlikte pulpa odası sıcaklık artışlarını karşılaştırmak.

Gereç ve Yöntemler: Çalışma, farlı ışık kaynakları ve farklı ışınlama süresine göre, her biri 10 deneyden oluşan sekiz grupla seksen Sınıf-V kavite üzerinde gerçekleştirilmiştir. Kısa süreli kürleme dört grupta gerçekleştirildi: kuvars-tungsten-halojen (QTH) 20 saniye (XL3000, 3M ESPE, St. Paul, MN, ABD); Woodpecker (WP) -15s (Lux V Işık Tedavi Ünitesi, WP, Apexion Dental Ürünleri); KR-10s (Demi Plus Dental Kür Işık, Kerr Dental); ve VL-3s (VALO ışık yayan diyot Kür Işık Xtra Güç modu, Ultradent Ürünler, South Jordan, UT, ABD). Aynı birimler, pozlama süresinin iki katı uzun süreli ışınlama için kullanılmıştır (yani, QHT-40s, WP-30s, KR-20s, VL-3+3s). Kompozit malzeme (Filtek Ultimate, 3M/ESPE) boşluklara yerleştirildi, ardından 1 mm mesafede ışıkla sertleştirme yapıldı. Pulpa odasındaki sıcaklık değişimleri kaydedildi. Kruskal-Wallis testi, ardından Mann-Whitney U çoklu karşılaştırma testi uygulanmış, istatistiksel analizlerde p<0,001 istatistiksel olarak anlamlı bulunmuştur.
Bulgular: Aynı ışınlama süreleri içerisinde ışınlama cihazları arasında önemli bir farklılık yoktu. Bununla birlikte, pulpa odası sıcaklıklarındaki artışlar, ışınlama süresine bağlı olarak önemli ölçüde değişmiştir (p<0,001). Tüm sertleştirme üniteleri, WP grupları hariç, uzun süreli ışınlama sürelerinde, önemli ölçüde daha yüksek sıcaklık değişikliklerine neden olmuştur (p<0,001). WP-30s, KR-20s ve VL-3+3s, > 5,5 °C kritik sıcaklık artışları sergiledi.
Sonuç: Daha uzun ışınlama süreleri, pulpa odasındaki kritik sıcaklık artışlarına yol açmıştır. Daha kısa ışınlama süreleri, pulpayı belirli bir eşiğin üzerindeki sıcaklık yükselmelerinin neden olduğu hasara karşı koruyabilir.

Introduction

The detrimental effects of increased temperature in pulp tissue during restorative treatment(s) have been a concern in dentistry for decades. The classical animal study by Zach and Cohen (1) demonstrated a threshold temperature for irreversible pulpal damage to a healthy tooth when external heat is applied. An intra-pulpal temperature increase of 5.5 °C led to necrosis in 15% of the tested pulps; an increase of 11 °C induced 60% damage; and an increase of 16 °C resulted in irreversible tissue damage to 100% of the pulps tested (1). The major factors leading to pulp tissue damage include: protoplasm coagulation; vascular injury in the tissue; and expansion of the lymph fluid in the dentin tubules (2). The photopolymerization process can also cause a significant increase in temperature. In previous *in vitro* and *in vivo* experiments, temperature increases in the pulp chamber were found to be as high as 20 °C depending on the method of tooth preparation, photoactivation, and the various materials used (3-7). In some earlier *in vitro* studies, it was also found that the photopolymerization of composite resin increased the temperature in the pulp chamber by only a few degrees due to the heat insulation of the hard tissue within the tooth (8,9). Temperature increases in the pulp chamber depend on the type of light source, the output power density, exposure time to the light, the distance between the tooth and the fiber optic tip, the composite color tone, the thickness of the composite material, and the remaining dentin thickness (3-5,10,11). The increase in temperature in the pulp chamber during curing represents the sum of light energy from the curing unit and the heat from the exothermic reaction during polymerization of the composite resin (12,13).

There are many factors that affect heat build-up in the dentin pulp complex, including the intensity and duration of heat applied to the tooth, the movement of the lymph fluid in the dentin tubules, pulpal blood microcirculation, and blood flow changes in the pulp depending on the nervous system of the pulp (14). Pulpal microcirculation flow is reported to be approximately 20-60 mL/per minumum (min) per 100 g of tissue in healthy teeth (15,16). The pulpal microcirculation is known to be the primary system that regulates heat distribution inside the pulp tissue and, thus, partially absorbs external thermal heat (14-17).

The primary objective of the present study was to determine the extent to which the total heat increase due to light curing and polymerization varies with the physiological heat of the pulp chamber at 37 °C and simulated pulpal microcirculation for different curing units and exposure times.

Materials and Methods

This study was approved by the Pamukkale University Ethics Committee of the Faculty of Medicine (approval number: 06, date: 20.04.2017). Before the tooth extraction, consent forms stating that the extracted teeth can be used in scientific research were signed by the patients.

Sample Preparation

The sample size was determined at a 95% confidence interval and a significance level of 0.001

(effect size =1.37), according to the study by Kodonas et al. (18) eighty extracted-human mandibular premolar tooth was used in the study. The specimens were stored in 0.1% thymol solution until beginning of the experments. The study was performed with 8 groups of 10 experiments each.

A standardized cavity (3×4×2 mm^3) with a Class-V preparation was used with a pulpal wall thickness of 1 mm. Pulpal dentin wall thickness was confirmed using radiography. The roots were sectioned using a water-cooled carborundum disk approximately 1 mm below the cemento-enamel junction upright to the long axis of the tooth. The coronal pulpal tissues were cleaned using an excavator (EXC17, Osung, USA), and the pulp chamber was irrigated with distilled water and gently dried with air. Access to the pulpal chamber was prepared, as needed, via the proximal surface of the sample to enable insertion of a 0.4 mm diameter thermocouple wire (Figures 1, 2). The intrapulpal temperature changes during the curing of the composite resin were determined with a device that simulated pulpal blood microcirculation (12,17,19). A K-type thermocouple (TT-K-30-SLE; Omega Engineering Inc. Stanford, CT, USA) was inserted into the pulp chamber until contact with the axial wall and sealed with thermal grease (ZM-STG2; Zalman Tech Co. Ltd. Dongan-gu, South Korea). The position of the thermocouple point was confirmed radiographically from two directions. The access hole of the thermocouple wire was filled with light-curing glass-ionomer cement (Ionoseal, VOCO, Cuxhaven, Germany) to avoid leakage from the system. The thermocouple cable was connected to a data logger (DT-3891G; CEM, Shenzhen, PRC), which was connected to a personal computer to monitor temperatures changes.

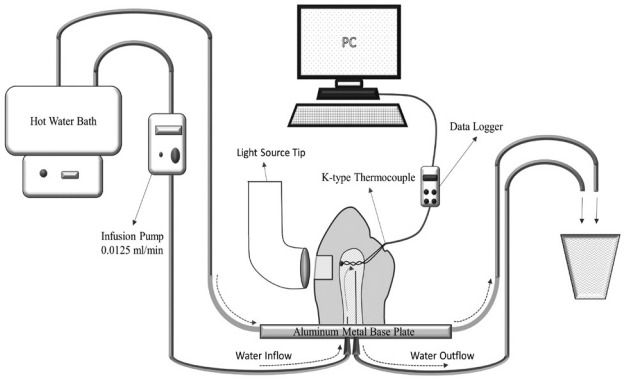

Figure 1. Schematic drawing of the mandibular premolar tooth and Class-V cavity with experimental microcirculation apparatus

Pulpal Microcirculation Simulation Model

The specimen was mounted on an experimental device made specifically for this study, which simulated pulpal blood microcirculation and regulated the temperature of the tooth to within physiological limits (37 °C ±1 °C). A standard infusion set (24.08000; Beybi Medical, İstanbul, TR) was attached to a digital water bath (WB-11; Daihan, Wonju, South Korea) holding distilled water. Two 25-gauge needles (8696569000777, Hayat Medical Co., İstanbul, Turkey) were placed to provide intrachamber microcirculation through the hole of a temperature-controlled aluminum base plate (TCAP), and used as an inflow/outflow pathway for the distilled water. Needles were attached to the drilled hole of the TCAP using cyanoacrylate adhesive. The specimens were fixed using a light-cured glass ionomer liner cement (Ionoseal; VOCO, Cuxhaven, Germany) on the TCAP in such a way that the needles lined-up with the inside of the pulp chamber. The flow rate of the distilled water was set at 0.0125 mL/min using an infusion pump set (IP12A; Biocare, Shenzhen, China), which was attached to the system (Figure 1). To provide physiological temperature in the pulp chamber, a 4 mm diameter, spiral-shape copper tube was attached under the TCAP using thermal grease and connected to a standard infusion set in a water bath. Hot water

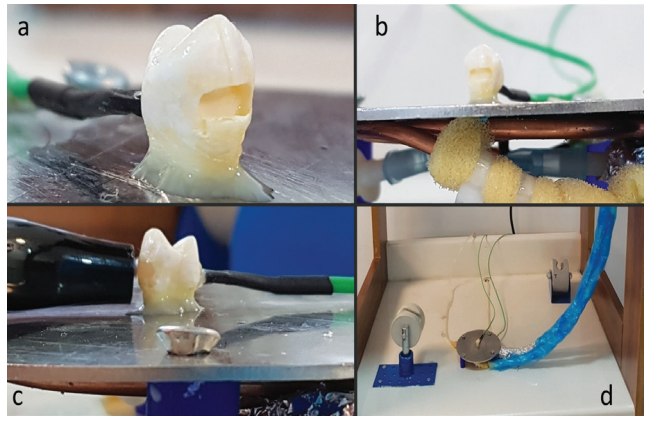

Figure 2. a: Experimental tooth Class-V cavity, b: bottom view of the "TCAP", c: the position of the tip of the irradiation device, d: top view of the test setup

TCAP: Temperature-controlled aluminum base plate

flowed from inside the copper tube to regulate physiological temperature to the TCAP (Figure 3).

Following the application of bonding agent (3M Scotchbond Multipurpose Plus, MN, USA) the cavities were restored with sufficient composite material (Filtek Ultimate, 3M/ESPE, Minneapolis, MN, USA) then light cured. Intrachamber temperature elevations were simulated by applying the various curing units with different time settings to the buccal direction of the Class-V cavity. The tips of the light sources were positioned 1 mm from the tooth. The curing units included a conventional halogen lamp and three high-intensity light-emitting diode (LED) lamps. Two different exposure times for each curing unit were tested in accordance with manufacturer's instructions. Four different curing units, their specifications, and time sets are listed in Table 1. The output power of the light-curing units were measured using a digital radiometer (Cure Rite; Caulk/Dentsply, Milford, DE, USA).

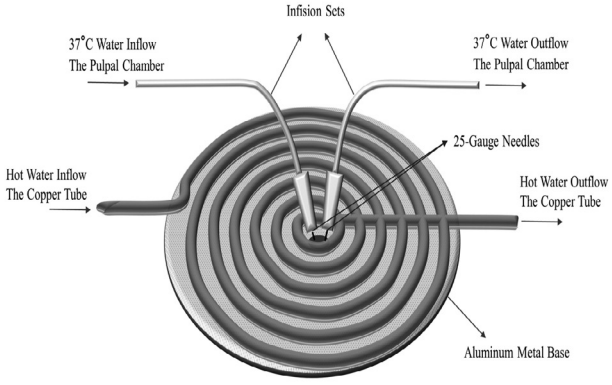

Figure 3. Bottom view of the temperature-controlled aluminum base plate, which is part of the experimental apparatus to regulate the tooth physiological temperature

Statistical Analysis

The changes between initial and maximum temperature in the pulp chamber axial wall (Δt) were recorded during the experiment, and the effect of light curing on temperature change was examined. The median changes in temperature from the initial physiological baseline are summarized in Table 1.

The Shapiro-Wilk omnibus normality test revealed that the data were not normally distributed. The Kruskal-Wallis test, followed by Mann-Whitney U multiple comparisons test, was used for statistical analyses (SPSS version 23.0, IBM Corporation, Armonk, NY, USA); $p<0.001$ was considered to be statistically significant.

Results

The median and interquartile ranges of maximum temperature increases according to the different light-curing sources and curing time sets are presented in

Table 1. Light-curing units used in the study and the median and interquartile ranges of temperature increases obtained from the test groups and intergroup comparisions

Groups	Light-curing units	Light type	Output of light tip (mW/cm²)	Curing time (s)	Temperature increase (Δt) (Median and IQR)
QTH-20 s	XL3000 (3M ESPE, St. Paul, MN, USA)	QTH	640	20	3.75 (3.3-4.5)[ab]
WP-15 s	Lux V Light Cure Unit (Woodpecker, Guangxi, China)	LED	1.000	15	4.45 (4-5)[ac]
KR-10 s	Demi™ Plus Dental Curing Light (Kerr Dental, USA)	LED	1.200	10	3.9 (3.7-4.3)[a]
VL- 3 s	VALO LED Curing Light Xtra Power mode (Ultradent, South Jordan, Utah)	LED	3.200	3	4 (3.8-4.9)[ae]
QTH-40 s	XL3000 (3M ESPE, St. Paul, MN, USA)	QTH	640	40	5.4 (5.1-5.9)[cde]
WP-30 s	Lux V Light Cure Unit (Woodpecker, Guangxi, China)	LED	1.000	30	5.85 (5.4-6.4)[d]
KR-20 s	Demi™ Plus Dental Curing Light (Kerr Dental, USA)	LED	1.200	20	5.85 (5.2-6)[cd]
VL-3/3 s	VALO LED Curing Light Xtra Power mode (Ultradent, South Jordan, Utah)	LED	3.200	3/3	5.85 (4.6-6.2)[cd]

*There are no significant differences between temperature increases with the same superscript letter

(Kruskal-Wallis test, Post-hoc Bonferroni test, $p<0.001$), IQR: Interquartile Ranges, mW/cm²: Millwatt/square centimeter, s: Second, Δt: Temperature changes, LED: Light-emitting diode, QTH: Quartz-tungsten-halogen, The same superscript letters are demonstrated no significant differences ($p<0.05$)

Table 1. There was no significant difference among all light curing units in both short- and long-term curing applications. However, pulp chamber temperature increases varied significantly depending on curing time [p<0.001 (Kruskal-Wallis test)]. All tested light-curing sources induced significantly higher intrachamber temperature changes in the long-term curing times, except for the WP group [p<0.001 (Bonferroni post-hoc test)].

The WP-30s, KR-20s and -3+3s groups demonstrated statistically significant critical temperature increases (i.e., >5.5 °C) (Table 2). The maximum temperature increase was observed on completion of light exposure (Figure 4).

Discussion

In deep restorations, the heat generated by light curing of the composite and the effect of this heat on the pulp are particularly important. Whitworth et al. (20) found that pulp damage was increased during deep restorations. When amalgam and composite deep restorations were compared, the incidence of pulp damage in the composite restorations was higher. Results of our model suggest that temperature increases in the pulp chamber and microcirculation during the curing of deep restorations varied with different curing units and at different times. It was clear that the increase in pulpal temperature varied according to the situation, and negatively affected the health of the pulp tissue; however, it was not clear at which temperature increment the pulp tissue was affected in the negative direction. Zach and Cohen (1) found that a temperature increase of 5.5 °C in the pulp tissue of rhesus monkey teeth caused necrosis in 15% of the pulp tissues. This was consistent with results reported by Pohto and Scheinin (19), and suggests that the irreversible damage of pulp tissue begins at 42-44 °C. The previous study by Zach and Cohen (1) demonstrated that pulp chamber temperature increased in a linear fashion because of the heat source used. However, in the present study, temperature increases due to the sum of the energy resulting from the light energy and the composite polymerization energy during light curing did not exhibit a linear progression (Figure 3). In a different study on human teeth reported that short-term temperature increases that were increased up to 14.7 °C did not cause degenerative changes in pulp tissue (21), while in another study, it was found that a temperature increase of 11-20 °C caused damage to the tissue (22). Safe temperature criteria for pulp tissue may be developed more accurately with the use of cultured cells and varying curing times (23,24). In this context, it is necessary to develop more detailed safety criteria based on temperature.

In vitro studies depend on many factors, such as temperature increase, the heat/light source, application time, thickness of the dental tissue, and microcirculation in the pulp chamber (12,17,18). In a previous study, the coronal pulp chamber volume of the mandibular premolar teeth was estimated to be 0.025 mL, and a serum infusion pump was set to a flow rate 0.0125 mL/min to simulate microcirculation in the pulp chamber (25). In several previous studies, the microcirculation did not take into account the contribution of regulating temperature in the pulp chamber (26-28). Many *in vitro* studies have been

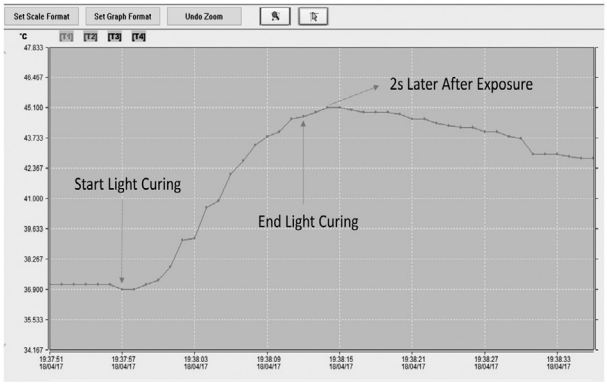

Figure 4. A graph which is showing the temperature increase during and after the 15 seconds exposure period

Table 2. Distributions of experiments below and above the critical temperature of 5.5 °C

Groups	Below 5.5 °C (n)	Above 5.5 °C (n)
QTH-20 s	10 (100%)	0 (0%)
WP-15 s	10 (100%)	0 (0%)
KR-10 s	10 (100%)	0 (0%)
VL-3 s	10 (100%)	0 (0%)
QTH-40 s	5 (50%)	5 (50%)
WP-30 s	1 (10%)	9 (90%)
KR-20 s	2 (20%)	8 (80%)
VL-3+3 s	2 (20%)	8 (80%)

QTH: Quartz-tungsten-halogen

performed using a 37 °C hot water tank. To achieve better clinical results, Kodonas et al. (18) simulated microcirculation flow in the pulp chamber by maintaining inflow at a constant 37 °C, which more accurately reflects *in vivo* conditions (29). In our pilot study, it was determined that the physiological temperature in the pulp chamber, which we attempted to adjust only by microcirculation, was very unstable. Therefore, the experimental conditions used to mimic periodontal tissues and regulate the temperature of the environment were adapted using a TCAP (Figure 2). Thus, the temperature in the pulp chamber was consistently maintained at 37±1 °C before the experiment.

In an *in vitro* study by Weerakoon et al. (30), Class-V composite restorations were cured for 40 seconds in premolar teeth. They found that LED light increased the temperature of the pulp chamber less than a halogen lamp. In our study, there was no significant difference in temperature increase between the quartz-tungsten-halogen (QTH) and LED groups in the short- and long-term curing settings (Table 1).

Exposure time is another crucial factor that influences temperature (8,29). During curing of the composite resin, the primary factor that increases temperature is light energy, while the secondary factor is the exothermic heat energy produced during polymerization of the composite resin (31). Total radiation exposures for the short-term setting groups were approximately 12.8, 15, 12, and 9.6 J/cm^2, and those for long-term irradiation groups were double these values. However, the temperature increases did not appear to follow the order of total radiation. This may be due to the fact that the total heat generated is regulated along with a microcirculation effect during different irradiation times. Almost all manufacturers that produce composite materials recommend 40 seconds of curing time. We selected two different exposure times set for each of the curing units, in accordance with manufacturer's instructions. Significant differences in pulp chamber temperature elevations were found among short-term and long-term exposure times in experiments with all curing units ($p<0.001$); this result is consistent with a previous study, except for the WP group (29). Long-term curing times for the QHT-40s, WP-30s, KR-20s and VL-3+3s units resulted in temperature increases >5.5 °C in the pulp chamber. To achieve optimum curing times for QHT-20s, WP-15s, KR-10s and VL-3s, temperature increases in the pulp chamber should be limited to under safe limits (i.e., <5.5 °C).

In this *in vitro* study, which simulated the microcirculation in the pulp chamber at 37 °C, various curing units were used at different time settings, and it was verified whether critical temperature increases in the pulp chamber had reached the 5.5 °C threshold. Although *in vitro* simulation closely approximates physiological conditions, it is not able to fully simulate the *in vivo* environment. Although the pulp microcirculation is kept constant in such *in vitro* studies, factors, such as age and gender, can actually influence pulpal microcirculation volume (32). In addition, the TCAPs that were used in this study to simulate the periodontal tissues may not simulate the *in vivo* environment. Because such studies are not ethical to perform *in vivo*, development of more realistic experimental simulation devices and protocols are anticipated to provide more accurate results.

Conclusion

Long-term curing times with LEDs exceeded critical temperature increases of 5.5 °C in the pulp chamber. Exposure time is an important risk factor for damage to the pulp tissue. Therefore, to prevent-or, at least mitigate-damage to the pulp tissue, short-term light exposure is the safer option.

Ethics

Ethics Committee Approval: This study was approved by the Pamukkale University Ethics Committee of the Faculty of Medicine (approval number: 06, date: 20.04.2017).

Informed Consent: Consent forms stating that the extracted teeth can be used in scientific research were signed by the patients.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: İ.F.E., Design: İ.F.E., Supervision: İ.F.E., Data Collection or Processing: B.Y., Analysis or Interpretation: İ.F.E., Materials: İ.F.E., Literature Search: İ.F.E., Writing: İ.F.E.

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

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Original Article / Özgün Araştırma

The Efficacy of Bismuth-based Quadruple and Sequential Therapies in *Helicobacter pylori* Eradication

Helikobakter pilori Eradikasyonunda Bizmutlu Dörtlü Tedavi ile Bizmutlu Ardışık Tedavinin Etkinliği

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Keywords
Helicobacter pylori, quadruple treatment, sequential treatment

Anahtar Kelimeler
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Abstract

Objective: New therapy regimens are needed for *Helicobacter pylori (H. pylori)* eradication because of increased resistance to the commonly used antibiotics. This study aimed to compare the efficacy of bismuth quadruple therapy versus sequential therapy for *H. pylori* eradication.

Materials and Methods: Patients who presented with dyspepsia complaints and were diagnosed with *H. pylori* infection by histopathologic examination of biopsies obtained by gastroscopy were evaluated retrospectively. Two hundred and five patients who received bismuth-based quadruple therapy and sequential therapy for *H. pylori* eradication were included in the study. Bismuth-based quadruple therapy group were given pantoprazole 2x40 mg, bismuth subcitrate 4x300 mg, amoxicillin 2x1000 mg and metronidazole 3x500 mg for 14 days. In the bismuth-based sequential therapy group, the protocol was as follows; pantoprazole 2x40 mg (14 days), bismuth subcitrate 4x300 mg (14 days), amoxicillin 2x1000 mg (first 7 days), metronidazole 3x500 mg (second 7 days) and levofloxacin 1x500 mg (second 7 days). Eradication was determined by stool *H. pylori* Antigen test six weeks after the treatment.

Results: A total of 102 patients in group 1 and 91 patients in group 2 completed the treatment and there was no significant difference between the two groups (p=0.310). *H. pylori* eradication rate showed no significant difference between the two groups (p=0.093), while group 1 attained a better eradication rate than group 2 on intention-to-treat, which was statistically significant (p=0.033).

Conclusion: We achieved better eradication rates with bismuth-based quadruple therapy compared to the sequential therapy. We recommend bismuth-based quadruple regimen as the first-line eradication therapy to avoid drug incompatabilities seen during the sequential regime.

Öz

Amaç: *Helikobakter pilori (H. pilori)* eradikasyon tedavisinde kullanılan antibiyotiklere karşı direncin artması nedeniyle yeni tedavi rejimlerine ihtiyaç duyulmaktadır. Bu çalışmada, *H. pilori* eradikasyonunda bizmutlu dörtlü tedavi ile bizmutlu ardışık tedavinin etkinliğini karşılaştırmayı amaçladık.

Gereç ve Yöntemler: Dispepsi yakınması ile başvuran, yapılan gastroskopi ile alınan biyopsilerde histolojik inceleme sonrasında *H. pilori* saptanan hastalar retrospektif

olarak değerlendirildi. Eradikasyon tedavisi olarak bizmutlu dörtlü tedavi ve bizmutlu ardışık tedavi alan 231 hasta çalışmaya alındı. Bizmutlu dörtlü tedavi alan gruba; 14 gün boyunca pantoprazol 2x40 mg, bizmut subsitrate 4x300 mg, amoksisilin 2x1000 mg, metronidazol 3x500 mg (14 gün) verilmişti. Bizmutlu ardışık tedavi alan gruba; pantoprazol 2x40 mg (14 gün), bizmut subsitrate 4x300 mg (14 gün), amoksisilin 2x1000 mg (ilk 7 gün), metronidazol 3x500 mg (ikinci 7 gün), levofloksasin 1x500 mg (ikinci 7 gün) verilmişti. Eradikasyon, tedaviden altı hafta sonra gaitada *H. pilori* Antijen testi ile ölçüldü.

Bulgular: Grup 1'de 102 hasta, grup 2'de 91 hasta tedaviyi tamamlamış ve her iki grup arasında anlamlı bir fark bulunmamıştır (p=0,310). Her iki grup arasında protokol başına eradikasyon oranı açısından anlamlı bir fark bulunmamış (p=0,093), grup 1 rejimi intention-to-treat'de grup 2'ye göre daha iyi eradikasyon oranına ulaşmış ve istatistiksel olarak anlamlı bulunmuştur (p=0,033).

Sonuç: Bizmut bazlı dörtlü tedavi ile ardışık tedaviye oranla daha iyi bir eradikasyon oranlarına ulaştık. Ardışık tedavi sırasında görülebilen ilaç uyumsuzluğu nedeniyle birinci basamak eradikasyon tedavisinde bizmut bazlı dörtlü tedavi rejiminin kullanılmasını önermekteyiz.

Introduction

Helicobacter pylori (*H. pylori*) infection is an important health problem in our country and worldwide. *H. pylori* is the main cause of chronic gastritis and peptic ulcer disease as well as a major risk factor for gastric cancer and mucosa-associated lymphoid tissue lymphoma (1,2). *H. pylori* is also associated with extraintestinal diseases such as idiopathic thrombocytopenic purpura, unexplained iron deficiency anemia, Diabetes Mellitus and atherosclerotic cardiovascular disease (3).

Worldwide prevalence of *H. pylori* infection is expected to be 4.4 billion in 2015, although itis decreasing in Western Europe and North America (4).

The efficacy of the recommended first line therapy for *H. pylori* infection, which was the triple therapy including proton pump inhibitors (PPIs), amoxicillin and clarithromycin, decreased in most countries, hence new treatment strategies are needed (5-7). The Maastricht V consensus meeting recommended bismuth-based quadruple therapy regimens in areas with high clarithromycin and metronidazole resistance (8). Moreover, sequential therapy regimes were also proposed as effective first-line treatment methods, in recent years (9). However, the complexity of the sequential therapy regime is a major disadvantage that threatens its feasibility and success rate.

In this study, we aimed to compare the efficacy of bismuth based quadruple therapy versus sequential therapy for the first-line *H. pylori* eradication therapy.

Materials and Methods

Study Design and Population

Patients admitted to two centers of gastroenterology department with dyspepsia complaints between October 2015 and October 2016 and who underwent endoscopic biopsy for *H. pylori* infection with the pre-diagnosis of non-ulcer dyspepsia and who were found positive for *H. pylori* infection in biopsy were retrospectively evaluated in the study.

Biopsy specimens from both antrum and corpus were sent for histopathological evaluation. *H. pylori* status was assessed by pathologists using hematoxylin-eosin and Giemsa staining procedures. Two hundred and five patients who received bismuth based quadruple therapy and sequential therapy for *H. pylori* eradication were included in the study. The written informed consent of the patients' was not received due to archival scan and retrospective study. Patient information was obtained from hospital information system and/or patient files. Patients who had stomach malignancy, gastric surgery history, previous *H. pylori* eradication therapy and who were under 18 years of age were excluded from the study. The study was approved by Non-invasive Clinical Research Ethics Committee of Adnan Menderes University Medical Faculty (protocol no: 2016/1009).

Treatment Procedures

Bismuth based quadruple therapy group (group 1) was given pantoprazole 2x40 mg (14 days), bismuth subcitrate 4x300 mg (14 days), amoxicillin 2x1000 mg (14 days) and metronidazole 3x500 mg (14 days). Bismuth based sequential therapy group (group 2) was given pantoprazole 2x40 mg (14 days), bismuth subcitrate 4x300 mg (14 days), amoxicillin 2x1000 mg (first 7 days), metronidazole 3x500 mg (second 7 days), levofloxacin 1x500 mg (second 7 days). Eradication was assessed in the stool by *H. pylori* Antigen test after six weeks of treatment, in the period without taking PPI. The stool assay used was the commercially available diagnostic *H. pylori* Antigen Rapid test

(Cer Test Biotec S.L. Zaragoza, Spain). Successful eradication was defined as Negative Stool test while eradication failure as Positive Stool test.

Statistical Analysis

Compliance of age with normal distribution was assessed by Kolmogorov-Smirnov test. Descriptive statistics were shown as mean (25^{th}-75^{th} percentile) since it did not show normal distribution. Mann-Whitney U test was applied to compare the groups for age variable. The descriptive statistics of categorical variables were expressed as number (percentage). *H. pylori* eradication rates were assessed with per-protocol (PP) and intention-to-treat (ITT) analyzes. Chi-square analysis was used for comparison with respect to the groups. P<0.05 was considered statistically significant.

Results

In our retrospective study, 193 (94.1%) of the 205 patients completed the provided treatment. Twelve patients (5.9%) failed to complete the treatment due to gastrointestinal system (GIS) intolerance and non compliance with drugs. GIS intolerance was detected in 4 patients of group 1 and 5 patients of group 2 while drug incompatability in 3 patients of group 2. Demographic data of the patients are shown in table 1. Hundred and two patients in group 1 and 91 patients in group 2 completed treatment and there was no significant difference between the two groups (p=0.310).

Eradication was achieved in 95 patients in group 1 and 78 patients in group 2. Eradication rates of *H. pylori* in ITT and PP analysis were 89.6% and 93.1% in group 1, 78.8% and 85.7% in group 2, respectively. There was no significant difference between the two groups in terms of PP eradication rate (p=0.093). Group 1 regimen reached a better eradication rate in ITT analysis than group 2 and this was statistically significant (p=0.033) (Table 2).

Discussion

H. pylori colonization was estimated to increase gastric cancer risk by approximately ten times and determined as a class I carcinogen by the World Health Organization (10). The most commonly used treatment regimen for *H. pylori* worldwide is the triple combination of PPI, amoxicillin, and clarithromycin. However, *H. pylori* eradication rates with this triple treatment regimens decreased significantly in recent years (11,12). *H. pylori* resistance rates against antibiotics are increasing in most parts of the world. The widespread use of clarithromycin against both *H. pylori* and other bacterial infections (especially upper and lower respiratory tract infections) raised concerns regarding clarithromycin resistance against *H. pylori*, which requires to reconsider the effectiveness of clarithromycin regimen for *H. pylori* eradication. In a study in Japan, the rate of resistance to clarithromycin increased from 8.7% to 34.5% from 1997 to 2008 (13). In various studies conducted in our country, amoxicillin resistance was reported as 0%, clarithromycin resistance as 18.2-41.9%, metronidazole resistance as 35.5-45.5%, tetracycline resistance as 0-9.1%, and levofloxacin resistance as 18.2-29.5% (14-17).

Table 1. Demographic data of the patients

Parameter	Bismuth based quadruple therapy group (Group 1)	Bismuth based sequential therapy group (Group 2)	p
Number of patients, n	106	99	-
Median age (years)	45.8±11.8	44±12.7	NS
Gender			
Male, n (%)	46 (43.4)	41 (41.4)	NS
Female, n (%)	60 (56.6)	58 (58.6)	
Number of patients who completed therapy, n (%)	102 (96.2)	91 (91.9)	NS
Reason to discontinue therapy			
GIS intolerance (n)	4	5	NS
Drug incompatability (n)	0	3	

n: Number, NS: Non-significant, GIS: Gastrointestinal system

Table 2. Comparison of eradication rates between study groups			
	Group 1	**Group 2**	**p**
ITT analysis	95/106	78/99	0.033
n/N			
%	89.6	78.8	
(95% CI)	(83.8-95.4)	(70.8-86.9)	
PP analysis	95/102	78/91	0.093
n/N1			
%	93.1	85.7	
(95% CI)	(88.2-98)	(78.5-92.9)	

ITT: Intention-to-treat, PP: Per-protocol, CI: Confidence interval, n: Number of patients who were eradicated, N: Total number of patients, N1: Number of patients who completed the study

In regions with a resistance rate of clarithromycin exceeding 15%, it is advised in the Maastricht V Consensus report that, triple treatment with PPI-clarithromycin should be discontinued unless clarithromycin resistance is verified by a preliminary susceptibility testing and bismuth quadruple or non-bismuth quadruple concomitant treatments should be given. In addition, bismuth quadruple therapy is recommended as the first-line treatment in areas with high dual resistance to clarithromycin and metronidazole (8).

Bismuth is known to have cytoprotective effects in addition to antimicrobial effects on *H. pylori* in the gastric mucosa. Up to now, resistance against bismuth has not been reported. Mechanisms of bismuth activity against *H. pylori* is not fully known. Bismuth shows its anti-helicobacter activity by forming complexes in the bacterial wall and periplasmic space, by inhibiting different enzymes of *H. pylori* such as urease, fumarase, phospholipase, by inhibiting adenosine triphosphate synthesis of bacteria, and by preventing adhesion of *H. pylori* onto the gastric mucosa (18). There is a synergy between bismuth salts and antibiotics. For example, when metronidazole and bismuth are used together, metronidazole-resistant *H. pylori* strains become susceptible (19). For this reason, we preferred to administer bismuth in both treatment groups. Different combinations of antibiotics are used in bismuth based quadruple treatments. In our study, we obtained ITT eradication rates of 89.6% and PP eradication rates of 93.1% with bismuth based quadruple treatment regimen, which is acceptable for *H. pylori* eradication. In different studies from our country, eradication rates for bismuth based standard quadruple treatment regimens were reported as 81.1% and 74.6% in ITT analysis and 86% and 75.6% in PP analysis, respectively (20,21). Unlike these studies, we preferred to use amoxicillin instead of tetracycline. Another study used the same therapy regimen as ours and obtained similar eradication rate of 93.9% in PP analysis (22). The period of treatment in these studies was 14 days. We also preferred a 14 days of treatment on both arms of our study. The Maastricht V Consensus report also recommends extending the duration of bismuth-based quadruple treatment to 14 days, unless 10-day treatments are locally proven effective (8).

There are various reports about levofloxacin-containing regimens, and the most important concern about the future of these regimens is the potential antibiotic resistance. Eradication success with sequential treatment regimens containing levofloxacin was reported as 81.3%and 78.0% in Korea (23) and as 98.4% and 96.8% in Italy (24), in PP and ITT analysis, respectively. Levofloxacin resistance in Turkey was reported between 18.2% and 29.5% (14-17). Using sequential levofloxacin therapy, Aydın et al. (25) achived 82.5% eradication with ITT analysis and 86.7% eradication with PP analysis while Polat et al. (9) achieved 90.2% and 86.6% eradication rates, respectiveley. In addition to these regimes, we used bismuth in group 2. Our eradication rates in group 2 were 78.8% with ITT analysis and 85.7% with PP analysis, which were similar to other studies. Four patients in group 1 and 5 patients in group 2 could not complete the treatment because of GIS intolerance

while 3 patients in group 2 due to drug incompatibility. However, the difference between the groups was not statistically significant. The main limitations of our study are the retrospective design and the absence of investigation on antibiotic resistance. Prospective studies involving also antibiotic susceptibility results may help to improve eradication success.

Conclusion

As a result, we achieved better eradication rate with bismuth quadruple treatment compared with bismuth sequential treatment in our study. Due to the drug incompatibility observed during sequential treatment, we recommend bismuth based quadruple therapy regimen as the first line eradication treatment.

Ethics

Ethics Committee Approval: The study was approved by Non-invasive Clinical Research Ethics Committee of Aydın Adnan Menderes University Medical Faculty (protocol no: 2016/1009).

Informed Consent: The written informed consent of the patients' was not received due to archival scan and retrospective study.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: A.C., Design: A.C., Data Collection or Processing: M.Ç., Analysis or Interpretation: M.Ç., Literature Search: A.K., Writing: A.C., A.K.

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

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Original Article / Özgün Araştırma

Investigating the Effect of Rosuvastatin, Paracetamol and Co-administration of Rosuvastatin and Paracetamol on Ocular Tissue

Statin, Parasetamol ve Statin-parasetamol Beraber Kullanımının Göz Dokularına Etkisinin Araştırılması

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IOP, acetaminophen, rational drug use, rat, statin

Anahtar Kelimeler
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Abstract

Objective: Statins and Paracetamol are two drugs that have a high prescription rate all over the world. Possible side effects can easily be augmented because they use the same cytochrome oxidase enzymes in liver. This study aimed to investigate the effect single or combined administration of these drugs on ocular tissues.

Materials and Methods: Twenty-eight 12- to 15-month-old rats were divided in four groups: Control, Rosuvastatin (10 mg/kg/day for 7 times a week), Paracetamol (50 mg/kg/day for 5 times a week) and Rosuvastatin (10 mg/kg/day for 7 times a week) + Paracetamol (50 mg/kg/day for 5 times a week) for 8 weeks. At the end of study, intraocular pressure (IOP) was measured and ocular tissues were obtained for histopathological evaluation under anaesthesia with Ketamine and Xylasine (50 mg/kg and 5 mg/kg, respectively).

Results: Rosuvastatin showed an IOP dropping effect and paracetamol did not prevent it. Histopathological evaluation mainly revealed retinal nerve fibre layer degeneration. Additionally, different pathological alterations such as corneal oedema and polypoid proliferation were observed in all the treated groups, although they were rare.

Conclusion: The IOP dropping effect of rosuvastatin shows that it is safe in glaucoma patients, but this beneficial effect was not observed with Paracetamol. Retinal nerve fibre layer degeneration with both drugs might be one of the reasons for visual disturbances in real life conditions.

Öz

Amaç: Statinler ve Parasetamol, tüm dünyada en çok reçete edilme oranına sahip ilaçlardan ikisidir. Olası yan etkileri, karaciğerde aynı sitokrom enzimini kullanmasına bağlı olarak kolaylıkla artabilir. Bu çalışmayla her bir ilacın ayrı ayrı ya da birlikte kullanımı sonucunda göz dokusuna olan etkisi incelendi.

Gereç ve Yöntemler: On iki - on beş aylık 28 sıçan 4 gruba ayrıldı: Kontrol, Rosuvastatin (10 mg/kg/gün, haftada 7 kez), Parasetamol (50 mg/kg/gün, haftada 5 kez) ve Rosuvastatin (10 mg/kg/gün, haftada 7 kez) + Parasetamol (50 mg/kg/gün, haftada 5 kez) 8 hafta boyunca uygulandı. Çalışmanın sonunda, Ketamine ve Ksilasin (50 mg/kg and 5 mg/kg) anestezisi altında, göz içi basıncı (GİB) ölçülüp, göz dokuları histopatolojik inceleme için alındı.
Bulgular: Statin GİB düşürücü etkiye sahiptir ve bu etki Parasetamol ile engellenmemektedir. Histopatolojik inceleme başlıca retinal sinir lifi tabakası hasarını ortaya çıkardı. Buna ek olarak, korneal ödem ve polipoid proliferasyon gibi farklı patolojik değişimler tüm tedavi gruplarında görüldü, ancak nadirdi.
Sonuç: Statin GİB düşürücü etkisi bu ilacın glokom hastaları için güvenli olduğunu gösterir, ancak bu faydalı etki Parasetamol'de belirlenmedi. Her iki ilaç ile belirlenen retinal sinirde hasar daha çok araştırma gerektirir; vizüel şikayetlerin bir nedeni olabilir.

Introduction

Statins, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, are widely prescribed for their cholesterol-lowering properties. In addition to lipid-lowering properties, it has been stated that statins have pleiotropic effects including anti-inflammatory, anti-apoptotic and antiproliferative effects (1,2). It has been suggested for several ophthalmic conditions including age-related macular degeneration, glaucoma, diabetic retinopathy and uveitis (2), although some ocular side effects has been reported such as blurred vision, visual impairment, visual field defect, reduced visual acuity, myopia, hypermetropia, presbyopia,and astigmatism which might be associated with muscle or liver problems (3).

Paracetamol (Acetaminophen, APAP) is one of the most common analgesics and antipyretics applied in health care and oral or intravenous routes can be administered at various stages of the pain treatment: pre-emptive, post-operative, and chronic pain (4). It is consumed as an over-the-counter medicine in many countries. Its responsible metabolism is cytochrome oxidase enzyme systems similar to statins and its highly toxic metabolite N-acetyl-p-benzoquinonimine (NAPQI) experimentally induced cataract formation has been reported (5).

Statin treatment is quite high in clinics and these patients have exposures many drugs together, such as APAP. Therefore, in this study we evaluated possible drug interactions between Rosuvastatin (RSV) and APAP on ocular tissues clinically and histopathologically.

Materials and Methods

A total of 28 male rats (12-15 months old) were obtained from Experimental Animal Center of University and all applicable international, national and institutional guidelines for the care and use of animals were followed.

The reason, statins are mostly used after middle age; we used 12-15 months old rats (their life span is approximately 26-28 months) for our study. The study mainly planned to assess possible hepatic and renal adverse drug interaction of RSV and APAP and supported by the grant of The Scientific and Technological Research Council of our country (TUBITAK 114S567). To decrease the animal number used in medical researches, the remained eye tissues have been evaluated after taking relevant ethical approval with this study. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution (HADYEK 64583101/2014/076).

The rats were randomized into 4 groups (seven in each group):

Control group; healthy, no drug was applied.

RSV group; was given 10 mg/kg RSV/daily with drinking water 7 times a week for 8 weeks.

APAP group; received 50 mg/kg paracetamol through intraperitonal injection, 5 days a week for eight weeks.

RSV+APAP group; was applied 50 mg/kg paracetamol through intraperitonal injection, 5 days a week for eight weeks and 10 mg/kg RSV with drinking water at the same time.

The rats were balanced every monday and the doses of RSV and APAP were adjusted for every cage. While giving the drugs, we did not want to use toxic doses, tried to mimic daily posology. At the end of experiment, under the anesthesia with Ketamine and Xylasine (50 mg/kg and 5 mg/kg, respectively), intraocular eye pressure (IOP) were measured with a Schiotz indentation tonometer three times for both eyes by the same masked researcher, and both eye tissues were harvested and kept in 10% formalin solution.

After routine tissue following, the obtained samples were sliced in 5 micrometer thickness and stained by hematoxylin-eosin. Histopathological examination was performed by using a light microscope (Zeiss Primo Star, Ankara, Turkey). Cornea, conjunctiva, retina and vascular structures were detected.

Statistical Analysis

The normality of IOP variable was analyzed with Kolmogorov-Smirnov test. The statistical evaluation was assessed with Kruskall-Wallis. Descriptive statistics were presented as median (interquartile range) and p values below 0.05 were considered significant.

Results

IOP of control rats were 11 (3.25) mm/Hg; RSV 7 (5) mm/Hg; APAP 14 (4) mm/Hg and co-administration of RSV+APAP 6.5 (4) mm/Hg. While APAP did not affect IOP (p=1.000), RSV dropped the IOP significantly (p<0.001) and this RSV effect did not prevented by co-administration of APAP (p<0.001). The comparison of IOP measurements between groups were summarized on Table 1.

Histopathological analyses of all groups did not show uniform pathological phenomenon. Some examples of findings have photographed in Figure 1. Control groups photographs of cornea and retina have been shown in Figure 1A (X40 magnification) and Figure 1B (X10 magnification). Corpora amylacea formation was present in all groups, one of examples in Figure 1C is belong to APAP group (X40 magnification). Degeneration of retinal nerve fiber layer has seen in RSV, APAP and RSV+APAP group (Figure 1D, E and F, respectively, X10 magnification). Neovascularization in Retinal nerve fiber layer (D X10 magnification) and cornea (G X10 magnification) with RSV; vascular endothelial polypoid proliferation in RSV+APAP (H X10 magnification), corneal edema in RSV (J X40 magnification) were the other rare findings. Corneal edema and thickness, conjunctival epithelial degeneration, retinal nerve fiber layer and vascular structure alteration numbers are as shown in Table 2.

Discussion

This study has been mainly planned to investigate APAP exposure during statin treatment as a "rational drug therapy" approach. Liver toxicity of statins is well known, but there was not any satisfactory report about adverse effects of co-administration of APAP which is another liver toxic agent. Meanwhile, we extended our study to ophthalmic effects. Highly toxic metabolite of APAP, NAPQI, consumes not only liver

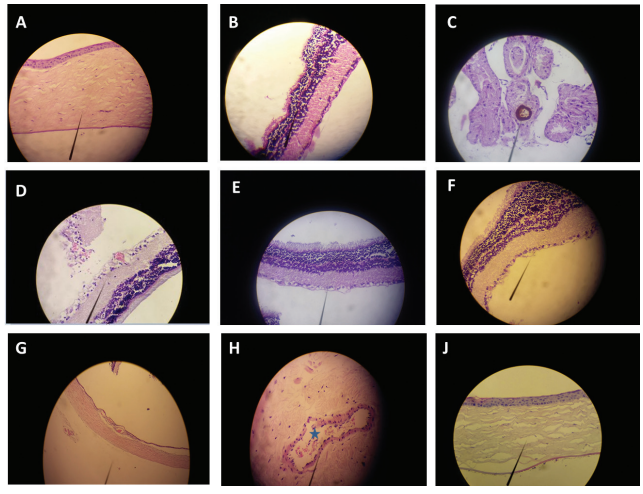

Figure 1. Some examples of histopathological findings in all groups. Control groups' cornea and retina in A (X40 magnification) and B (X10 magnification) respectively; Corpora amylacea formation in APAP group (C X40 magnification); Retinal nerve fiber layer degeneration in RSV, APAP and RSV+APAP group (D, E and F, respectively, X10 magnification); Neovascularization in RSV (G X10 magnification), vascular endothelial polypoid proliferation (blue star) in RSV+APAP (H X10 magnification), corneal edema in RSV (J X40 magnification)

RSV: Rosuvastatin; APAP: Paracetamol (Acetaminophen)

Table 1. Intraocular pressure evaluation of ocular tissues in all experimental groups

Group vs Group	IOP			p
Control vs Rosuvastatin	11 (3.25)	vs	7 (5)	<0.001
Control vs APAP	11 (3.25)	vs	14 (4)	1.000
Control vs Rosuvastatin + APAP	11 (3.25)	vs	6.5 (4)	<0.001
Rosuvastatin vs APAP	7 (5)	vs	14 (4)	<0.001
Rosuvastatin vs Rosuvastatin + APAP	7 (5)	vs	6.5 (4)	1.000
APAP vs Rosuvastatin + APAP	14 (4)	vs	6.5 (4)	<0.001

APAP: Paracetamol (Acetaminophen), IOP: Intraocular pressure

Table 2. Histopathological evaluation of ocular tissues in all experimental groups. Numbers show the pathological sample numbers

	Control	Rosuvastatin	APAP	Rosuvastatin + APAP
Corneal thickness	0	1	1	2
Corneal edema	3	7	4	5
Conjunctival epithelial degeneration	0	3	0	0
Retinal nerve fibril layer degeneration	0	6	5	2
Endothelial polypoid proliferation	0	0	0	1
Corpora amylacea in lacrimal gland	2	2	2	2

APAP: Paracetamol (Acetaminophen)

glutathione, but also tissue levels as well. It has been shown that there is not any significant difference of pharmacokinetic parameters between concentrations of Paracetamol and Paracetamol-glucuronide in the plasma and aqueous humour (4). On the other hand, one of the earlier studies demonstrated morphological changes in the retina of rats following direct intra vitreal injection of lovastatin (0.25 micromol in 7.5 microlitre of 10 mM, Tris-HCl, pH 7.4). This study described degeneration of photoreceptor inner and outer segments, rosette formation, and the appearance of debris-filled macrophages (6); it led us to expand our research on ocular tissue and performed histopathological analyses as well. Therefore, the first question was to understand individual side effect of APAP and statin, and second question was the outcome of these agents co-administration on ocular tissues in chronic usage.

Here, the prominent clinical finding of this study is RSV treatment has 32.84% IOP decreasing effect on healthy rats; this beneficial effect was not obtained with APAP and APAP did not prevent statins' IOP dropping effect. Contrary, a clinical study demonstrated that 1 g orally given APAP for 2 weeks significantly reduced IOP of 9 open angle glaucoma patients (7); but, Jampel HD study supports our findings. He planned a small randomized clinical trial with 10 glaucoma patients, he gave 650 mg APAP four times a day for 7 days, did not get any significant IOP lowering effect of APAP (8). On the other hand, statins IOP dropping effect is previously reported clinical glaucoma study (1). Our study have shown that this effect can be seen on healthy subjects too, without depending on lipid lowering or any suggested impact on vascular system; at some point, statin treatment might have prophylactic effect if the patient takes any statin therapy for any reason.

Searching of the literatures, histopathological side effects of these two medications are very rarely studied and reported. In our study, the most important histopathological finding is degeneration of retinal nerve fiber layer in all treatment groups. For statin, this finding is contradictory of previous experimental and observational studies that it has been suggested neuro-protective on ocular diseases (1). A report for Lovastatin and Simvastatin with 150 patients was provided good evidence that there was not any toxicity finding on lens and other ocular tissues (9). Contrary, a detailed retrospective analyses of statin users indicate that 1.6% (301/18.395) RSV patients developed ocular side effects, including blurred vision, visual impairment and reduced visual acuity between 1988 and 2013, but this study could not performed an adjustment of age (3). The prevalence of visual disturbances of statin seems highly low, but at some point this might be explained with shrinking of retinal nerve fiber layer that we have found in our study. On the other hand, for APAP, Nassini et al. (10) proved that NAPQI metabolite is responsible of releasing sensory neuropeptides that mediates neurogenic inflammation in conjunctiva at therapeutic doses. This paper might support our observations that chronic APAP treatment results in degeneration of retinal nerve fiber layer of six animals out of seven. So, damaging of retinal nerve fiber layer with both drugs should take more attention; it can bring some clinical important side effects.

Corpora amylacea formation has seen in all groups as a common findings and this thought to be because of the animals' age. Although, edema has seen even

in control group, the medications are increased its forming too. Previously, histopathological analysis of rabbit eyes treated with topical application of APAP shown an edematous cornea (11), we have given systemic administration and detected four rats have corneal edema and one rat with corneal thickness in out of seven. Conjunctival epithelial degeneration was only seen with RSV in three animals out of seven. Endothelial polypoid proliferation has determined only in one rat from the combine treatment group. Considering that we have determined neovascularization in retinal nerve fiber layer in one rat and in cornea in another rat with RSV, this endothelial polypoid proliferation might be related with this structural changing. However, we would like to emphasize that these pathological findings are really rare, therefore we are not sure about their presence are due to the medications. Beside the beneficial reports of statins, retrospective 95 case reports analyzed, although, some patients also received medications known to increase bleeding times, it is suspected ocular hemorrhage is ''possibly'' due to statin therapy (12). We could not determine any bleeding focus on the ocular tissue layers. APAP metabolite NAPQI, has induced cataract formation experimentally (5), we could not detect any lens tissue changing as well with the chronic administrations of medications.

Conclusion

Statin IOP dropping effect shows this medication is safe in glaucoma patient, but we cannot see this beneficial effect with Paracetamol. Retinal nerve fiber layer degeneration with both drugs needs more attention; it might be one of the reasons of visual disturbances.

Ethics

Ethics Committee Approval: Aydın Adnan Menderes University for Animal Experiments (HADYEK 64583101/2014/076).

Informed Consent: This is an animal experiment study.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: A.G., B.D., Design: A.G., B.D., Data Collection or Processing: A.İ.A.Ü., F.K., B.D., Analysis or Interpretation: A.İ.A.Ü., F.K., Y.Ö., B.D., Literature Search: A.İ.A.Ü., F.K., B.D., Writing: A.İ.A.Ü., B.D.

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Original Article / Özgün Araştırma

The Relationship Between Monocyte Count to High-density Lipoprotein Ratio and Severity of Inflammation in Aggressive Periodontitis: A Retrospective Analysis

Agresif Periodontitis Hastalarında Enflamasyon Şiddeti ile Monosit-yüksek Yoğunluklu Lipoprotein Oranı Arasındaki İlişki: Retrospektif Analiz

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Abstract

Objective: Hyperlipidemia is a known cardiovascular disease risk factor. A common biological mechanism between systemic diseases, such as cardiovascular diseases, and periodontal diseases has been suggested. This study aimed to examine the association between blood lipid profile and the severity of aggressive periodontitis (AgP).

Materials and Methods: A total of 32 systemically healthy patients with AgP (24 females and 8 males; mean age 34.78±6.42 years) were consecutively selected. The patients were divided into 2 groups as localised AgP (LAgP) and generalised AgP (GAgP). Monocyte counts, high-density level (HDL) count and monocyte/HDL ratios (MHR) were recorded from the patients' blood samples. Mean Plaque index, bleeding on probing, periodontal pocket depth and clinical attachment level were recorded before treatment. The relationship between monocyte to HDL ratios and clinical attachment level between the groups were analysed by Spearman correlation.

Results: Sixteen GAgP and 16 LagP patients were included in the study. The mean MHR values for the LAgP and GAgP groups were 9.87±2.85 and 13.43±4.64, respectively; there was a statistically significant difference between the groups ($p<0.05$).

Conclusion: The increased MHR values in patients with severe periodontitis may be one of the pathogenic mechanisms underlying the link between periodontal and cardiovascular diseases. Although we observed statistically significant differences between the MHR values in both groups, a larger sample size is needed to confirm the results of this study in the future.

Öz

Amaç: Hiperlipidemi, kardiyovasküler hastalıklar için bilinen bir risk faktörüdür. Kardiyovasküler hastalıklar gibi sistemik durumlar ile periodontal hastalıklar arasında ortak bir biyolojik mekanizma olduğu gösterilmiştir. Bu çalışmanın amacı, kan lipid profili ile agresif periodontitis (AgP) şiddeti arasındaki ilişkiyi incelemektir.

Gereç ve Yöntemler: AgP tanısı almış toplam 32 sistemik sağlıklı hasta (24 kadın ve 8 erkek; ortalama yaş: 34,78±6,42) çalışmaya dahil edildi. Hastalar lokalize (LAgP) ve generalize agresif periodontitis (GAgP) olarak iki gruba ayrıldı. Hastalardan alınan kan örneklerinde monosit sayıları, yüksek yoğunluklu lipoprotein seviyesi (HDL) sayımı ve monosit/HDL oranları (MHR) kaydedildi. Ortalama plak indeksi, sondlamada kanama, periodontal cep derinliği ve klinik ataşman seviyesi tedavi öncesinde kaydedildi. Monosit ile HDL oranları arasındaki ilişki ile gruplar arasındaki klinik bağlanma düzeyi arasındaki ilişki Spearman korelasyonuyla analiz edildi.
Bulgular: On altı GAgP ve 16 LagP hastası çalışmaya dahil edilmiştir. LAgP ve GAgP grupları için ortalama MHR değeri sırasıyla 9,87±2,85 ve 13,43±4,64 olarak bulunmuştur ve gruplar arasında istatistiksel olarak anlamlı bir fark bulunmuştur ($p<0,05$).
Sonuç: Şiddetli periodontitisli hastalarda MHR değerlerinde artış, periodontal hastalık ve kardiyovasküler hastalıklar arasındaki bağlantıların altında yatan patojenik mekanizmalardan biri olabilir. GAgP grubu arasında MHR değerlerinde istatistiksel olarak anlamlı farklılıklar gözlemlemiş olsak da, bu çalışmanın sonuçlarını doğrulamak için daha büyük bir örneklem büyüklüğü ile gelecekte yapılacak araştırmalara ihtiyaç vardır.

Introduction

Periodontitis is a chronic inflammatory disease caused by microbial-induced host-related inflammation, resulting in connective tissue and alveolar bone loss. Periodontal pathogens produce a local inflammatory response, including the exudation and migration of a large number of leukocytes (1,2). Inflammatory response in the first line of defense against bacterial pathogens, such as monocytes/macrophages, neutrophils, lymphocytes that are amplified due to pro-inflammatory mediators produced by various cells. The release of these substances into the bloodstream can trigger a host response with significant systemic inflammation (3).

Aggressive periodontitis (AgP) can lead to an improved host response and metabolic changes, such as increased C-reactive protein (CRP) serum level. In addition, chronic systemic inflammatory stimulation by untreated periodontal disease has been shown to increase neutrophil and lymphocyte levels in patients (3).

Studies have shown that systemic inflammation associated with periodontitis may be associated with the pathogenesis of inflammatory diseases such as atherosclerosis and cardiovascular disease (CVD) (4,5). Although chronic inflammation plays a clear role in the pathogenesis of both diseases, it has been reported that changes in lipoproteins and lipoprotein metabolism may be important. Periodontal disease modifies lipoprotein metabolism to provoke atherosclerosis and CVD (6). Although chronic inflammation causes various changes in serum lipid and lipoprotein levels, one of the most known changes is a decrease in high-density lipoprotein cholesterol (HDL-C) level (7). In addition to the reduction in HDL-C level, chronic inflammation also causes changes in the composition of HDL-C (8). Changes in inflammation-induced HDL-C reduce the ability of HDL-C to participate in reverse cholesterol, low-density lipoprotein (LDL) transported and protected from oxidation. Generalized AgP (GAgP) patients have a higher atherogenic lipoprotein profile than control groups (9).

The increased number of monocytes is associated with atherosclerosis and the HDL-C molecule may prevent the activation and proliferation of monocytes. It has been shown that the monocyte count/HDL-C ratio (MHR) may be superior to the individual monocyte count or HDL-C level, and that increased MHR may be a predictive marker for the development and progression of atherosclerosis and therefore for cardiovascular events (10). Stable coronary artery disease (CAD) in order to estimate the severity of coronary atherosclerosis in patients with CAD, the newly introduced MHR can be used to predict the severity of inflammation, however, there is no data on the relationship of this parameter to the severity of periodontitis in the literature review.

AgP is a serious form of periodontitis characterized by rapid attachment loss and bone destruction in healthy adolescents and young adults (11). Research has shown some mechanisms in the pathogenesis of AgP, such as specific microbial environments, hypersensitive monocytes and macrophages that increased inflammatory mediator levels, specific genetic risk factors and altered host defense are not associated with a specific systemic marker with periodontal destruction.

The aim of this study was to determine the relationship between the severity of periodontal inflammation and MHR in AgP patients, as a result of the established relationship between periodontitis and atherosclerosis and the severity of periodontitis.

Materials and Methods

Between May 2018 and November 2018, AgP patients admitted to Kütahya University of Health Sciences Turkey, Faculty of Dentistry, Department of Periodontology were included in the study. Patients were enrolled according to the following criteria: at least 18 years of age, no pregnancy, lack of systemic disease (endocrinological disorders such as autoimmune disease, diabetes) and bisphosphonates. Before the study, clinical procedures and blood sampling were explained and all subjects gave written consent in accordance with the 1975 Declaration of Helsinki, which was revised in 2000. The study protocol was approved by the Local Ethic Committee (decision no: 2019/03).

Blood values and Periodontal index values that were requested before treatment were evaluated retrospectively on the data of AgP patients who applied to our clinic in 2018. Periodontal indices and orthopantomograph data were retrospectively analyzed. Periodontal clinical measurements included the following indices: plaque index (PI) (12), probing bleeding (BOP), probing depth (PD) and clinical attachment level (CAL). Clinical parameters were measured with a Williams probe calibrated in millimeters at four sites per tooth (mesio-buccal, buccal, disto-buccal and lingual).

Patients with periodontitis were diagnosed with AgP according to the 1999 Consensus Periodontal Disease Classification in accordance with the latest Consensus report on the identification of cases of periodontitis (11-13). We have identified patients with AgP as evidence of a healthy condition (except periodontitis), familial aggregate, rapid binding loss and bone destruction. AgP patients were classified as localized AgP (LAgP) or GAgP with the number of affected teeth (LAgP: first molar/cutter tooth presentation localized with interproximal binding loss on at least two permanent teeth, one of which was the first molar) and not including more than two teeth except for the first molar and incisor teeth: GAgP: loss of generalized interproximal attachment affecting at least three permanent teeth other than the first molar and incisor teeth.

Statistical Analysis

The data were analyzed using the software program (SPSS for Windows v. 20.0 Statistical Package, USA). All parameters were analyzed by Kolmogorov-Smirnov test to determine normal or abnormal distribution. Student's t-test was used for normal distribution and Mann-Whitney U test was used for abnormal distribution. The relationships between PD, CAL parameters and MHR values were evaluated using Pearson correlation coefficients (r). To demonstrate the sensitivity and specificity of MHR, as well as the cut-off value to estimate the severity of the periodontal disease, the recipient study characteristics [receiver operating characteristic (ROC)] curve was used. p value less than 0.05 was considered statistically significant. The power was calculated 80% when there was 17 patient per each AgP group, using the α value as 0.05 (14).

Results

Initially, 33 patients with AgP were included in this study. A subject was excluded from the study because of the diagnosis of Von Willebrand's disease. A total of 32 systemic healthy patients (24 females and 8 males; mean age 34.78±6.42 years) with AgP, 16 with LAgP and 16 with GAgP were consecutively selected. Two patients were smoking in each AgP group. Table 1 summarizes the demographic data of the patients included in the study groups.

There were statistically significant differences between the LAgP and GAgP groups for PI, BOP, PD and CAL. The GAgP group had more severe clinical index measurements than the LAgP group. Patients with GAgP had significantly higher monocyte counts than patients with LAgP. HDL-C levels did not show a statistically significant difference between the groups (Table 2).

The mean MHR values for the LAgP and GAgP groups as shown in Table 3 were 9.87±2.85 and 13.43±4.64, respectively. For this value, there was a statistically significant difference between the AgP groups (p=0.015).

Table 1. Demographic data

Group	GAgP (n=16)	LAgP (n=16)
Age (year)	35.19±5.97	34.38±7.01
Female/male (%)	12/4 (75%/25%)	12/4 (75%/25%)
Smoking (10> per a day)	2	2

GAgP: Generalized aggressive periodontitis, LAgP: Localized aggressive periodontitis

ROC analysis was performed to determine MHR cut-off value. This value in the admission for the severity of predictive disease in the study group was 9.88 with 0.75% sensitivity and 0.5% specificity (area below the curve was 0.734, p=0.024) (Figure 1).

Spearman correlation analysis did not show a significant relationship between the CAL parameter and the MHR value (r=0.315, p=0.080).

Discussion

Monocytes are the most important sources of pro-inflammatory and antioxidant cytokines in inflammatory sites (10). In contrast, HDL-C has anti-inflammatory and anti-oxidant effects. It is thought that MHR may be used as a new marker for predicting CVD severity due to combining two detrimental processes such as inflammation and oxidative stress (10,15,16). MHR is positively correlated with CRP levels and its role in systemic inflammation is supported by studies presented (10,17). Recently, MHR has been reported to be a new marker for major adverse outcomes in heart disease (15,18).

To our knowledge, this study is a pilot study showing the relationship of MHR with AgP severity. In this cross-sectional study, the association of MHR values with disease severity in generalized and localized AgP patients was evaluated. In the GAgP group, MHR values were significantly higher and MHR values greater than 9.88 were found to be a risk markers for GAgP.

One study reported that increased PD is significantly associated with elevated CRP concentrations, and that the surface area or volume of periodontal lesion in AgP patients is the most determinant of serum CRP levels and therefore a correlation between periodontal destruction and systemic inflammation is supported (2).

According to Nibali et al. (19) , both the leukocyte counts and the serum lipid levels of the subjects participating in the study were related to their periodontal status. A trend was observed for dose dependence of periodontitis as an exposure: higher inflammatory (leukocyte counts) and metabolic markers (LDL levels) appeared in people with more general disease. D'aiuto et al. (3) in patients with periodontitis, the number of leukocytes increased in relation to the presence of deep periodontal pocket.

Table 2. Clinical and biochemical measurements on baseline

	GAgP (n=16)	LAgP (n=16)	p
PI	1.56±0.56	1.07±0.59	0.022*
PD	4.17±0.69	3.02±0.49	0.000*
BOP (%)	89.37±10.28	63.06±23.48	0.000*
CAL	5.12±0.69	3.60±0.97	0.000*
Monocyte (x10^3)	0.58±0.17	0.43±0.09	0.017*
HDL-C (mg/dL)	45±10.31	45.38±10.02	0.918

PI: Plaque index, PD: Probing depth, BOP: Bleeding on probing, CAL: Clinical attachment level, HDL-C: High-density lipoprotein Count, GAgP: Generalized aggressive periodontitis, LAgP: Localized aggressive periodontitis Mann Whitney U analyes were used

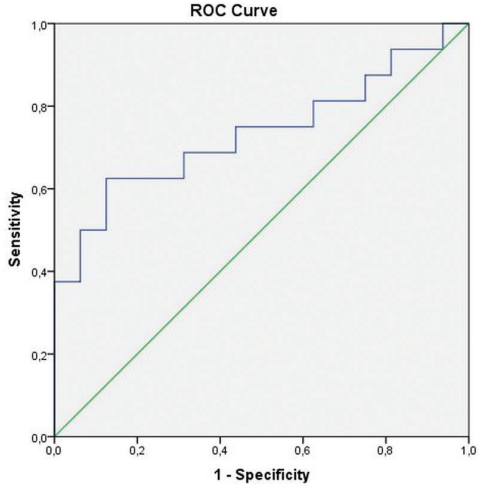

Figure 1. Area under the curve analysis
ROC: Receiver operating characteristic

Table 3. Monocyte count to high-density lipoprotein ratio

	GAgP (n=16)	LAgP (n=16)	p	95% confidence interval of the difference	
				Lower	Upper
MHR ratio	13.43±4.64	9.87±2.85	0.015	6.3675	0.7474

GAgP: Generalized aggressive periodontitis, LAgP: Localized aggressive periodontitis, MHR: Monocyte count to high-density lipoprotein ratio

According to these findings, significantly higher monocyte counts were observed in the GAgP group of our study.

Gao et al. (20) showed that HDL-C levels of patients with GAgP were significantly lower than those of healthy controls, whereas Davies et al. (21) reported that serum lipid concentrations were not significantly different in individuals with AgP compared to controls. The discrepancy between these studies may be a result of the severity of periodontal inflammation and differences in age and ethnicity distribution. HDL-C levels were not significantly different in our study groups.

Bacterial lipopolysaccharides leading to hypersensitive phenotype resulting from the production of proinflammatory cytokines may increase serum lipid levels. Increased proinflammatory cytokines in periodontitis may contribute to the pathogenesis of hyperlipidemia (22). Kırzıoğlu et al. (23) in this study, HDL-C levels in the periodontitis group were higher than in the periodontal healthy group. It has been reported that HDL-C may become dysfunctional and its anti-inflammatory and anti-thrombotic functions decrease in inflammatory conditions and chronic diseases. Fentoglu et al. (24) showed that HDL-C levels were higher in gingivitis than in periodontitis and this difference was attributed to the role of inflammation rather than infection.

Our study has some limitations, first of all, the study had a cross-sectional design, so this study could not give a definite conclusion about the role of MHR in severe periodontal diseases. Secondly, since the effect of body weight on both inflammatory and metabolic markers is clear, we see that this variable is not a limitation in our study. Third, the number of patients was relatively small and larger studies were needed to clarify the relationship between MHR and periodontal status. Fourth, study design did not have periodontal healthy controls to compare MHR values. The lack of information on CVD and HDL-related family history, obesity, physical activity and dietary habits is an limitation of the study. Considering these conditions in future studies on MHR may give more explanatory information about obtaining MHR values.

The assumption that a relationship between AgP and CVD may be caused by genetic susceptibility to hyperinflammatory responses led our study population to the form of AgP (25). Furthermore, it was concluded that the genetic susceptibility locus was shared by both AgP and heart diseases. above mentioned study.

The result of this study, in which MHR may be affected by AgP as a new cardiovascular risk factor, may lead to significant clinical results. These findings will not only facilitate identification of persons at risk of losing their teeth due to severe periodontal disease, but will also allow early interventions to prevent or reduce the severity of CVD. Future studies will focus on the effect of periodontal treatment on MHR values.

Conclusion

MHR values in patients with severe periodontitis may be one of the underlying pathogenic mechanisms of the connections between periodontal disease and CVD. This may be particularly alarming in young individuals represented by AgP patients who may contribute to early or faster CVD in susceptible patients with periodontal disease-associated higher MHR values.

Although we observed statistically significant differences in the MHR values between the GAgP groups, a larger sample size is needed to confirm the results of this study.

Ethics

Ethics Committee Approval: The study protocol was approved by the Kütahya University of Health Sciences Turkey, Faculty of Medicine non-interventional Ethic Committee (decision no: 2019/03).

Informed Consent: Participants signed the written informed consent form.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: K.O., B.G., Design: K.O., B.G., Data Collection or Processing: B.G., K.O., Analysis or Interpretation: B.G., Literature Search: E.D., B.G., Writing: E.D.

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Original Article / Özgün Araştırma

Evaluation of Cemental Tear Frequency Using Cone-Beam Computed Tomography: A Retrospective Study

Semental Ayrılma Sıklığının Konik Işınlı Bilgisayarlı Tomografi ile Değerlendirilmesi: Retrospektif Bir Çalışma

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Cone-beam computed tomography, prevalence, cemental tear

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Abstract

Objective: Cemental tear is a clinical term referring to partial or complete separation of the cementum from the root surface. Unnecessary treatment can be applied due to its low prevalence and difficulty in diagnosis. This study aimed to determine the frequency and distribution of cemental tear using cone-beam computed tomography (CBCT).

Materials and Methods: A total of 813 CBCT images were evaluated in this retrospective study. Root fragments that were separated from the root surface on CBCT images were accepted as cemental tear. The frequency of cemental tear, tooth region, tooth type, previous treatment and periapical/periodontal lesions were assessed. A chi-square test was performed to determine the relationship between the categorical variables.

Results: The frequency of cemental tear was determined to be 1.85%. Of the patients, 51.3% were males (n=417) and 48.7% (n=396) were females. There was no significant relationship between the frequency of cemental tear and patient age, gender, tooth region, tooth type and previous treatment ($p>0.05$). In teeth with periapical/periodontal lesions, significantly more frequent cemental tears were observed ($p<0.05$).

Conclusion: Early diagnosis is essential to prevent advanced bone loss and extraction of teeth with cemental tear. Clinicians may misdiagnose teeth with cemental tears without adequately evaluating all predisposing factors, clinical features and available radiographs. Further studies with larger sample sizes could be useful to more precisely determine the predisposing factors and clinical features of cemental tear.

Öz

Amaç: Semental ayrılma, sementin kök yüzeyinden tamamen veya kısmen ayrılması anlamına gelen klinik bir terimdir. Düşük prevalansı ve teşhis zorluğu nedeniyle gereksiz tedavi uygulanabilmektedir. Bu çalışmada, semental ayrılma sıklığı ve dağılımının konik ışınlı bilgisayarlı tomografi (KIBT) kullanılarak belirlenmesi hedeflenmiştir.

Gereç ve Yöntemler: Toplam 813 KIBT görüntüsü bu retrospektif çalışmada değerlendirildi. KIBT görüntüleri üzerinde, kök yüzeyinden ayrılmış kök parçaları

semental ayrılma olarak kabul edildi. Semental ayrılma sıklığı, diş bölgesi, diş tipi, önceden uygulanan tedaviler ve periapikal/periodontal lezyon varlığı değerlendirildi. Kategorik değişkenler arasındaki ilişkiyi belirlemek için ki-kare testi kullanıldı.
Bulgular: Semental ayrılma sıklığı %1,85 olarak belirlendi. Hastaların %51,3'ü erkek (n=417) ve %48,7'si (n=396) kadındı. Semental ayrılma sıklığı ile hasta yaşı, cinsiyeti, diş bölgesi, diş tipi ve önceki tedavi arasında anlamlı bir ilişki saptanmadı (p>0,05). Periapikal/periodontal lezyonlu dişlerde anlamlı olarak daha fazla semental ayrılma gözlendi (p<0,05).
Sonuç: Semental ayrılma bulunan dişlerde ileri kemik kaybını ve dişin çekimini önleyebilmek için erken teşhis çok önemlidir. Klinisyenler tüm predispozan faktörleri, klinik özellikleri ve mevcut radyografları yeterince değerlendirmeden semental ayrılma bulunan dişlere yanlış tanı koyabilirler. Semental ayrılmanın predispozan faktörlerini ve klinik özelliklerini kesin olarak belirleyebilmek için daha büyük örneklem büyüklüğü ile yapılacak ileri çalışmalar yararlı olabilir.

Introduction

Cemental tear is a clinical term that refers to complete or partial separation of the cementum from the root surface along the cementodentinal interface or incremental line (1). Although the exact cause of cemental tear is not fully clear, it has been associated in the literature with increased age, traumatic occlusion, trauma, previous periodontal treatment, and previous root canal treatment (2,3). It has been reported that cemental tear can be confused with an endodontic or periodontal lesion and can lead to advanced bone loss if left untreated (3). Additionally, cemental tear can be confused with vertical root fracture (VRF) in endodontically treated teeth. Both pathologies are characterized by deep or isolated periodontal pockets, periodontal or periapical bone loss, gingival abscess, and the presence of a sinus tract (3-5). However, while VRF is frequently observed in endodontically treated teeth and more frequently in molars, cemental tear is observed in both vital and endodontically treated teeth and, more frequently, in the anterior teeth. In addition, while VRF is observed more frequently in the bucco-lingual direction, cemental tear is frequently observed on proximal surfaces (4-8). When a cemental tear is diagnosed accurately, it can be treated by removing the fractured part through periodontal curettage and root planning, periodontal surgery, and/or apical surgery (9). In addition, its low prevalence reduces the likelihood of noticing and diagnosing a cemental tear (3,5). In the literature, it is reported that cemental tear is often misdiagnosed, and root canal treatment is applied to the teeth due to the clinical features of cemental tear mimicking periodontal and periapical lesions (6,7). It is reported that cemental tear causes an increase in existing periodontal and periapical bone destruction when left untreated or when improperly treated (8,9). Therefore, accurate diagnosis of cemental tear is critical in the prognosis of the affected tooth (10). A definitive diagnosis of cemental tear can be made by histopathological examination of the removed fragment (9). However, studies and case reports in the literature show that prevalence was determined by periapical and panoramic radiographs (10,11). Despite the fact that the presence of a thin radiopaque fragment on the root surface suggests cemental tear on radiograph, it is difficult to make an accurate diagnosis with two-dimensional imaging (5,10). Although cone-beam computed tomography (CBCT) is recommended in the literature (11) to screen cemental tear, there is no research on this subject. Therefore, the purpose of this study is to determine the frequency of cemental tear using CBCT images of patients who were admitted to a University Dental Hospital in the Aegean region of Turkey over a period of one year and to evaluate the relationship of cemental tear with the gender, age, tooth type, and previous treatments of the patients.

Materials and Methods

Aydın Adnan Menderes University Clinical Research Ethics Committee approved this study (protocol number: ADUDHF 2019/074, date: 20.11.2019). CBCT images (Planmeca Promax 3D Mid, Helsinki, Finland) of patients who applied to our faculty's Oral and Maxillofacial Radiology Department between January 2015 and January 2019 were used in the study. Images that do not include both jaws or have poor image quality were excluded. A total of 813 CBCT images were evaluated retrospectively. Patient consent was not obtained due to the retrospective evaluation of CBCT images.

In addition to age and gender information for the patients, previous treatment experiences were obtained from the patient records. Groups were classified as aged 18-39 years, 40-59 years, and 60

years and over. Images of patients younger than 18 years old were not included in the study. The presence or absence of cemental tear in all teeth was determined through CBCT images. Root fragments that were completely or partially separated from the root surface were considered to be cemental tears (Figure 1). The frequency, mesiodistal location (buccal/labial, palatal/lingual, or proximal) and apicocoronal location (cervical one-third, middle one-third, or apical one-third) of the cemental tear as well as tooth region (maxilla or mandible), tooth type (incisor, premolar, or molar), presence or absence of previous treatment, and periapical/periodontal lesions were evaluated by an experienced oral radiologist. Evaluations were repeated by the same examiner two weeks later.

Statistical Analysis

Data were analyzed using the statistical software package SPSS v25.0 (IBM Corp., Armonk, NY). Descriptive statistics and frequency tables were used to evaluate the data. To determine the relationship between categorical variables, chi-square tests were performed. P<0.05 was considered statistically significant. The intra-examiner agreement was evaluated using the Kappa coefficient.

Results

In this study, 15,886 teeth of 813 patients were evaluated, and 1.85% (n=15) of the patients were diagnosed with cemental tear. The intra-examiner agreement for CBCT evaluations was 0.92, demonstrating almost perfect agreement. Of the patients, 51.3% were male (n=417), and 48.7% (n=396) were female; 53.3% of the patients in whom cemental tear was detected were male (n=8), and 46.7% were female (n=7). There was no statistically significant difference between the genders in terms of cemental tear occurrence (p>0.05). The average age of the 15 patients with cemental tears was 49.20±16.4 years. The age group with the greatest number of cemental tears was 18-39 years (40.3%); however, no statistically significant difference was found between age and the occurrence of cemental tear (p>0.05). Of the teeth with cemental tear, 53.3% were detected in the maxillary region (n=8), and 46.7% were detected in the mandibular region (n=7). The demographic and clinical features of the patients are provided in Table 1. A statistical analysis revealed that there was no significant difference between the maxillary and mandibular region in terms of cemental tear occurrence (p>0.05). The teeth more frequently affected by cemental tear were incisors (53.3%). However, the effect of tooth type on cemental tear was not statistically significant (p>0.05). Prosthetic restoration was detected in 33.3% of the teeth with cemental tear (n=5); however, the presence or absence of prosthetic restorations had no statistically significant effect on cemental tear (p>0.05). No previous root canal treatment was observed in 11 of the 15 teeth with cemental tear; however, no statistically significant effect was detected in the presence or absence of previous root canal treatment on cemental tear (p>0.05). In 12 of the 15 cases, periapical/periodontal bone loss was detected; the presence of periapical/periodontal bone loss had a statistically significant effect on cemental tear (p<0.05). Proximal surfaces were the most frequently observed direction detected with cemental tear. There was a statistically significant difference in the occurrence of cemental tear only between proximal surfaces and lingual surfaces (p<0.05). There was no statistical difference between apicocoronal locations (p>0.05). No patients with multiple cemental tears were observed. The clinical and radiological characteristics of cemental tear cases are provided in Table 2.

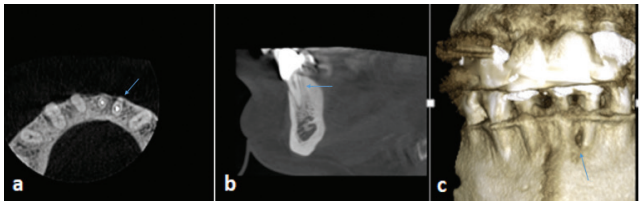

Figure 1. Axial (a), sagittal (b), and three-dimensional reconstruction (c) views of cemental tear cases on CBCT

CBCT: Cone-beam computed tomography

Discussion

Due to its low frequency, cemental tear can be confused with other endodontic and periodontal lesions (3,12). Little information is found in the literature about the frequency of cemental tear. In the present study, the frequency of cemental tear was found to be 1.85%. Keskin and Güler (10) reported a frequency of 0.89% for cemental tear in their study, which was performed in a different region of Turkey. These different findings may be the result of

differences in the radiological methods used and in the populations involved in the studies. In the literature, the use of CBCT is recommended to diagnose cemental tear instead of two-dimensional imaging in order to differentiate cemental tear from endodontic/periodontal lesions (11,13). Two-dimensional imaging cannot diagnose cemental tears in the buccal/labial or lingual/palatal directions of the teeth (10).

No gender or age predilection was observed in the present study in terms of cemental tear occurrence, similar to the report by Keskin and Güler (10). In two different studies, Lin et al. (3,5) reported that cemental tear is more common in men and in patients older than 60 years. In addition, the results of the present study revealed that clinical characteristics and conditions of the teeth such as tooth region, tooth type, presence of prosthetic restoration, and previous root canal treatment did not affect the occurrence of cemental tear. Only the presence of periapical/periodontal bone loss was found to be related to the formation of cemental tear, similar to the findings of Lin et al. (3) and Keskin and

Table 1. Demographic and clinical features of the patients

	Gender, n (%)		Mean Age	Patient (n)	Maxillary incisor	Maxillary premolar	Maxillary molar	Mandibular incisor	Mandibular premolar	Mandibular molar
	Female	Male								
Number of patients and teeth examined	396 (48.7%)	417 (51.3%)	36±12.6	813	3.455	1.816	2.548	3.701	1.481	2.885
Number of patients and teeth with cemental tear	7 (46.7%)	8 (53.3%)	49.20±16.4	15	3	3	2	5	1	1

Table 2. Clinical and radiological characteristics of cemental tear cases

CBCT examination	Analyzed sample	p
Prosthetic restoration		
Yes	5	p>0.05
No	10	
Previous root canal treatment		
Yes	4	p>0.05
No	11	
Periapical/periodontal bone loss		
Yes	12	p<0.05
No	3	
Mesiodistal direction		
Proximal[a]	9	p<0.05
Lingual[b]	2	
Buccal[ab]	4	
Apicocoronal location		
Cervical one-third	3	p>0.05
Middle one-third	4	
Apical one-third	8	

[a, b]; significant differences are represented by different superscript letters, CBCT: Cone-beam computed tomography

Güler (10). On the other hand, both of these studies (3,10) suggested that the presence of cemental tear is more common in the maxillary region. While Lin et al. (3) declared that the type of teeth is important in the formation of cemental tear, Keskin and Güler (10) reported that it is not. In the present study, most of the cemental tears were detected in the proximal root surfaces, which is similar to the findings of Lin et al. (5). However, while the most common location was the middle one-third in their study, in our study, the most common location was in the apical one-third. It is thought that these differences may be due to the different study populations, study designs, and the sample sizes of the researches.

No patient with multiple cemental tear was detected in this study. There are few case reports in the literature of multiple cemental tears in a single patient (6,11,12). Watanabe et al. (12) declared that multiple cemental tears may result from the structural weakness of the cementum.

In this retrospective study, CBCT images were used to detect cemental tears. Thanks to the use of three-dimensional imaging, the limitations of two-dimensional imaging were eliminated. CBCT images can detect the location of cemental tears and positively affect treatment outcomes (11). Although CBCT offers high resolution three-dimensional imaging, the exact diagnosis of cemental tear can be made only by histopathological examination of the removed fragment (3,5,9,13). Since this study was retrospective, histopathological examination was not performed, and the possible causes of cemental tear, such as occlusion disorders, trauma, and bruxism, could not be evaluated. In addition, the treatment interventions for all teeth with cemental tear were not available in the patient records.

The prognosis of untreated or improperly treated teeth with cemental tear will inevitably become worse because of increasing periodontal and periapical bone destruction. Several treatment options exist for cemental tear, including periodontal treatment, root canal treatment, apical surgery, and tooth extraction (9-16). Appropriate treatment can vary depending on whether the cemental tear is diagnosed early or late as well as according to clinical or radiological signs. Lin et al. (5) emphasized that cemental tear should be completely removed to improve prognosis. Watanabe et al. (12) recommended the management of occlusion to prevent recurrence if the main cause is occlusal trauma. The authors recommended the extraction of the tooth in the case of a structural defect, which can lead to recurrence. Chou et al. (14) suggested periodontal surgery and a proper maintenance regimen for the treatment of a tooth with cemental tear. Pilloni et al. (15) reported that after removal of the cemental tear, the teeth can be successfully treated using hyaluronic acid and an absorbable collagen membrane. Qari et al. (16) declared that local, conservative curettage is the best treatment approach in cases in which periodontal probing is negative, tooth vitality is positive, and the lamina dura is destroyed. Lin et al. (9) reported that most teeth with cemental tears can maintain their function in the mouth with nonsurgical and surgical periodontal and endodontic interventions.

Conclusion

Cemental tear may occur in association with various predisposing factors reported in the literature. In the present study, only periapical/periodontal bone loss contributed to the formation of cemental tear. The frequency of cemental tear was determined to be 1.85%. Due to its rare occurrence, early and accurate diagnosis of the lesion is crucial to ensure a successful treatment approach. A prospective study conducted with a larger sample size and including histopathological examination of the removed fragment will be more decisive in determining the frequency and characteristics of cemental tear.

Ethics

Ethics Committee Approval: Aydın Adnan Menderes University Clinical Research Ethics Committee approved this study (protocol number: ADUDHF 2019/074, date: 20.11.2019).

Informed Consent: Patient consent was not obtained due to the retrospective evaluation of CBCT images.

Peer-review: Externally peer-reviwed.

Authorship Contributions

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

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Original Article / Özgün Araştırma

The Effect of Different Irrigation Solutions on the Accuracy of Two Electronic Apex Locators in Locating Artificial Root Perforations

Yapay Kök Perforasyonlarını Belirlemede Farklı İrrigasyon Solüsyonlarının İki Elektronik Apeks Bulucunun Doğruluğuna Etkisi

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Keywords
Electronic apex locator, irrigant, raypex 6, root perforation, root ZX mini

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Abstract

Objective: This study aimed to assess the accuracy of the Raypex 6 (VDW GmbH, Munich, Germany) and Root ZX mini (J. Morita Corp., Tokyo, Japan) electronic apex locators (EALs) with regard to detecting root perforations in dry conditions and in the presence of irrigation solutions [2.5% sodium hypochlorite (NaOCl), 17% ethylenediaminetetraacetic acid (EDTA) and Qmix].

Materials and Methods: Twenty single-rooted human teeth were perforated artificially with a 1 mm diameter in the middle region. The actual canal lengths up to the perforation site were determined, and then the teeth were embedded in an alginate mold. The electronic measurements of the perforations were obtained using a size #20 K-file for each EAL in various conditions.

Results: There were significant differences between the different canal conditions in the Raypex 6 group ($p<0.05$), but no significant differences in the Root ZX mini group ($p>0.05$). There was a statistically significant difference ($p<0.05$) between the two EALs in the presence of EDTA solution, but no statistically significant difference between the accuracy of two EALs in the presence of other solutions and in the dry canal condition ($p>0.05$).

Conclusion: Under the conditions of this study, both apex locators detected root canal perforations within a clinically acceptable (range of -0.18 to 0.31mm) distance from the coronal border of the perforation region. Irrigation solutions within the root canal affected the accuracy of Raypex 6, but not of Root ZX mini.

Öz

Amaç: Bu çalışmanın amacı kök perforasyonunu tespit etmede kuru koşullarda ve irrigasyon solüsyonları varlığında [%2,5 sodyum hipoklorit (NaOCl), %17 etilendiamintetraasetik asit (EDTA) ve Qmix] Raypex 6 (VDW GmbH, Münih, Almanya) ve Root ZX mini (J. Morita Corp., Tokyo, Japonya) elektronik apeks bulucularının (EAB) doğruluğunu değerlendirmektir.

Gereç ve Yöntemler: Yirmi adet tek köklü insan diş kökünün orta bölümünde 1 mm çapında yapay perforasyon oluşturuldu. Perforasyon alanına kadar olan gerçek

kanal uzunlukları belirlendi ve sonra dişler bir aljinat kalıbına gömüldü. Her koşulda her bir EAB için #20 K boyutunda bir eğe ile perfore alana kadar olan elektronik ölçümler elde edildi.
Bulgular: Raypex 6 grubunda farklı kanal koşulları arasında anlamlı fark vardı ($p<0,05$), ancak Root ZX mini grubunda ($p>0,05$) anlamlı fark yoktu. EDTA çözeltisinin varlığında iki EAB arasında istatistiksel olarak anlamlı bir fark vardı ($p<0,05$), diğer çözeltilerin varlığında ve kuru kanal koşulunda iki EAB'nin doğruluğu arasında istatistiksel olarak anlamlı bir fark yoktu ($p>0,05$).
Sonuç: Bu çalışmanın koşulları altında, her iki apeks bulucu da, perforasyon bölgesinin koronal sınırından klinik olarak kabul edilebilir sınırlar içinde (-0,18 ila 0,31 mm aralığında) kök kanal perforasyonlarını saptamışlardır. Kök kanalı içindeki irrigasyon çözeltileri, Raypex 6'nın ölçüm doğruluğunu etkiledi, ancak Root ZX Mini'nin ölçüm doğruluğunu etkilememiştir.

Introduction

Root perforations are clinical conditions that joint the root canal system with the external root surface and surrounding tissues by destroying the cementum layer, which is the outermost part of the root (1). The etiology of root perforations (pathological, iatrogenic, idiopathic, etc) varies widely. If not correctly diagnosed and treated, the prognosis is poor and may result in related tooth extraction (1,2).

In teeth with root perforation, the materials used during endodontic treatment and the debris produced during preparation are at high risk of contact with the perforated region (3). This may lead to irritation and contamination risk during endodontic treatment in relation to the area affected (4). In addition, the incorrect detection of the perforation area also increases the likelihood of procedural errors such as over instrumentation and overfilling. For this reason, accurate detection of the perforation area is important for the prognosis of endodontic treatment (5). Various techniques such as operation microscopy, endoscopy, optic coherence tomography, digital radiography, and electronic apex locator (EAL) can be used to detect root perforations, as well as direct observation of the bleeding in the perforated area and indirect evaluation with a paper point (2,6). In the presence of bleeding in the perforation area, it may not always be possible to detect the perforation region directly or indirectly with the help of a paper point. In addition, for the detection of the perforation area may be misleading because of its limitations (7,8).

EALs are also useful to determine the area of perforation in endodontic treatment applications. They are easy to use, produce immediate results, and reduce exposure to radiation. Thus, these devices provide more acceptable treatment for both the clinician and the patient (7). The Root ZX mini (J. Morita Corp., Tokyo, Japan) is an EAL with a compact, easily portable design, working with proportional method developed by modifying Root ZX (9). Raypex 6 (VDW GmbH, Munich, Germany) is an EAL that works with the multi-frequency method and is the latest member of the Raypex series (10).

Studies have shown that fluids (irrigants, blood, pulp, exudates, etc) in the root canal could affect the accuracy of EAL (10). The aim of this study is to evaluate the accuracy of two different EALs in the detection of the perforation area in teeth with artificial root perforations in the presence of various root canal irrigants. The null hypothesis of the study is that there are no differences with regard to determining the root perforation between the solutions and the EALs used.

Materials and Methods

The research design was approved by Bolu Abant İzzet Baysal University Clinical Researches Local Ethics Committee (approval number: 2018/26, date: 24.05.2018). The current study was carried out in accordance with the World Medical Association Declaration of Helsinki and written informed consent was obtained from all participants. Based on a previous study (5) a power calculation was performed using G*Power 3.1 software (Heinrich Heine University, Dusseldorf, Germany). The calculation indicated that the sample size for each group should be at least six teeth.

Sample Selection

In this study, 20 mandibular premolar teeth with a single root and single canal extracted for orthodontic and periodontal reasons were used. Root canal anatomy was evaluated by taking periapical radiographs from buccal-lingual and mesio-distal directions for each tooth. The teeth with immature roots, calcification, fractures, and root canal treatment were replaced with new ones. After the sample selection, the teeth were disinfected in 2.5% sodium hypochlorite (NaOCl) solution for 48 hours. The teeth were then

washed under water and stored in distilled water until use. Conventional endodontic access cavities were prepared using diamond round burs (Dentsply Maillefer, Ballaigues, Switzerland). The apical patency was checked using a #10 K-file (Dentsply Maillefer). The crowns of the teeth were removed with diamond burs (Diatech, Charleston, USA) under water cooling as to ensure that the root length was 15 mm. Thus, a constant and flat reference point was obtained for the measurements. After the tip of #10 K-file became visible in the apical foramen of the teeth, the working length of each root canal was determined to be 1 mm shorter than this measurement.

Canals were prepared using a #15 K-file (Dentsply Maillefer). After preparation, the canals were irrigated with 2 mL of 5.25% NaOCl (CanalPro; Coltene-Whaledent, Allstetten, Switzerland) followed by 2 mL of distilled water and were dried with paper points (DiadentGroup International Inc, Chongju, Korea). The 010 size round diamond burs (Dentsply Maillefer) was placed on the proximal surface of the roots at a 90-degree angle to create artificial perforation areas 1 mm in diameter at a distance of 5 mm from the apical foramen. The diameter of the perforation areas was checked by measuring using digital calipers.

Before measuring the electronic length (EL), the actual lengths (AL) of the canals up to the perforation area were recorded with a 20 K-file (Dentsply Maillefer) at 20X magnification under a stereomicroscope. The teeth were then embedded in alginate (Blueprint, Denstly, England) and the lip clip was contacted with the alginate during the measurement. Measurements were made in 5 different conditions, dry and in the presence of NaOCl, ethylenediaminetetraacetic acid (EDTA), Qmix, and chlorhexidine digluconate (CHX) solutions.

For EL measurement, Root ZX mini (J Morita Corp., Tokyo, Japan) and Raypex 6 (VDW, Munich, Germany) were used in accordance with the manufacturer's recommendations. A #20 K-file was used for the measurements by placing it in the canal and advancing it to the apical. When the EAL gave the apex exit signal, the stopper of the file was brought to the reference point and this measurement was recorded using the endoblock (Dentsply Mini-Endobloc). All measurements were performed after root canal irrigation with 2.5 mL of solution of the corresponding group. To completely remove the previous solution between different groups, canals were irrigated with 5 mL distilled water and dried with paper points. All irrigation procedures were performed with a double side-port needle (31 gauge NaviTip Sideport; Ultradent Products Inc, South Jordan, UT, USA).

Measurements were repeated 3 times in each canal, and the average of these 3 values was determined as the raw length. All measurements were made by the same operator experienced in the use of EALs. The difference between the EL and the AL of the perforations was calculated for each sample. A negative value indicates a shorter measurement, while positive value indicates a longer measurement than the AL. If the value is 0, this means that the AL and the EL are equal.

Statistical Analysis

All statistical analyses were performed using SPSS for Windows (version 16.0, SPSS Inc., Chicago, IL, USA). The Friedman and Wilcoxon signed-rank tests were used to analyze the data. The significance was determined at $p<0.05$.

Results

The mean difference between the EL and AL of the perforation and the standard deviation (SD) of each EAL in different canal conditions is shown in Table 1. In the Raypex 6 group, there was a significant difference between the measurements in the presence of EDTA

Table 1. The mean difference between the electronic length and the actual length of the perforation with the standard deviation for each electronic apex locator in different canal conditions (mm)

	Dry Mean ± SD	NaOCl Mean ± SD	EDTA Mean ± SD	CHX Mean ± SD	Qmix Mean ± SD
Raypex 6	0.22 ± 0.36^{Aa}	-0.04 ± 0.37^{Abc}	-0.18 ± 0.32^{Ab}	0.20 ± 0.35^{Aac}	0.22 ± 0.45^{Aca}
Root ZX mini	0.22 ± 0.28^{Aa}	0.12 ± 0.39^{Aa}	0.18 ± 0.31^{Ba}	0.11 ± 0.39^{Aa}	0.31 ± 0.30^{Aa}

Different superscript uppercase (A, B, C) letters in the same column indicate a statistically significant difference ($p<0.05$), different superscript lowercase (a, b, c) letters in the same row indicate a statistically significant difference ($p<0.05$), SD: Standard deviation, EDTA: Ethylenediaminetetraacetic acid, CHX: Chlorhexidine digluconate, NaOCl: Sodium hypochlorite

and in the presence of CHX, Qmix, and a dry canal; also, between the measurements with NaOCl and measurements made in dry canal and a CHX presence ($p<0.05$). Electronic measurements were shorter than the AL in the presence of NaOCl and EDTA solutions, while measurements were longer than the AL in other conditions. There were no significant differences among the different canal conditions in the Root ZX mini group ($p>0.05$). While a significant difference was noted among the two EALs in the presence of EDTA solution ($p<0.05$), there was no statistically significant difference between the accuracy of two EALs in the presence of other solutions and in dry canal conditions ($p>0.05$).

Discussion

Successful treatment of root perforations depends on the covering of the perforation area by a biocompatible material that will not impair the health of the periodontal ligament (11). For this reason, it is important to determine the perforation area correctly for an appropriate treatment (11).

Radiographs are widely used to detect the perforation area. However, periapical radiographs are not always sufficient in determining the root perforation region, because of the superposition of anatomical structures and provide a 2-dimensional image of a 3-dimensional anatomy (11). Cone-beam computed tomography (CBCT) has been shown to be a more reliable method for detecting perforation than periapical radiography (11,12). On the other hand, in a previous study, it was concluded that CBCT had a higher risk of misdiagnosis in the detection of strip perforation (13).

EALs have been described as a highly reliable method of locating root perforation in many studies (2,5,14). It has also been shown that EAL gives more accurate results than periapical radiographs (15). In EAL measurements, electrical principles are more important than biological properties of surrounding tissue (16). For this reason, *in vitro* studies investigating the accuracy of EALs use materials that simulate the electrical resistance of periodontal ligament (PDL). Saline, alginate, agar agar, and gelatin are used to simulate the electrical resistance of PDL (5). In the literature, it has been reported that there is no statistical difference between studies investigating the accuracy of EAL in *in vivo* and *in vitro* conditions, so that *in vitro* models have yielded reliable results (17). However, *in vitro* models have the disadvantage of not fully reflecting *in vivo* studies (18).

In order to simulate the electrical resistance of the PDL, the alginate model which has been proved to be a reliable method, was used in this study because the construction phase is simple, the operator does not see the file tip, and it provides consistent measurements (19). Since alginate is a material that may deform over time, it was renewed in each group of measurements in the present study.

In the present study, the size of root perforation was standardized to 1 mm in all teeth. In studies evaluating the accuracy of EALs, *in vitro* study models were developed by creating perforation areas smaller or larger than 1 mm in size (20,21). In the literature, one study reported that perforations of 1 mm size and larger do not fully reflect the clinical condition (20). However, perforations of 1 mm diameter can be caused by iatrogenic or pathological reasons such as post placement, post removal, or root resorption.

There is no consensus on the effects of different canal conditions on the accuracy of EAL in the literature. Li et al. (22) reported that the accuracy of Propex, Raypex 5, and Root ZX was not affected when detecting root perforations (1 mm size) under different canal conditions. However, Venturi and Breschi (23) revealed that the accuracy of Apex Finder and Root ZX were influenced by intra-canal conditions. In the present study, it was observed that the accuracy of Root ZX mini was not affected in the determination of root perforation in different canal conditions, whereas the accuracy of Raypex 6 was affected by such conditions. When the literature was reviewed, no in vitro study had been conducted to evaluate the accuracy of Raypex 6 under different canal condition. The difference between the two devices may be due to differences in working principles and technology (9,10). In addition, the differences in the results of these studies can be explained by methodological differences, and by the ability and experience of the operator with regard to using the EAL.

In the present study, there was no significant difference in the Raypex 6 measurements when NaOCl compared with EDTA and Qmix; and CHX compared with Qmix. On the contrary, the EL determined in the presence of CHX was statistically different from that determined in the presence of NaOCl and EDTA. There

was also a statistically significant difference between the Qmix and EDTA groups in Raypex 6 measurements. In the literature, there was no study investigating the effect of Qmix on the accuracy of EAL measurements. For this reason, the results of this study were not directly compared with the results of the studies related to this subject in the literature. It has been reported in the literature that the accuracy of apex finders is affected by the electroconductive properties of the solutions (5,8,18). Therefore, the reason for the differences between the solutions can be explained by the differences in the electroconductive properties.

In some studies, investigating the accuracy of the EAL, ±0.5 mm was accepted as a tolerable error range (16,24), while in some studies a ±1 mm range was accepted (25). In a study conducted by D'Assuncao et al. (2) with perforation detection using Mini Apex Locator, Root SW, and Root ZX II devices, the mean values were 0.005, -0.007 and -0.008, respectively. In the present study, the mean distance from the tip of the file to the root canal perforations was in the range of 0.11 to 0.31 for Root ZX mini and 0.22 to -0.18 for Raypex 6. These differences may be due to methodological differences, including the mounting model which was used by D'Assuncao et al. (2) to minimize the procedural errors during measurements. However, according to the literature, the results obtained in this study show that both EAL measurements are within the acceptable range.

It has been reported that the SD is more important than the difference between AL and EL, and that a low SD is closely related to the reliability of the device when evaluating the accuracy and reproducibility of the EAL measurements (26,27). In the present study, the findings obtained using the Root ZX mini in dry condition and in the presence of EDTA and Qmix were more consistent than those using the Raypex 6 device. Duran-Sindreu et al. (27) also found higher SD in the presence of NaOCl and CHX with Root ZX compared with iPex.

Conclusion

Under the conditions of this study, both devices detected the root canal perforations within a clinically acceptable range of -0.18 to 0.31 mm distance from the coronal border of the perforation site. The content of the root canal affected the accuracy of Raypex 6 but did not affect the accuracy of Root ZX mini.

Acknowledgments

This study was presented as oral presentation at the 8th International Endodontics Symposium in Adana on May 10-13, 2018.

Ethics

Ethics Committee Approval: The research design was approved by Bolu Abant İzzet Baysal University Clinical Researches Local Ethics Committee (approval number: 2018/26, date: 24.05.2018).

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

Peer-review: Externally peer-reviewed.

Authorship Contributions

Supervision: D.A., Concept: Z.U.A., D.A., Design: Z.U.A., D.A., Data Collection or Processing: B.M., Z.U.A., Analysis or Interpretation: Z.U.A., Literature Search: D.A., Writing: Z.U.A., D.A.

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

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Original Article / Özgün Araştırma

Evaluation of the Efficacy of 2% Chlorhexidine in Combination with Passive Ultrasonic Irrigation on *Enterococcus faecalis* Biofilm

%2'lik Klorheksidinin Pasif Ultrasonik İrrigasyon Eşliğinde Kullanımının Enterococcus faecalis Biyofilmi Üzerine Etkinliğinin Değerlendirilmesi

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Agitation, biofilm, chlorhexidine, *Enterococcus faecalis*, passive ultrasonic irrigation

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Abstract

Objective: This study aimed to evaluate the combined effectiveness of 2% chlorhexidine (CHX) and passive ultrasonic irrigation (PUI) against *Enterococcus faecalis (E. faecalis)* biofilm.

Materials and Methods: The root canals of 66 single-rooted extracted human mandibular premolar teeth (n=66) were enlarged up to a size of 40/0.06 taper and autoclaved. Fifty-nine roots were inoculated with *E. faecalis* and incubated for 4 weeks, whereas 7 roots served as the negative control group and were filled with sterile brain heart infusion broth. The remaining specimens (n=59) were assigned into 4 experimental groups (n=13) and a positive control group (n=7): CHX via standard needle irrigation (SNI), sodium hypochloride (NaOCl) via SNI, CHX via PUI, NaOCl via PUI and non-treated positive controls. Bacteriological samples were collected before and after the intervention, and microbiological analysis was performed by counting the colony forming units. Reduction in colony count (RCC) between before and after the intervention in each group were compared using One-Way ANOVA.

Results: The highest RCC was determined in NaOCl/PUI and the lowest in CHX/SNI ($p<0.05$). Regarding RCC, CHX/PUI showed statistically similar results with NaOCl/PUI and NaOCl/SNI ($p>0.05$).

Conclusion: A PUI of 2% CHX induced a statistically similar amount of RCC with both a PUI of 2.5% NaOCl and SNI of 2.5% NaOCl. PUI combined with 2% CHX can be used in secondary endodontic infections and during routine endodontic treatment due to increased antibacterial efficiency against *E. faecalis*.

Öz

Amaç: Bu çalışmanın amacı %2'lik klorheksidin (CHX) ve pasif ultrasonik irrigasyonun (PUİ) kombine kullanımının *Enterococcus faecalis (E. faecalis)* biyofilmine karşı etkinliğini değerlendirmektir.

Gereç ve Yöntemler: Bu çalışmada 66 adet (n=66) tek köklü insan alt küçük azı dişine ait kök kanalları 40/0,06'lık genişlik ve konisiteye kadar şekillendirildi ve dişler otoklavlandı. Dişlerden 59 tanesi *E. faecalis* suşu ile enfekte edilerek 4 hafta inkübasyona bırakıldı (n=59), kalan 7 adet dişin kök kanalları steril beyin kalp infüzyon sıvı besiyeri ile dolduruldu ve negatif kontrol grubuna alındı (n=7). Elli dokuz adet diş, 4 deney grubu (n=13) ve 1 pozitif kontrol grubuna (n=7) ayrıldı. Standart şırınga irrigasyonu (SŞİ)/CHX, SŞİ/Sodyum hipoklorit (NaOCl), PUİ/CHX, PUİ/NaOCl deney gruplarını oluşturdu ve pozitif kontrol grubuna irrigasyon işlemi uygulanmadı. İrrigasyon işleminden önce ve sonra olmak üzere bakteri örnekleri kök kanallarından toplandı ve kültürde gelişen koloni oluşturan birimler sayıldı. Her grupta irrigasyon işleminin neden olduğu koloni cinsinden azalma (KCA) tek yönlü varyans analizi kullanılarak karşılaştırıldı.
Bulgular: En yüksek KCA, NaOCl/PUİ grubunda saptanırken en düşük KCA, CHX/SNI grubunda tespit edildi ve bu gruplar arasında istatistiksel açıdan anlamlı fark gözlendi (p<0,05). KCA miktarları açısından CHX/PUİ grubunda, NaOCl/PUİ ve NaOCl/SŞİ grupları ile istatistiksel açıdan benzer sonuç elde edildi (p>0,05).
Sonuç: %2'lik CHX solüsyonunun PUİ eşliğinde kullanımı; %2,5'lik NaOCl'nin PUİ ve SŞİ eşliğinde kullanımıyla istatistiksel açıdan benzer KCA'ya neden olmuştur. %2'lik CHX solüsyonunun PUİ eşliğinde kullanımı *E. faecalis*'e karşı üstün antibakteriyel etkinliği nedeniyle ikincil endodontik enfeksiyonlarda ve rutin endodontik tedavi sırasında tercih edilebilir.

Introduction

One of the main actions that affects the success of endodontic treatment is reducing the intensity and diversity of bacterial populations to a threshold level that induces periradicular healing (1) because there is no way to completely eliminate the bacteria from the root canal system (RCS) (2).

Enterococcus faecalis (E. faecalis) is a gram-positive anaerobic cocci that has been associated with endodontic failures in previous studies (3). It can penetrate deep into the dentinal tubules and adhere to the collagen matrix in dentin (4). It can also be located in the isthmus and ramification areas (5), which cannot be adequately reached by some irrigants. After the obturation process, even though its nutrient sources decrease, *E. faecalis* can survive as a single microorganism (6) with prolonged survival capacity (7) that is capable of biofilm formation (8). Bacteria in biofilm are more resistant to antimicrobial agents than bacteria in planctonic form (9). They produce a specific polysaccharide matrix, and in this way, a physical barrier against disinfecting agents can be constructed (10). Moreover, bacterial biofilm provides nutrients to bacteria and enables bypassing of the immune system's defensive mechanisms (11).

A cationic biguanide, chlorhexidine (CHX), shows broad-spectrum antimicrobial activity against endodontic bacteria during root canal treatment (12). It exhibits long-lasting and residual antibacterial effects on dentinal walls (13); its optimum concentration is 2% (14) as a root canal irrigant.

Passive ultrasonic irrigation (PUI), enhances the effectiveness of endodontic irrigants and delivers the irrigants throughout the RCS, including all anatomic irregularities (15). Ultrasound energy inducts acoustic streaming in the root canal and facilitates removal of intraradicular biofilm (16). Moreover, the energy released during ultrasonic movement is converted into heat energy in the root canal space (17), which is expected to increase efficiency when used with sodium hypochloride (NaOCl) (18). To the best of our knowledge, there is a gap in the literature regarding the investigation of the antibacterial activity of 2% CHX via PUI in human root canals infected with *E. faecalis in vitro.* The objective of the present study is to investigate the efficiency of four irrigation protocols in eliminating experimental *E. faecalis* biofilms in root canals.

Materials and Methods

Sample Preparation

G*Power 3.1 software was used to compute the required sample size for One-Way ANOVA testing. The required sample size for experimental groups was calculated to be when the power of the test was 0.80, the effect size was 0.40, the type I error rate was 0.05 and the type II error rate was 0.20. Ethical clearance was obtained from the Ethical Committee of Mersin University, Mersin, Turkey (number: 2018/404, Clinical Research Ethics Committee dated: 17 October, 2018) as an *in vitro* study, no informed consent was required. Human extracted lower premolar teeth with similar root size and anatomy were collected for this study. Sixty-six (n=66) of samples with straight roots and round shaped canals were randomly selected. Mesiodistal and buccolingual dimensions of selected

samples were examined on radiography and round shaped root canals were confirmed. Root canal treated, carious, cracked and calcified teeth are set aside and also teeth with multiple canals and root curvatures were excluded. The coronal portion of the teeth were removed with an Isomet 5.000 saw (Buehler, Lake Bluff, IL, USA) and the length of the root samples was standardized as 14 mm. The working length (WL) was considered 1 mm short of the apical foramen. Root canals were shaped to the WL using ProFile rotary instruments (Dentsply Tulsa Dental, Tulsa, OK, USA). Each canal was shaped to a size 40/0.06 taper. Root canals were irrigated with 2 mL 2.5% NaOCl (Merck KGaA, Darmstadt, Germany) using a 30-gauge endodontic needle (Sybron Endo, Orange, CA, USA) after each instrument. In all experimental groups, root canals were then irrigated with 5 mL 5% ethylenediaminetetraacetic acid (Merck) followed by 5 mL 2.5% NaOCl and 5 mL saline solution. Apical foramina of each root and the root surfaces were covered with 2 layers of a nail varnish. Each root was taken into 1.5-mL Eppendorf tubes filled with sterile brain heart infusion (BHI) broth (Merck KGaA, Darmstadt, Germany) and autoclaved inside these tubes. Teeth were kept in an incubator for two days at 37 °C in order to check for bacterial contamination.

Contamination of the Roots with *E. faecalis*

Seven roots served as a negative control group; and were filled with sterile BHI broth. Fifty-nine roots were infected with *E. faecalis* pure culture, that was cultivated in the BHI agar for 24 hours. A 1 McFarland suspension was prepared in BHI broth and then diluted 30-fold to obtain an initial bacterial suspension of $1x10^7$ colony-forming units (CFUs) per milliliter. Each root canal was completely filled with 10 µL *E. faecalis* suspension using sterile micropipettes; also it was attempted to deliver the bacterial suspension along the entire root canal length with sterile size 15 hand files. Roots were incubated at 37 °C and 95% humidity for 1 month, during this period the BHI was removed and replenished every 48 hours (h) under laminar flow. Two bacterial samples were collected from the root canals, before (S1) and after (S2) final irrigation. Before final irrigation with 2% CHX or 2.5% NaOCl with irrigant delivery techniques, the root canal was rinsed with 1 mL sterile 0.85% saline solution to remove unattached cells, and two sterile size 15 paper points were used sequentially at the WL for 1 minute (min) to soak up the canal contents.

Experimental Groups and Procedures

After 4 weeks, samples were removed from the inoculation tubes that had been placed in biosafety cabinets to prevent sample contamination. The root canals were disinfected using four different irrigation protocols, as described below.

Group 1: CHX via standard needle irrigation (SNI) (n=13): Five mL of 2% CHX irrigation was performed as the final step. A 30-gauge side-vented needle was placed within 2 mm of the WL and moved in a vertical motion to avoid the needle being locked in the canal. To ensure length control, a stopper was placed on the needle at the required length.

Group 2: NaOCl via SNI (n=13): Five mL of 2.5% NaOCl irrigation was performed as the final step. All the procedures, except for the final solution, were the same as for group 1.

Group 3: CHX via PUI (n=13): Root canals were rinsed with 5 mL of 2% CHX. An ultrasonic tip with a noncutting end (Irri-Safe tip K20/21 mm; Acteon, Mt. Laurel, NJ) mounted in a piezoelectric ultrasonic device (P5; Satelec Acteon, Merignac, France) was inserted to 1 mm less than the WL and activated at a power setting of 4 for 20 seconds. The rinsing and ultrasonic activation were repeated for 3 cycles.

Group 4: NaOCl via PUI (n=13): Root canals were rinsed with 5 mL of 2.5% NaOCl. All the procedures, except for the final solution, were the same as for group 3.

Group 5: Negative (sterile) control (n=7): Non-contaminated root canals were irrigated with 5 mL of 2% CHX via SNI technique.

Group 6: Positive (infected) control (n=7): The root canals were infected but received no further treatment.

In each group (except the positive control group), after final irrigation either with CHX or NaOCl via various irrigant delivery techniques, 1 mL 10% sodium thiosulfate was injected into the root canals by a 30-G syringe to neutralize both NaOCl and CHX and remained in the root canals for 30 seconds. Finally, a second bacterial sample from the root canal was taken (S2) with size 25 paper points (Dentsply Sirona), as described previously.

Quantification of the Bacterial Load

The paper points were transferred to the tubes containing 1 mL of 0.85% saline solution and vortexed for 1 min. After 10-fold serial dilutions in sterile

saline, 0.1 mL aliquots of each diluted sample were plated onto BHI agar plates and incubated at 35±2 °C for 24 h. The cultivated CFUs were counted and then transformed into actual counts based on the previously determined dilution factors.

Statistical Analysis

The data were analyzed using SPSS statistics 25.0 software (IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY). The normal distribution of the data for quantitative variables was evaluated by the Shapiro-Wilk normality test and Q-Q graphs. The Wilcoxon signed-rank test was used to evaluate the reduction in colony count (RCC) between before and after intervention in each group. Bacterial count comparisons of independent groups with more than two subcategories were evaluated using ANOVA. A Tamhane test was used for multiple comparisons according to the homogeneity of group variance tests. In residual bacterial count (RBC) comparisons, Kruskal-Wallis analysis was used according to the normality test results, and the Dunn-Bonferroni post-hoc method was used for multiple comparisons. Significance level was set at 0.05.

Results

Table 1 represents the RCC based on the mean counts before and after the intervention. The highest percentage RCC was determined in NaOCl/PUI and the lowest in CHX/SNI ($p<0.05$). CHX/PUI showed statistically similar results with NaOCl/PUI and NaOCl/SNI ($p>0.05$). There were no significant differences between CHX/PUI and CHX/SNI ($p>0.05$).

Table 2 shows the logarithmic CFU values of the RBC after intervention in all experimental groups and also in the non-treated (positive control) group. There were significant differences between all experimental groups and the positive control group ($p<0.05$), whereas no statistical difference was observed among experimental groups ($p>0.05$).

Discussion

Today, to achieve predictable results, clinicians and investigators aim to reduce the number of bacteria in RCSs to below the threshold level in order to eliminate pathogen species and remove biofilm associated with the root canal. Ma et al. (19) stated that techniques that should produce at least one logarithmic step decrease in CFUs. *E. faecalis* is a key pathogen identified in failing endodontic cases (20), as this high biofilm producer has the ability to form biofilm within 48-72 h (21), to organize biofilms through its aggregation products and to engage dentine and other bacteria (22). In experimental conditions, the incubation time, which is an important factor for biofilm development, was set to 4 weeks. Stojicic et al. (23) reported that during the first hour, bacteria are mostly planktonic and that, in the first 2 weeks, biofilm bacteria are sensitive to NaOCl (1%), CHX (2%) and iodine (0.2/0.4%); after 3 weeks, however, they become very resistant to the same agents. In the present study, we used *E. faecalis* to evaluate the combined effectiveness of PUI with 2% CHX and 2.5% NaOCl in extracted human teeth.

In the present study, human mandibular premolars with single-rooted, straight and round root canals were standardized to 14 mm root length. Thus, similar dimensions of root canal volumes were incubated with *E. faecalis* strain. In a previous study (24),

Table 1. Tamhane post-hoc Analysis from One-Way ANOVA shows the mean difference, p value, and 95% CI of log (CFU/mL) data between each pair of experimental groups regarding RCC

Group A	Group B	Mean difference (A-B)	p	95% CI	
				Lower bound	Upper bound
NaOCl via SNI	NaOCl via PUI	-0.67	0.342	-1.68	0.34
	CHX via SNI	1.65*	0.043	0.04	3.25
	CHX via PUI	0.22	0.999	-1.26	1.70
NaOCl via PUI	CHX via SNI	2.32*	0.002	0.78	3.86
	CHX via PUI	0.89	0.375	-0.51	2.30
CHX via SNI	CHX via PUI	-1.42	0.190	-3.24	0.39

*indicate that there was statistically difference between values $p<0.05$, NaOCl: Sodium hypochloride, SNI: Standard needle irrigation, PUI: Passive ultrasonic irrigation, CHX: Chlorhexidine, CI: Confidence interval, CFU: Colony-forming unit, RCC: Reduction in colony count

Table 2. The number of residual bacterial count after the intervention (log_{10}) (CFU/mL)				
Groups	**Median (Q_1-Q_3)**	**p**	**Post-Hoc**	**p**
NAOCl via SNI (1)	1 (1-1)	<0.001	1-2:1.000	1-3:1.000
NaOCl via PUI (2)	1 (1-1)		1-4:1.000	**1-5<0.001**
CHX via SNI (3)	1 (1-2,65)		2-3: 1.000	2-4: 1.000
CHX via PUI (4)	1 (1-1)		**2-5<0.001**	3-4: 1.000
Positive control (5)	6.60 (6.47-7.69)		**3-5<0.001**	**4-5<0.001**
NaOCl: Sodium hypochloride, SNI: Standard needle irrigation, PUI: Passive ultrasonic irrigation, CHX: Chlorhexidine, CFU: Colony-forming unit				

variations in root canal size and selection of tooth model were assumed as parameters that had affected the diversity of results in different studies. Samples were more easily collected and better controlled in the straight root canals of single-rooted teeth used in the current study, and sterile paper points were used to collect bacteria with the same technique described in previous studies (25,26).

According to Rôças et al. (27), both 2.5% NaOCl and 2% CHX can be used as root canal irrigant in infected teeth. A few studies (4,28,29) in the literature compared the effectiveness of NaOCl and CHX at various concentrations on *E. faecalis* and reporsted no significant differences. However, in a previous study, Vianna et al. (30) stated that 2.5% NaOCl was significantly more effective than 2% CHX gel in reducing bacterial populations in root canals. In the present study, a higher RCC has been observed in the NaOCl/PUI and NaOCl/SNI groups compared to the CHX/SNI group, while no statistical difference was found between the CHX/PUI and CHX/SNI groups. It is clear that in studies where no difference was found between NaOCl and CHX, different concentrations of NaOCl and CHX were used (28) and that instead of counting bacterial growth in CFU/mL, the minimum contact times for inducing negative cultures were compared (4,29). Although a non-culture dependent methodology was used (30), the same concentrations of NaOCl and CHX were used in the present study and Vianna et al.'s (30) study. Moreover, both of the studies were quantitative, which is why our findings are in accordance with theirs (30).

In previous studies, ultrasonic agitation of irrigants showed better efficacy of cleaning and disinfecting in RCS than in SNI alone (31,32). Nevertheless, Siqueira et al. (33) reported that NaOCl with PUI was not superior to NaOCl with SNI when the efficacy of NaOCl was compared using different delivery techniques. In their study (33), a turbidity test was used to compare the effectiveness of irrigants and delivery techniques, and a number of negative and positive cultures were recorded. In the current study, a quantitative culture-based methodology is used, and it has shown a superior RCC with PUI compared to with SNI, but the difference was not significant. In one study, NaOCl was accepted as the main endodontic irrigant due to its antibacterial properties and its capacity to dissolve organic tissue residues (34). That is why the results in the present study for NaOCl with both PUI and SNI are so similar and are not statistically different. In an *ex vivo* study (35), the sequential use of PUI and a final rinse with CHX was suggested as the best approach, over PUI alone and CHX alone. However, in the current study, no statistical difference was found between CHX/PUI and CHX/SNI, and both NaOCl groups (NaOCl/PUI and NaOCl/SNI) induced a larger RCC than did CHX/SNI. The discrepancy may have occurred because of the usage of NaOCl instead of CHX (35) during the ultrasonication process in the previously mentioned study (35).

According to the results of the current study, in all experimental groups, RBCs (after intervention) were statistically different from the positive control group (Table 2). Ruiz-Linares et al. (36) claimed that 2.5% NaOCl was the most effective irrigant against endodontic pathogens in their examination and evaluation of a multispecies mature biofilm model in human dentine. They found (36) the mean percentage of live cells to be 4.26% in the 2.5% NaOCl group. Although mixed bacterial flora exist in endodontic infections, monospecies biofilm with *E. faecalis* was used in the present study. A higher sensitivity and the almost eradication of biofilm in response to the same

concentration of NaOCl in the second samples may be related with this fact.

As in previous studies (25,26), samples in the present study were taken with the aid of sterile paper points before and after interventions, and cultures were grown for both scenarios. Absorbent paper points can collect bacterial samples from the main canal and from the smooth-surfaced root canal walls but may not be able to pick up bacteria located in more distant areas and in anatomical complexities such as dentinal tubules, isthmuses, recesses and lateral canals. This limitation may be one of the reasons for the small number of bacterial colonies recorded after treatment in all experimental groups. In the future, the results of the present study should be confirmed by a molecular study that provides quantitative results.

Conclusion

A PUI of 2% CHX induces a statistically similar amount of RCC with both a PUI of 2.5% NaOCl and an SNI of 2.5% NaOCl. Thus, we have identified the positive effects of PUI combined with 2% CHX in the elimination of *E. faecalis* in the root canal and recommend it for routine endodontic treatment.

Ethics

Ethics Committee Approval: Ethical clearance was obtained from the Ethical Committee of Mersin University, Mersin, Turkey (number: 2018/404, Clinical Research Ethics Committee dated: 17 October, 2018) as an *in vitro* study.

Informed Consent: No informed consent was required.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: B.K., H.S.T., S.T.Ü., Design: B.K., H.S.T., S.T.Ü., B.S., Data Collection or Processing: B.K., S.T.Ü., N.K., Analysis or Interpretation: B.K., S.T.Ü., H.S.T., G.A., Literature Search: B.K., Writing: B.K., S.T.Ü.

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

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Original Article / Özgün Araştırma

Should Discectomy Performed Without Replacement be the First Choice in Patients with Pain and Reduced Mouth Opening?

Yerine Bir Şey Konulmaksızın Yapılan Diskektomi Ameliyatı Ağrı ve Limitli Ağız Açıklığı Olan Hastalarda İlk Tercih Olmalı mıdır?

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Abstract

Objective: In this study, we evaluated the pain and functional development of patients with internal derangements before and after discectomy.

Materials and Methods: This was a retrospective cohort study in which patients with internal derangement underwent discectomy without replacement. The main primary variables were pre- and post-operative maximum mouth opening (MMO) and pain according to the Visual Analogue scale (VAS). Paired t-test was used to compare means to determine the differences between pre- and post-operative mouth opening and pre- and post-operative VAS.

Results: Among the 20 patients included in this study, six and 14 underwent bilateral and unilateral discectomy without replacement, respectively. Paired sample t-test results revealed that there was a difference in MMO pre- and post-operatively, whereas the VAS scale showed no difference in terms of pain. According to the Wilkes classification, the difference between pre- and post-operative MMO of the patients was recorded. Based on these findings, no difference was observed between the mean pre- and post-operative MMOs of Wilkes stage III and IV patients, whereas a significant difference was found between Wilkes stage V and III patients ($p<0.05$). No significant difference was found between Wilkes stage IV and V patients ($p>0.05$).

Conclusion: In Wilkes stage III and IV subjects, discectomy without replacement is a good treatment option to relieve pain and improve functions in cases where conservative treatment fails.

Öz

Amaç: Bu çalışmada; diskektomi operasyonu öncesi ve sonrası iç düzensizlikleri olan hastaların, ağrı ve fonksiyonel gelişimi değerlendirildi.

Gereç ve Yöntemler: Bu retrospektif kohort çalışmada internal düzensizliği olan hastalara yerine bir şey konulmaksızın diskektomi uygulanan hastaların ağrı ve fonksiyonel gelişimi değerlendirildi. Birincil ana değişkenler ameliyat öncesi ve sonrası maksimum ağız açıklığı (MAA), Görsel Analog skalasına (VAS) göre ağrı idi.

Ortalamaları, farklılıkları, ameliyat öncesi ve sonrası ağız açıklığı ile ameliyat öncesi ve sonrası VAS'si belirlemek için eşleştirilmiş örnekelem t-testi kullanıldı.
Bulgular: Altısı bilateral ve 14'ü unilateral olmak üzere yerine bir şey konulmaksızın diskektomi uygulanan toplam 20 hasta çalışmaya dahil edildi. Eşleştirilmiş örneklem t-testi sonuçları; ameliyat öncesi ve sonrası MAA'da bir fark olduğunu, VAS ölçeğinde ise ağrı açısından fark olmadığını göstermiştir. Wilkes sınıflandırmasına göre hastaların ameliyat öncesi ve sonrası MAA değerleri arasındaki fark hastaların dosya verilerinden kaydedilmiştir. Bu bulgulara dayanarak, evre III ve IV'teki hastaların ameliyat öncesi ve sonrası ortalama MAA'ları arasında fark gözlenmezken, evre V ve III'teki hastalar arasında anlamlı bir fark görülürken ($p<0,05$); Wilkes evre IV ve V arasında anlamlı fark bulunmamıştır ($p>0,05$).
Sonuç: Wilkes III ve IV hastalarında yerine bir şey konulmaksızın diskektomi, konservatif tedavinin başarısız olduğu durumlarda ağrıyı hafifletmek ve fonksiyonları iyileştirmek için iyi bir tedavi seçeneğidir.

Introduction

Internal derangement of temporomandibular joint (TMJ) refers to the abnormal relationship between the articular disc and mandibular condyle, fossa, and articular tubercle (1). Internal derangements of TMJ are traumatic-origin and progressive organic lesions. Since progressive degenerative conditions may occur, its effective clinical evaluation has become important (2). Internal derangements of TMJ have a significant place among the joint disorders and are the most difficult cases treated by maxillofacial surgeons (3). These derangements are in close relationship with the displacement of the disc. Symptoms are usually pain in muscles, preauricular or TMJ region. Besides the pain, TMJ sounds called crackling or crepitus are often heard in patients with TMJ as well as limitation and deviation during mandibular movements (4,5). Some patients experience a reduced joint dysfunction following a short joint crackling and popping history and although symptoms of displaced disc or osteoarthritic changes and similar clinical symptoms are seen in most of these patients, they can continue their daily life activities without any limitation (6). Asymptomatic patients are prone to have unilateral distress in the joint and are at a lower stage of Wilkes classification compared to symptomatic patients, but in both cases, symptomless joint disc displacement is possible to occur (7). Patients usually have such symptoms as pain, dysfunction, having difficulties while opening their mouths and exhaustion. Making the differential diagnosis of TMJ disorders and defining the disease correctly are very important for the treatment. Symptoms of the disease can be easily confused with relatively simple muscle spasms. Anamnesis, a detailed clinical examination and advanced imaging techniques should be used in the diagnosis of these disorders and the data should be carefully evaluated.

Numerous methods have been followed in the classification of internal TMJ derangements. There are many classifications that many researchers have created by their own methods. Although there are many classifications by researchers such as Helkimo and the classifications used with the modifications made on them, the most commonly used classification is the Wilkes classification. Wilkes classified this disorder according to the degenerative changes described during surgery (2).

For the last 40 years, various techniques have been used for joint surgery. These include repositioning of the disc, eminectomy, high condylectomy, discopexy, discectomy, modified condylectomy, arthroscopic lysis and lavage. Studies with long-term follow-up duration have reported several complications and high success rates for the patients who underwent discectomy alone without replacement (8,9). Of these, discectomy is the most widely used procedure for joint pain (10), which has the best long-term data results (11,12). Other signs include different underlying pathologies, their relation, the ability of the surgeon to interpret these symptoms because the symptoms of internal derangement of the joint are often confusing, and the selection of surgical technique (13,14).

This study aimed to evaluate the pre- and post-operative pain and the efficacy of discectomy used to improve the mandibular function in patients with internal derangement as well as evaluating the success of discectomy in patients at different stages of the Wilkes classification.

Materials and Methods

The study included patients who underwent discectomy without replacement to TMJ between

2013 and 2018 at Aydın Adnan Menderes University Faculty of Dentistry (Aydın, Turkey). This retrospective cohort study was performed by two surgeons with similar experience and ethics committee approval was obtained and was conducted in accordance with the guidelines of the World Medical Association Declaration of Helsinki and the Local Ethics Committee of Adnan Menderes University (Aydın, Turkey) approved it with 2018/049 ethical number. An informed consent was obtained from all patients.

The inclusion criteria were as follows: 1) Patients above 18 years of age with painful mandibular movement and with a maximal mouth opening (MMO) of less than 35 mm. 2) Patients whose clinical examination was performed at least once, those who underwent magnetic resonance imaging (MRI) before the surgery, those who were diagnosed with internal derangement and classified according to the Wilkes classification. 3) Patients who had previously been diagnosed with internal derangement and did not respond to non-surgical medical treatments applied including hot application, soft diet, acupuncture, transcutaneous electrical nerve stimulation, massage, physiotherapy, occlusal splint, and arthrocentesis. Only subject 8,9,10,11 and 19 who had normal MMO but discectomy had been done from violent pain.

Surgical indication was decided considering the clinical diagnosis, findings and symptoms. For all patients, modified endaural approach, described by Al-Kayat and Bramley, was used for discectomy (Figure 1). When the lateral joint surface was reached, local anaesthesia was performed for haemorrhage in the upper joint cavity and the horizontal incision was applied and the disc was isolated from all surfaces and removed through blunt dissection. The mandibular MMO was manipulated and joint surfaces were observed to ensure an easy condylar rotation and translation. Then, hyaluronic acid was injected into the region to provide lubrication in the mandibular movement and the wound was closed with all layers. All patients were given 1 gr cefazolin and 8 mg dexamethasone intravenously.

Study Variables

The primary main variables were pre and post-operative MMO, pain according to the visual analogue scale (VAS). A standard compass was used to measure the MMO from the incisal angles of the maxillary and mandibular incisors. In addition to these main variables, pain in the pre and post-operative movements according to the VAS, clicking, muscle pain and TMJ pain in palpation were also evaluated. All patients were graded according to the Wilkes classification before the operation, and the degree of relief was recorded after these operations.

Statistical Analysis

Paired t-test was used to determine means, differences, pre and post-operative mouth opening, and pre and post-operative VAS. Statistical analysis was performed using SPSS software and a p value of <0.05 was considered statistically significant.

Tukey test was used to identify the variables in the groups to evaluate whether they caused the differences. The significance levels were assessed by a difference of less than 0.05 ($p<0.05$).

Results

There were 20 patients with complete files in this retrospective study. Of these patients, six and fourteen underwent bilateral and unilateral discectomy without replacement, respectively. Stages were determined according to Wilkes classification; six were at stage III, nine were at stage IV, and five were at stage V. These patients had many subjective

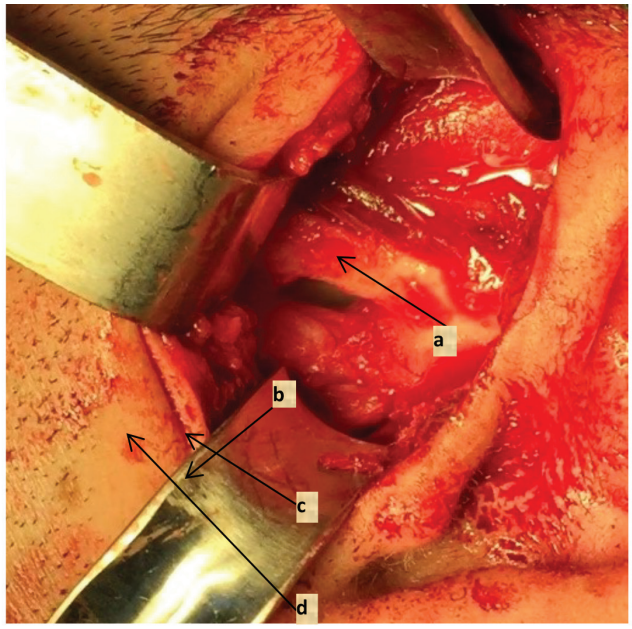

Figure 1. Intraoperative view of modified endaural approach and discectomy
a) temporalis superficialis facia b) disc c) fossa mandibularis d) eminence

Table 1. Patients clinical outcome variables after discectomy

Patient	Surgery	Age	Preop cliking	Postop cliking	Preop MMO	Postop MMO	Preop VAS	Postop VAS	Preop muscle pain	Postop muscle pain	Preop TMJ pain	Postop TMJ pain	Preop masticatory efficiency (VAS)	Postop masticatory efficiency (VAS)	Wilkes stage
1	Left	46	+	-	28	37	10	1	+	-	+	-	10	1	3
2	Left	74	+	+	32	30	10	7	-	-	+	-	10	8	5
3	Right	34	-	+	29	34	7	5	-	-	-	+	7	5	4
4	Right	58	+	-	30	37	10	4	+	-	+	-	10	4	3
5	Right	56	+	-	20	34	9	1	+	-	+	-	8	1	4
6	Right/Left	58	+	-	25	33	8	7	-	-	+	-	9	8	4/5
7	Right	61	+	-	34	35	8	3	+	-	+	-	8	3	4
8	Right/Left	24	+	+	40	41	10	3	-	-	+	-	10	3	5/3
9	Right	46	+	-	42	42	10	2	+	-	+	-	8	1	4
10	Right	32	+	-	42	35	1	8	-	+	-	+	2	6	4
11	Right/Left	39	-	-	23	35	10	3	+	-	+	-	10	2	4/3
12	Left	27	-	-	15	35	7	0	-	-	+	-	8	1	3
13	Left	44	+	+	32	39	9	1	+	-	+	-	10	2	3
14	Left	55	+	+	25	42	3	1	+	-	-	-	4	1	4
15	Right/Left	41	+	+	20	20	8	7	+	+	+	+	9	8	5/5
16	Left	55	-	-	27	36	10	1	-	-	+	-	10	1	3
17	Right	35	+	-	15	37	10	2	+	-	+	-	10	2	3
18	Right	51	+	-	20	36	8	1	+	-	+	-	8	1	4
19	Right/Left	34	+	+	45	37	8	1	-	-	+	-	9	2	5/4
20	Right/Left	69	+	+	25	32	8	4	+	+	+	+	8	5	4/3

MMO: Maximum mouth opening, VAS: Visual Analogue scale, TMJ: Temporomandibular joint

symptoms, including joint pain, pain in chewing muscles and stiffness in the neck, clicking, pain in the mandibular movement, pain in palpation in the chewing muscles, opened or closed locks, limitation in chewing, and headache (Table 1).

The MMO and VAS scale in the patients' files were examined. Paired sample t-test results revealed that there was a difference in MMO before and after surgery, while VAS scale showed no difference in terms of pain (Table 2).

According to the Wilkes classification, the difference between pre and post-operative MMO of the patients was recorded. Tukey's test was used to determine whether the difference was significant according to the Wilkes classification. Mean pre and post-operative MMOs of the patients, who were at Wilkes stage III, were 24 mm and 36.71 mm, respectively. Mean pre and post-operative MMOs of the patients, who were at Wilkes stage IV, were 29.12 mm and 35.75 mm, respectively. Mean pre and post-operative MMOs of the patients, who were at Wilkes stage V, were 32.4 mm and 33.2 mm, respectively. Based on these findings, no difference was observed between the mean pre and post-operative MMOs of patients at stage III and IV, while a significant difference was found between the patients at stage V and III ($p<0.05$). No significant difference was found between Wilkes stage IV and V ($p>0.05$) (Table 3).

Three patients at Wilkesstage V andonepatient at Wilkesstage IV underwent modified condylectomy. The total number of condylectomies was four. Improvement was observed in terms of painand MMO

in all of these patients who underwent a second surgery. Subject 2,6,15 classified Wilkes stage V and subject 10 classified Wilkes stage IV had an increase in the VAS from preoperative score a post-operative score (Table 1). This subjects also had a decrease in mouth opening. This subject 2,6,10,15 required further treatment and was the treatment condylectomy. Sixteen of 20 patients did not require a second surgical procedure.

Discussion

In this retrospective study, patients with different rates of joint degeneration according to the Wilkes classification was treated with discectomy without replacement. There are studies in which a large number of displaced and deformed discs are reshaped and repositioned. There are also studies in which the degenerated disc is removed and replaced with temporary or permanent different disc materials. In another perspective, extracapsular approach as well as eminectomy and modified condylectomy without removing the risk are also preferred. There is no golden standard in the literature for any surgical technique with a potential of low morbidity and high success. Although many long-term studies have proven that discectomy without replacement provides successful results (15-17). In a study by Dimitroulis (18), discectomy performed with dermal graft has been reported to cause less crepitation sound during joint function, compared to discectomy. In a study by Holmund et al. (19), 24 chronic closed locks patients and 27 patients with reciprocal clicking underwent discectomy without replacement. As a result of the six-month follow-up of the patients, it was found that their MMO were 35 mm and above, functional development was at least 40%, and scoring downed below four in the VAS scale. In our study, among the patients, who underwent discectomy without replacement, MMO of those who were at Wilkes stage III increased by an average of 12.71 mm, while MMO of the patients at Wilkes stage IV and V increased by 6.63 mm and 0.8 mm on average, respectively. Among these patients, the status of those who were at Wilkes stage III and IV were found to be improved in terms of clicking, locking, pain in the palpation of muscles, and pain in mandibular movement. However, those who were at Wilkes stage V (subject 2,6,15) and IV (subject 10) underwent a high condylectomy due to the fact that their pains were not relieved. Following this, improvement was observed in terms of pain and MMO in four of these patients who underwent a second surgery. All of thesepatients (subject 2,6,10,15) who underwent a high condylectomy were relieved post-operatively.

Table 2. Comparision of meanchanges in the clinic outcome variables

	N	Correlation	Sig.
Preop cliking & postop cliking	26	-0.052	0.800
Preop muscle pain & postop muscle pain	26	0.220	0.279
Preop TMJ pain & postop TMJ pain	26	-0.374	0.060
Preop MMO & postop MMO	26	0.534	0.005*
Preop VAS & postop VAS	26	-0.243	0.233
Preop masticatory efficiency & postop masticatory efficiency (VAS)	26	-0.068	0.741

MMO: Maximum mouth opening, VAS: Visual Analogue scale, TMJ: Temporomandibular joint, *Paired sample t-test

Table 3. According to the Wilkes classification, the difference between pre and post-operative maximum mouth opening of the patients

		Mean difference	Standard error	Significant	Lower bound	Upper bound
Wilkes 3	Wilkes 4	4.83838	2.80937	0.219	-2.1972	11.8740
	Wilkes 5	9.61111	3.29428	0.020*	1.3611	17.8611
Wilkes 4	Wilkes 3	-4.8383	2.80937	0.219	-11.8740	2.1972
	Wilkes 5	4.77273	3.17223	0.307	-3.1716	12.7171

*Tukey's test

Six of the patients had bilateral discectomy, while 14 patients underwent unilateral discectomy. Among those patients who had bilateral discectomy, three of the patients, whose at least one side was at Wilkes stage V, underwent a second surgery (subject 2,6,15). And those patients who had unilateral discectomy, one of the patients, whose only one side was at Wilkes stage IV, underwent a second surgery (subject 10) .We attributed this to the fact that patients who underwent bilateral discectomy with at least one side at Wilkes stage V had less improvement after discectomy, compared to other patients (those who were at Wilkes stage III and IV and who underwent unilateral discectomy).

As stated by Miloro et al. (17), if the jaw is responsible for the reduction of jaw functions and if the anatomical problem cannot be solved by non-surgical and minimally invasive procedures, the disc must be removed without being inserted again. If the morphology of the disc is abnormal and it provides a poor TMJ disc-condyle-fossa relationship, mandibular movement will be restricted in which the disc must also be removed (17). In particular, minimal invasive surgeries such as arthrocentesis and arthroscopy can be tried to treat abnormalities in the disc position or morphology of Wilkes stage I to III. However, as stated by Zhang et al. (20), minimal invasive surgeries do not provide a permanent solution for Wilkes stages IV and V due to the potential risk of relapse. Furthermore, a temporary vascular tissue formation can be induced in the region by using a dermis-fat graft of the disc, temporal muscle flap or fat tissue following the discectomy. However, jaw movements can be restricted since fat is too soft and easily shredded and it creates potential tissue for scar. Dermis-fat graft is used to fill the joint space after discectomy (21). Dermis-fat grafts are a physical barrier for heterotypical bone formation and fibrosis (22,23), but do not always protect the condyle from degenerative changes like osteoarthritis (22). In a study by Dimitroulis (22), joint replacement and condylectomy were required due to the development of osteoarthritis in eight of 123 patients. In the studies by Hansson et al. (24) and Takaku et al. (25), MRI findings of the patients, who underwent discectomy without replacement, have revealed that the intervening soft tissue having direct contact with the thick articular surfaces has filled the joint space. Therefore, the advantages of filling of the joint space by abdominal dermis-fat graft or fat graft after discectomy in these patients may not neutralize the disadvantages of creating a second surgical site, post-operative pain, and morbidity. In this case, if there is a possibility of a degenerative change in the condyle in the cases whose joint space is filled, should discectomy performed without replacementbe the first choice in patients in terms of pain and morbidity?

Nyberg et al. (16) included only the patients with unilateral internal derangement for whom they applied discectomy without replacement. In a study by Miloro et al. (17), 23 patients who underwent discectomy without replacement (11 were unilateral; 6 were bilateral) were included. In our study, bilateral discectomy and unilateral discectomy were applied for six and 14 patients, respectively. A limitation of our study may be the combined evaluation of patients who underwent unilateral and bilateral discectomy. We believe that patients who have operated for the second time may have changed the result since the first surgeries were considered in the pre and post-operative evaluation of pain in the VAS. Because in our clinical evaluations, post-operative records of patients who had unilateral discectomy were positive.

Success criteria for TMJ meniscectomy, recommended by second Goss (26) and Holmlund et al. (8) are as follows:

i. Mild, short and sparse pain without any concern

ii. Vertical mandibular movement of more than 35 mm; protrusive and lateral mandibular movement of more than 6 mm

iii. Being able to consume the routine diet easily by avoiding hard foods at worst

iv. Stability of changes in possible degenerative images in imaging

v. Absence of significant complications

vi. Absence of symptoms for at least two years

In a study by Hansson et al. (24), MMO of all patients was increased during the one-year follow-up period, and no pain was reported in 20 of 28 patients, however, four patients reported residual pain one year later. Takaku et al. (25) reported no restricted mouth opening in 31 of 35 patients followed by long-term MRI, who underwent discectomy without replacement. Additionally, 32 of the patients reported no pain while three had pain only in occlusal movements. Based on i,ii,iii and v no success criteria,

we believe that we have achieved 80% success in 16 of 20 patients without requiring any second surgery. No patients at the Wilkes stage III was required to be re-operated; our success rate was 100%. However, four of our patients with Wilkes stage V on one side and Wilkes stage V or IV on the other were required to be operated for the second time. This meant 20% success. However, after performing modified condylectomy in these patients, our success rate increased to 100%. We believe that the use of discectomy in cases where conservative treatment fails is significant. However, we would like to state that case-adapted treatment is significant for the patients at Wilkes stage V.

Conclusion

We believe that discectomy surgery without replacement is a good treatment option to relief the pain and improve the functions in cases where conservative treatment fails. Future studies with a larger patient population evaluating the relationship between the condyle and fossa in details for longer period of time will provide a better understanding of the results.

Ethics

Ethics Committee Approval: This retrospective cohort study was performed by two surgeons with similar experience and ethics committee approval was obtained and was conducted in accordance with the guidelines of the World Medical Association Declaration of Helsinki and the Local Ethics Committee of Adnan Menderes University (Aydın, Turkey) approved it with 2018/049 ethical number.

Informed Consent: Informed consent was obtained from all patients.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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Case Report / Olgu Sunumu

Non-familial Multiple Trichoepithelioma: A Case Report

Non-familyal Multipl Trikoepitelyoma: Olgu Sunumu

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Abstract

Trichoepithelioma is a well-differentiated benign follicular tumour. Clinically, it may either be solitary or multiple. Multiple trichoepitheliomas are often located on the face and its vicinity. Here, we present this case due to the increasing number of lesions over ten years, particularly with the lesions blocking the external auditory canal. This case is presented with clinical, histopathological and immunohistochemical features, with differential diagnoses. The patient was a 70-year-old female with localised papules on the external auditory canal, ear lobule, tragus, neck and lower lip. The microscopic examination of the excisional biopsy from the external auditory canal revealed a tumoural formation surrounded with stromal fibrosis and mononuclear infiltration, which is composed of basaloid cells showing peripheral palisading and keratinocytes with infundibular keratinisation. Upon immunohistochemical studies, the surrounding stroma of the tumour showed diffuse cytoplasmic positivity for CD34, and focal cytoplasmic positivity for BerEP4. There was no significant family history in this case. This pathology must be kept in mind in the differential diagnosis of tumours of the epidermis and skin appendages which occur on the face and its vicinity.

Keywords
Non-familial, multiple, trichoepithelioma, external ear canal

Öz

Trikoepitelyoma, iyi diferansiye benign folliküler tümördür. Klinik olarak tek olabileceği gibi multipl olarak da izlenebilir. Multipl trikoepitelyoma olguları sıklıkla yüz ve yüz çevresinde yerleşim gösterir. Bu çalışmada, 10 yıldır sayısı giderek artan lezyonları, özellikle dış kulak yolunu tamamen kaplaması ile ortaya çıkan klinik bulguları nedeniyle klinik, histopatolojik, immünohistokimyasal ve ayırıcı tanı özellikleri ile bir olgu sunulmaktadır. Yetmiş yaşında kadın hastanın lezyonları papüller şeklinde olup dış kulak yolu, kulak lobulü, tragus, boyun, alt dudakta lokalizedir. Olgunun dış kulak yolundan alınan eksizyonel biyopsilerin mikroskopik incelemesinde; stromal fibrozis ve mononükleer enfiltrasyonun çevrelediği, periferde palizad yapan bazaloid hücreler ve enfundibular keratinizasyon gösteren keratinositlerden oluşan tümöral oluşum görülmüştür. Olguya uygulanan immünohistokimyasal çalışmada; tümörü çevreleyen stromada CD34 ile yaygın sitoplazmik boyanma, BerEP4 ile fokal sitoplazmik boyanma görülmüştür. Aile öyküsü bulunmayan bu hastada lezyonların çok sayıda olması ve klinik bulguları nedeniyle trikoepitelyoma olgusu sunulmuştur. Yüz ve yüz çevresi yerleşimli epidermis ve deri eki kaynaklı tümörlerde bu antite ayırıcı tanıda düşünülmelidir.

Anahtar Kelimeler
Non-familyal, multipl, trikoepitelyoma, dış kulak yolu

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Introduction

Trichoepithelioma was first described in 1892 by Brooke and Fordyce under the name ‘‘epithelioma adenoides kystique’’ (1,2). Trichoepithelioma is a rare hamartomatous skin tumor that develops from the germinative cells of the folliculo-sebaceous-apocrine unit and shows follicular differentiation (3). It is a type of tumor that occurs as papules with the same color as the skin, especially in the face area. They are clinically seen as solitary or multiple (4-6). Multiple trichoepithelioma shows autosomal dominant inheritance (7). The histological features of these two types are the same. In the current case, a patient with multiple trichoepithelioma obstructing hearing due to completely covering the outer ear canal around the face is presented.

Case Report

A 70-year-old female patient was admitted to the clinic due to the increasing number of skin lesions which were localized in the the outer ear canal, the ear lobule, tragus, neck, lower lip and periorbital area within the 10-year-period. The tumor completely covered the outer ear canal which also caused hearing problems (Figure 1,2). Several excisional biopsies were taken from this area. In the microscopic evaluation, a tumoral formation consisting of mainly basaloid cells that made peripheral palisading of nuclei with keratinocytes showing infundibular keratinization have been observed. Horn cysts were common (Figure 3-5).

Figure 1,2. Multiple papules that completely fill the outer ear canal in the right and left ear

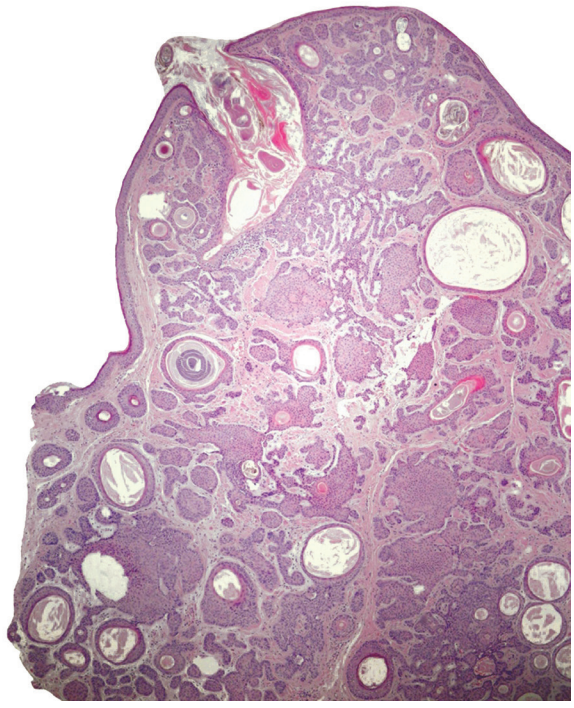

Figure 3. Tumoral formation consisting of mainly basaloid cells that made peripheral palisading of nuclei with multiple horn cysts, H&E, X100

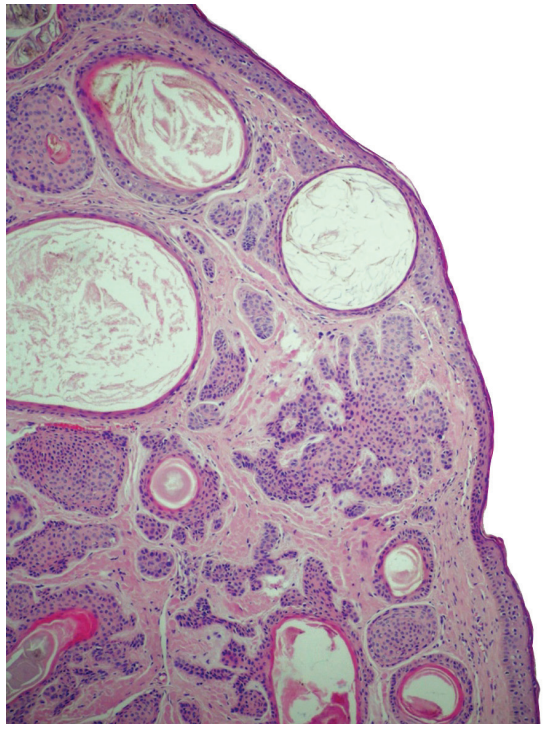

Figure 4. Tumoral formation consisting of mainly basaloid cells that made peripheral palisading of nuclei with multiple horn cysts, H&E, X200

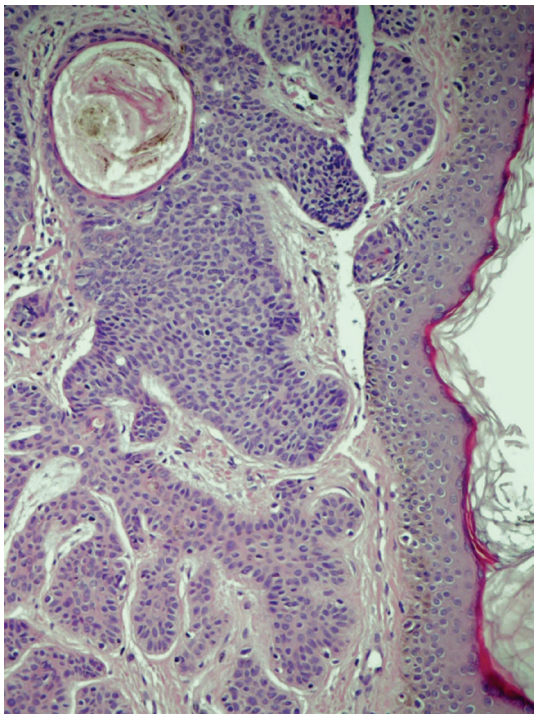

Figure 5. Tumoral formation consisting of mainly basaloid cells that made peripheral palisading of nuclei with multiple horn cysts, H&E, X400

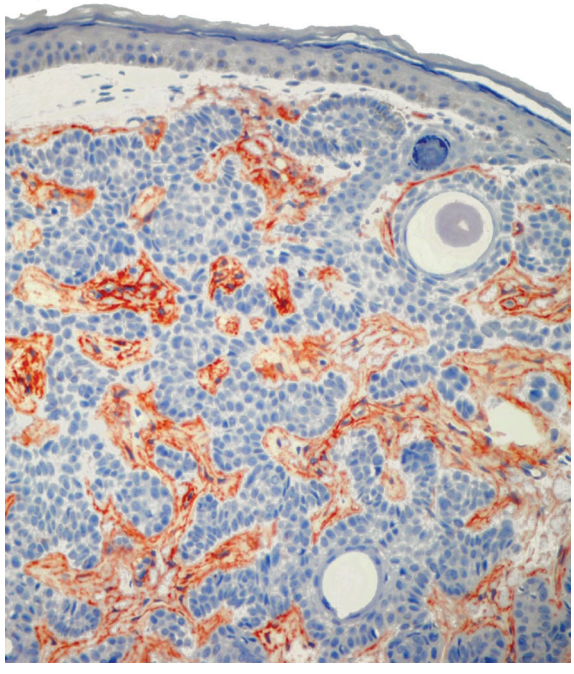

Figure 6. Diffuse cytoplasmic staining with CD34 in the stroma surrounding the tumor, X200

In the immunohistochemical study applied to the case; widespread cytoplasmic staining was observed with CD34 in the stroma surrounding the tumor which supported the diagnosis of trichoepithelioma (Figure 6) wheras BerEp-4 which favors basal cell carcinoma was focally stained in a very limited area. There is no family history in the case. During the clinical follow-up, new lesions have been occurring in the similar area.

Discussion

Trichoepithelioma is a well differentiated benign tumor originating from the hair follicle (3-8). It can be clinically solitary or multiple (4-6). Lesions are most often located in the form of flesh-colored papules located around the face. In this case, skin papules were seen around the face, especially the periorbital area, lower lip, and tragus. The number of the lesions gradually increased in 10 years. However, atypically it caused hearing problems due to the large number of lesions in the outer ear canal. Trichoepithelioma cases show symmetrical location (9). In our case, the lesions were also located within a certain symmetry.

Trichoepithelioma may be familial or may occur sporadically (4,6). There was no family history in this case.

Although there is no genetic difference between women and men, trichoepithelioma frequently occurs in women (10). Lesions complete the formation processes between the ages of 50-70. Our patient was a 70-year-old female patient, and her lesions increased especially in the last 10 years. In terms of diagnosis, it is necessary to take a biopsy and evaluate it histopathologically (11). The most characteristic features of trichoepithelioma are keratin cysts and basaloid cell groups in the form of solid and adenoid formations surrounding them (6). Similar histopathological findings were observed in our case.

Basal cell carcinoma and trichofolliculoma are important in the differential diagnosis of trichoepithelioma. In adults, trichofolliculoma is developing from hair follicles, which are mostly dome-shaped lesions that appear as solitary in the facial region (5). In this study, although our case is similarly located around the face, it differs from trichofolliculoma by being in the form of multiple papules.

It is difficult to differentiate with basal cell carcinoma in immature trichoepithelioma cases where keratin cysts are not seen (3). At this point, immunohistochemical studies are useful in reaching the diagnosis. The stroma between basaloid areas are CD34 positive in trichoepithelioma is positive, while it is negative in basal cell carcinoma (11). In our case, a similar staining profile was observed. Widespread cytoplasmic staining was observed with CD34 in the stroma surrounding the tumor. BerEp-4 was stained in a very focal area.

Malign transformation is rare in trichoepithelioma (12,13). In our case, although the lesions increased in the last 10 years, there was no evidence in favor of malignancy in multiple biopsies taken from the lesions.

In our case, the lesions were seen as multiple papules at different points of the face, especially the outer ear canal and periorbital area. It caused hearing loss due to the large number of occurrence in the outer ear canal.

Local excision is the most significant treatment method, if the lesion is solitary. The surgical approach is not meaningful in the presence of multiple tumors (5). It has been observed that lesions located in the facial region show recurrence after surgical correction by dermabration or laser treatment (14). In our case, the lesions in the outer ear canal were excised locally. During the follow-up, new lesions occured in the similar area in the meantime.

Our case was presented because of the high number of lesions and the clinical appearance that these lesions caused especially in the outer ear canal. The absence of a family history makes it difficult to reach the diagnosis in such sporadic cases, but it is recommended to keep in mind in the differential diagnosis.

Ethics

Informed Consent: Informed consent was obtained.

Peer-review: Internally peer-reviewed.

Authorship Contributions

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

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Case Report / Olgu Sunumu

Extracorporeal Membrane Oxygenation Experience in the Patient with Acute Respiratory Distress Syndrome After *Candida pneumonia*

Candida Pnömonisi Sonrası Akut Solunum Sıkıntısı Sendromu Hastasında Ekstrakorporyal Membran Oksijenasyonu Deneyimi

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Abstract

Acute Respiratory Distress syndrome (ARDS) is an acute, diffuse, inflammatory lung injury that leads to increased pulmonary vascular permeability, increased lung weight and loss of ventilated tissue. Since the definition of ARDS, more than 40% of the mortalities is caused by it despite the significant developments in the management of severe ARDS. The patients who used extracorporeal membrane oxygenation (ECMO) for severe ARDS treatment had lower morbidity and mortality rates. In this case report, a patient with coronary artery disease in whom severe ARDS and septicemia were developed after *Candida pneumonia* and was treated with ECMO was presented.

Öz

Akut Solunum Sıkıntısı sendromu (ARDS) pulmoner vasküler geçirgenlikte artış, akciğer ağırlığının artması ve havalanan doku kaybına yol açan akut, yaygın, enflamatuvar bir akciğer hasarıdır. Ağır ARDS, ilk tanımlanmasından bu yana yönetimindeki önemli gelişmelere rağmen, %40'tan fazla mortaliteye neden olmaya devam etmektedir. Ağır ARDS tedavisinde ekstrakorporeal membran oksijenasyonu (ECMO) yapılan hastalar daha düşük morbidite ve mortalite oranlarına sahiptir. Bu olguda koroner arter hastalığı olan *Candida* pnömonisi sonrası ARDS ve septisemi gelişen hastada ECMO tedavisi uygulanmıştır.

Keywords
Candida pneumonia, ARDS, ECMO

Anahtar Kelimeler
Candida pnömonisi, ARDS, ECMO

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Introduction

Acute Respiratory Distress Syndrome (ARDS) is an acute, diffuse, inflammatory lung injury that leads to increased pulmonary vascular permeability, increased lung weight, and loss of ventilated tissue. ARDS diagnostic criteria include new or worsening respiratory symptoms within a week, bilateral opacities that are compatible with pulmonary edema and cannot be explained by other reasons in chest X-ray or computerized tomography (CT) in the lack of heart failure or loading of excessive fluid to explain respiratory failure and the rate of arterial oxygen pressure (PaO_2)/fraction of inspired oxygen (FiO_2) ≤300 when positive end-expiratory pressure (PEEP) is ≥5 cmH_2O. When PEEP is ≥5 cmH_2O with mechanical ventilation, if PaO_2/FiO_2 is ≤100, it is defined as severe ARDS (1). Despite the significant developments in the management of severe ARDS since its definition, it causes more than 40% of the mortalities (2).

Extracorporeal membrane oxygenation (ECMO), which is a kind of extracorporeal life support (ECLS), provides oxygenation and carbondioxide elimination in one of two main configurations, either venoarterial (VA) or venovenous (VV). VV ECMO is used mostly in adult patients and represents 78% of ECLS use in this population (3). The patients who used ECMO for severe ARDS treatment had lower morbidity and mortality rates (1). In this case report, a patient with coronary artery disease in whom ARDS and septicemia were developed after *Candida pneumonia* and who was treated with ECMO was presented. However, the patient died on the 7th day of her admission to the intensive care unit.

Case Report

A 78-year-old woman with diabetes mellitus, hypertension, coronary heart disease, coronary stent and thyroidectomy was admitted to the intensive care unit. Her general condition was poor, she was confused, Glascow Coma Scale (GCS) score was 11 and the oxygen saturation was low (SpO_2: 80-90). Informed consent was obtained from the patient and relatives. There were bilateral diffuse ground-glass infiltrations in the chest X-ray. The auscultation of the lungs revealed bilateral coarse crackles.

The arterial blood gas (ABG) showed pH: 7.2, pO_2: 35 mmHg, pCO_2: 67 mmHg and SaO_2: 57, she was intubated and followed-up with synchronous intermittent mandatory ventilation in pressure support mode on FiO_2: 100% PEEP: 15 cmH_2O in mechanical ventilator.

A 12 F central venous catheter was placed and the patient was anaesthetized with 0.4 mg/kg/hour propofol infusion. In the following hours, the mode was switched to pressure-regulated volume control and recruitment maneuver was applied to the patient. Noradrenaline infusion of 0.15 mcg/kg/min was added to the treatment of the hypotensive patient and in addition to piperacillin + tazobactam treatment, levofloxacin was added.

According to the trans-thoracic echocardiography, pulmonary arterial pressure was 60 mmHg. The patient who was considered to have right heart failure and pulmonary hypertension was started on clopidogrel and sildenafil treatment. The culture from the bronchoalveolar lavage taken with bronchoscopy reported *Candida* growth. Anidulafungin was started to the patient with multiorgan failure such as hepatic and renal failure.

We decided to start VA ECMO support when the ABG of the patient showed pH: 7.15, pO_2: 50.9 mmHg, pCO_2; 72 mmHg, SaO_2: 82. ECMO catheters were placed to the left femoral artery and vein. After the heparine bolus of 80 u/kg, the maintenance infusion was started at the dose of 18 u/kg/hr. The efficacy of the administered heparine was controlled with activated clotting time (ACT). The ACT was tried to be kept between 150-200. In the follow up, anisocoria was detected in the patient. The GCS was 3-4 so cranial CT was obtained. In cranial CT, a probably acute hemorrhage area with focally characterized diffuse areas of parenchymal hemorrhage which reached to 7.5 cm at the widest diameter, which was hypodense centrally with a peripheral hyperdence rim that was considered to belong to the hemorrhage and which was at the level of corpus callosum in the middle line and extending partially to the opposite hemisphere, which occurred on the basis of a possible infection or old hemorrhage was observed in left frontal region. There was acute hematoma in the left at the level of basal ganglia and diffuse hemorrhage in the ventricules. There was diffuse hemorrhage also within the cerebellar folia, bilateral sylvian fissures with the right side being more prominent and in the

subarachnoid spaces in the bilateral fronto-parietal regions.

Discussion

ARDS was firstly defined in 1967. Ashbaugh et al. (4) reported a mortality rate of 58% in 12 patients with ARDS. Gille and Bagniewski (5) collected data from 7 countries, 233 ARDS patients were treated with ECMO by 90 health care providers. The cumulative survival rate was 15%.

Between 1986-2006, Brogan et al. (6) examined the registry of Extracorporeal Life Support Organization, which is an international network, to create the first major international multicenter database of ECMO for severe ARDS. That registry included 1.473 patients with a mean age of 34 years and their 78% were treated with VV-ECMO for the mean duration of 154 hours. The discharge rate and overall survival rate were 50%. Mortality associated risk factors were advanced age, decrease in the patient's weight, prolonged ventilation time before ECMO, arterial blood pH being lower than 7.18, underlying reason of respiratory failure and the complications of ECMO. VV ECMO was associated with increased survival compared to VA ECMO. About 9% of patients had radiographic findings related to cerebral infarction, bleeding or brain death (6).

The most important complication of ECMO in the first years was bleeding. Knoch et al. (7) showed surface-heparinized ECMO circuits and membranes reduced daily blood loss and required intravenous heparin dose. The survival rate was higher in patients treated with heparinized systems. During ECMO treatment, in a study using standard anticoagulation therapy with unfractionated heparin as continuous infusion followed by repeated measurements of ACT or activeted partial thromboplastin time (APTT), ACT values were found to be within the range of 180-220 second and the target APTT level was desired to be at least 1.5 times higher than the upper limit of the normal reference interval (8). Heparin and albumin coated cannulas were used in our patient. Heparin dose was regulated with ACT follow-ups and it was kept between 150-200.

Intracerebral hemorrhage or infarction in ECMO is a devastating and often fatal complication that occurs 10% to 15% of patients with ARDS (9). In the the Australian and New Zealand-ECMO series, 43% of deaths were associated with intracranial hemorrhage (ICH) (10). Seventy-four adults who received VA ECMO at a single center, the ICH rate was 18.9%. ICH risk increases with female gender, heparin use, renal insufficiency, dialysis need and most importantly, in correlation with thrombocytopenia. Mortality was 92.3% in patients with ICH whereas 61% in non-ICH patients (11). The emergence of ICH in the patient who fulfilled all criteria increased the risk of mortality.

The most important complication may be infection and sepsis. The large surface of the synthetic material is prone to colonization and infection. Of ECMO patients, 79% died from multi-organ failure or septic shock (12). The risk of bacteremia and fungemia is high in ECMO patients, because blood is in contact with artificial surfaces on which bacteria and fungi can easily spread. Cannulas allow skin bacteria to enter the bloodstream. There are no guidelines regarding prophylactic antibiotic or antifungal therapy for ECMO (13). Coagulase-negative *Staphylococci* and *Candida* species are common causes of ECMO-associated bloodstream infection, and the risk of infection with *Stenotrophomonas maltophilia* and Aspergillus species may increase in patients with long-term ECMO treatment (14). Despite the initiation of the treatment for *Candida pneumonia* in our case, perhaps the ECMO treatment has increased the risk of septicemia.

The incidence of right ventricular failure (RVF) in ARDS was reported as 9.6%-25%. The main reason for the development of RVF in ARDS is the increased pulmonary vascular resistance (PVR). Mechanical ventilation with PEEP also causes increase of PVR (15). In the studies on hemodynamic variables of pulmonary hypertension related RVF and survival with pulmonary arterial hypertension, low cardiac index and high mean right atrial pressure were observed to be associated with poor survival (16). When RV failure develops, VV ECMO can be changed to VAV mode to evacuate RV volume and RV shear stress, or VAV mode is preferred for the treatment of RVF and ARDS (17). VA ECMO mode was preferred in our patient with pulmonary hypertension and RVF.

Severe ARDS is associated with high morbidity and mortality rates in patients treated with mechanical ventilation in intensive care units. In recent years, the marked advances in ECMO technology have led to safer initiation and maintenance of this life-saving

treatment and reduced its risks regarding the patients. However, further well-designed, randomized clinical trials are needed before widespread use of treatment is accepted in suitable patients with severe ARDS.

Ethics

Informed Consent: Informed consent was obtained from the patient and relatives.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: E.S.Ö., A.C., M.O., Design: E.S.Ö., A.C., M.O., Supervision: M.O., Fundings: E.S.Ö., A.C., M.O., Materials: E.S.Ö., A.C., Data Collection or Processing: E.S.Ö., A.C., Analysis or Interpretation: E.S.Ö., A.C., M.O., Literature Search: E.S.Ö., A.C., M.O., Critical Review: M.O., Writing: E.S.Ö., A.C.

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Case Report / Olgu Sunumu

A Reason of Facial Diplegia: Guillain-Barré Syndrome

Bir Fasiyal Dipleji Nedeni: Guillain-Barré Sendromu

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Abstract

Guillain-Barré syndrome (GBS), an acute demyelinating inflammatory poliradiculopathy, is characterised by rapidly progressive, symmetrical, ascending weakness and areflexia. The disease usually occurs after 1-3 weeks of infection, vaccinations, trauma, surgical interventions and immunosuppression. Facial diplegia and paraesthesia which is a rare GBS variant has no weakness whereas, GBS is present with weakness which usually begins from lower extremities. In this article, three cases were presented to emphasise the importance of GBS in patients with facial diplegia.

Keywords
Facial diplegia, Guillain-Barré syndrome, paraesthesia

Öz

Guillain-Barré sendromu (GBS); hızlı progresif, simetrik, asendan yayılımlı güçsüzlük ve arefleksi ile karakterize akut, demiyelinizan, enflamatuvar poliradikülopatidir. Hastalık çoğunlukla 1-3 hafta önce geçirilmiş enfeksiyon, aşılanma, travma, cerrahi girişimler ve immünsupresyon gibi durumlar sonrasında ortaya çıkmaktadır. Çoğunlukla alt ekstremitelerden başlayan güçsüzlükle prezente olurken nadir görülen bir GBS varyantı olan fasiyal dipleji ve parestezide ise motor kayıp yoktur. Bu yazıda; fasiyal dipleji ile gelen hastalarda GBS'nin de önemini vurgulamak için üç olgu sunulmuştur.

Anahtar Kelimeler
Fasiyal dipleji, Guillain-Barré sendromu, parestezi

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Introduction

Guillain-Barré syndrome (GBS); acute demyelinating inflammatory poliradiculopathy, is characterized by rapidly progressive, symmetrical, ascending weakness and areflexia. GBS has become the most common disease causing acute generalized flask paralysis after the decrease in the frequency of poliomyelitis.

The incidence is 1-2/100,000. Generally patients apply to the clinic with a complaint of weakness. Weakness may begin in the lower extremities and spread to the arm, face, and oropharyngeal muscles. Rarely, there may be start an onset of extremity proximals or facial weakness (1). In this article, in order to emphasize the importance of GBS among the differential diagnoses in facial weakness, 3 cases of GBS who presented with facial diplegia were presented.

Case Reports

Case 1

A male aged 33 years had neck pain applied to the another hospital but no significant findings were detected in the examination. Five days later, he presented to our hospital with difficulty in swallowing and speaking. Systemic examination was normal. In the neurological examination of the patient, he was fully orientated. Lack of gaze was not detected. There was significant bilateral facial weakness on the right side of the patient.

Speech of the patient was dysphonic. Neck flexion was 4+/5, extension was 5/5, and lower and upper extremities were 5/5 on the patient's muscle strength examination. Sensory deficit was not detected in the patient. Deep tendon reflexes (DTRs) were hypoactive at the upper and lower extremities and no clonus and extensor plantar response. The patient was hospitalized with a initial diagnosis of GBS. There was no history of infection, trauma and vaccination in the anamnesis of the patient recently. No pathological findings were detected in whole blood tests, biochemistry tests and infectious markers. Cranial magnetic resonance imaging (MRI) was taken to exclude demyelinating disease, mass and vasculitic lesions was evaluated as normal. In the lumbar puncture test, cerebrospinal fluid (CSF) did not have any cells and the CSF protein was found to be 761 mg/dL. In the Electromyography (EMG) test, findings compatible with sensorimotor polyneuropathy with demyelinating feature were obtained. The patient was diagnosed with GBS and intravenous immunoglobulin (IVIG) treatment was administered at a dose of 0.4 g/kg/day for five days. After the treatment, the patient's speech improved, eye closing capacity improved, swallowing difficulties decreased. It was observed that facial weakness on the right side of the patient decreased after 16 months of treatment.

Case 2

A 36-year-old male patient complained of numbness in his hands and feet 15 days ago. After 10 days, the patient was admitted to with us the complaints of weaked eye closing and speech impairment. Systemic examination was normal. In the neurological examination of the patient, he was fully orientated. Lack of gaze was not detected. There was peripheral type facial paralysis on the right side of the patient and eye closing was weak on the left side. In the examination of muscle strength, neck flexion was 4/5, bilateral 4+/5 in the distal upper extremities, proximal and distal 5/5 in the lower extremities. DTRs were not seen at the lower, while biceps and triceps reflexes were not seen at the upper extremities, and styloradial reflex was hypoactive. There was no sense of deficit. No pathological reflex was detected. The patient was hospitalized with a initial diagnosis of GBS. In his anamnesis, it was learned that he had a history of upper respiratory infection about 3 weeks ago. In whole blood tests and biochemistry tests, no pathological findings were detected in infectious markers. Cranial MRI was taken to exclude demyelinating disease, mass and vasculitic lesions was evaluated as normal. In the lumbar puncture test, CSF did not have any cells and the CSF protein was found to be 70 mg/dL. In the EMG test, findings compatible with sensorimotor polyneuropathy with demyelinating feature were obtained. The patient was diagnosed with GBS and IVIG treatment was administered at a dose of 0.4 g/kg/day for five days. After the treatment, the patient's eye closing was better and in the examination of muscle strength the neck flexion became 5/5. At the control of the patient after 12 weeks, it was observed that the examination findings completely improved.

Case 3

A 49-year-old male patient complained of pain in the waist and legs 10 days ago, and steroid therapy was initiated due to peripheral type facial paralysis due to the development of weakness in the left facial half. He applied to our hospital after the patient feel weakness in the right half of the face a few days later. Systemic examination was normal. In the neurological examination of the patient, he was fully orientated. Lack of gaze was not detected. There was significant bilateral facial weakness on the left side of the patient. In the examination of muscle strength, there was no deficit. DTRs were not seen at the upper and lower extremities. Sensory deficit and pathological reflex were not detected. Nerve transmissions were detected normally in EMG. While F responses were normal at the upper extremities, tibial F response was extended at the left lower extremities. The findings were considered significant in terms of early polyneuropathy. Thepatient was hospitalized with a initial diagnosis of GBS. No pathological findings were

detected in whole blood tests, biochemistry tests, Lyme test and infectious markers. Cranial MRI taken to exclude demyelinating disease, mass and vasculitic lesions was evaluated as normal. In the lumbar puncture test, CSF did not have any cells and the CSF protein was found to be 113 mg/dL. It was observed that F responses disappeared in the control EMG performed 10 days later. The patient was diagnosed with GBS and IVIG treatment was administered at a dose of 0.4 g/kg/day for five days. It was observed that the patient's examination findings completely regressed at the 2nd month control after discharge.

Discussion

Peripheral facial paralysis is one of the most common neuropathies, but it is often idiopathic. Bilateral involvement is seen in 0.3% of facial paralysis and there is an underlying cause. In facial diplegia; conditions such as Lyme disease, Mobius syndrome, GBS, sarcoidosis, infectious mononucleosis, leukemia, meningitis, head trauma, multiple sclerosis should be considered. Although GBS is often seen as weakness starting from the lower extremities and spreading upwards, there are varying variants of starting and progression. Facial diplegia and paresthesia is one of the rarely seen GBS variants, and it appears as facial diplegia and paresthesia in the extremities and no motor loss is expected (2).

The majority of patients have a history of infection 1-3 weeks ago as seen in GBS. Less frequently, it can occur after vaccination, trauma, surgical procedures, and immunosuppression. Cytomegalovirus, Epstein-Barr virus, varicella-zoster virus, hepatitis A virus, hepatitis B virus, haemophilus influenza, campylobacter jejuni are some microorganisms that have been linked to GBS. Symptoms reach the highest level in the first 2-4 weeks in GBS. Rapid progression of the disease, bulbar involvement, development of autonomic dysfunction, accompanying facial diplegia are signs of poor prognosis. Approximately 70% of patients with bulbar involvement require a mechanical ventilator. In patients with autonomic dysfunction, deaths due to cardiac arrhythmias can be observed, so if possible, patients should be monitored closely in intensive care conditions. Significant improvement was observed in the prognosis of GBS with the improvement of intensive care conditions. Mortality rates, previously which were up to 33%, it had declined to 5%.

The diagnosis is made with clinical findings. While there is no cell increase in CSF examinations, protein increase is an expected finding. However, the lack of an increase in protein does not exclude the diagnosis of GBS, it may be related to its early stage. The most common electrophysiological abnormalities include prolongation of distal motor responses and F wave latency, absence or loss in F waves, conduction block, decrease in distal compound muscle action potential amplitudes and slowing in motor transmission rates (3).

In treatment, IVIG and plasmapheresis are administered. Studies have shown that neither treatment method is superior to each other. However, because of the experienced center requirement for plasmapheresis and higher risk of developing complications, IVIG; It has become the first choice in GBS treatment due to its ease of use. In patients administered IVIG, complications such as anaphylaxis, aseptic meningitis, congestive heart failure, thrombotic events, and acute renal failure can be observed (4). Therefore, plasmapheresis is recommended in cases such as hyperviscosity, congestive heart failure, chronic kidney failure and congenital IgA failure (5).

As a result, facial diplegia is a rare condition and the underlying causes need to be investigated. In cases such as accompanying paraesthesia or weakness, GBS should be carefully examined and diagnosis should be supported by electrophysiological and CSF examination.

Ethics

Informed Consent: Informed consent was obtained from the patients.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: Z.A., Design: A.A., N.K., Data Collection or Processing: Z.A., A.A., N.K., Analysis or Interpretation: A.A., N.K., Literature Search: Z.A., Critical Review: N.K., Writing: Z.A.

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Case Report / Olgu Sunumu

Ultrasonographically Diagnosed Bicipital Fasciitis: The Importance of Ultrasonography for Early Diagnosis and Emphasis on Aseptic Technique

Ultrasonografi ile Tanı Konulan Bisipital Fasiit: Erken Tanıda Ultrasonografinin Önemi ve Aseptik Tekniğe Vurgu

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Keywords
Bicipital fasciitis, diagnosis, ultrasonography, aseptic technique

Anahtar Kelimeler
Bisipital fasiit, tanı, ultrasonografi, aseptik teknik

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Abstract
Here, we present a case of bicipital fasciitis after intraarticular injection of the shoulder joint. Bicipital fasciitis was diagnosed using real-time ultrasonography. The infection was probably due to non-compliance with aseptic conditions. Therefore, we also want to emphasise the importance of using an aseptic technique.

Öz
Bu makalede gerçek zamanlı ultrasonografi ile tanı koyduğumuz, eklem içi enjeksiyon sonrası gelişen bir bisipital fasiit olgusu sunduk. Enfeksiyon muhtemelen aseptik koşullara uyulmaması nedeniyle gerçekleşmişti. Bu yüzden bu yazıda aseptik tekniğin önemini de vurguladık.

Introduction

There are few publications on the bicipital fascia infections in the literature, most of them are on necrotizing fasciitis (NF) of the fascia. It is reported that bicipital fascia infections leads to confusion in differential diagnosis (1). There is a paucity of signs seperates necrotising fasciitis from non-necrotising soft tissue infection or inflammation. Patients complain of pain, erythema, and swelling in both conditions, but tense oedema and/or bullae or skin necrosis are reported as diagnostic for necrotising fasciitis (2). Making early diagnosis of NF and making differential diagnosis from non-necrotising fascia infection is important because NF is a condition with significant mortality rate. Mortality for NF has been observed to be approximately 30% and it is reported that delay in definitive treatment because of failure to recognise and diagnose the condition at first presentation is the most common cause

of mortality (3,4). We reported here a case of bicipital fasciitis diagnosed at early stage using real-time imaging with ultrasonography (US) who underwent ultrasound guided aspiration simultaneously. We think, infection was most likely related to non-compliance with aseptic conditions. So, we also want to emphasize the importance of aseptic technique.

Case Report

A 53-year-old female patient was admitted with a complaint of severe pain spreading from her right shoulder to her elbow. Intraarticular sodium hyaluronate injection had been performed one month before due to right shoulder pain. Two days after the injection, the pain began spreading from the shoulder to the volar side of the arm and swelling developed in the volar aspect of the forearm.

The patient had not been able to sleep because of the pain for the past week and was suffering from sweating and fever at night. She did not describe a trauma to the arm or an infection. Locomotor system examination of the neck and upper limbs revealed the following: Right shoulder flexion was limited and painful in the middle of the joint range of motion. Passive movements of the elbow passive were in normal limits but painful. The voluntary flexion and supination of the elbow could not be made due to pain. She had a slight reddening on the skin over the biceps brachii muscle and a 10 cm x 8 cm swelling which was painful in palpation.

The patient underwent ultrasonographic evaluation. Ultrasonographic examination shows intense effusion around the biceps tendon. When the probe was moved towards the biceps muscle, the appearance of the effusion around the tendon disappeared, but a little later, a collection of liquids measuring about 11cm x 6 cm and 15 mm in depth was observed in the biceps fascia (Figure 1A, 1B). Inflammatory tissue gave ++ Doppler signal. Real-time US guided aspiration was performed. Because of the purulent nature of the aspirated liquid (Figure 2), the patient was referred to infectious diseases clinic.

Methicillin sensitive *Staphylococcus aureus* (*S. aureus*) was isolated from the aspirate culture. The patient was hospitalized. Laboratory findings were as follows: Erythrocyte sedimentation rate: 87 mm/h; C-reactive protein: 7.78 mg/dL, white blood cell count: 18,300/mm^3, urine culture: contaminant, biochemical analysis: normal, and brusella agglutination test: negative. 4x1.5 gr/day Sulbactam + Ampicillin treatment was started to the patient. After 11 days, significant improvement was observed and the patient was discharged with oral Amoklavin 2x1 gr/day treatment. At 3 weeks after discharge from hospital, his clinical symptoms declined; pain decreased and range of motion of the shoulder joint increased significantly. Signed consent form was obtained from the patient.

Discussion

The causes of necrotising soft tissue infection in the extremities are usually related to trauma, chronic wound infections, diabetes, obesity, chronic liver disease, immune suppression, alcoholism, intravenous injections, etc. It is usually a polymicrobial rather

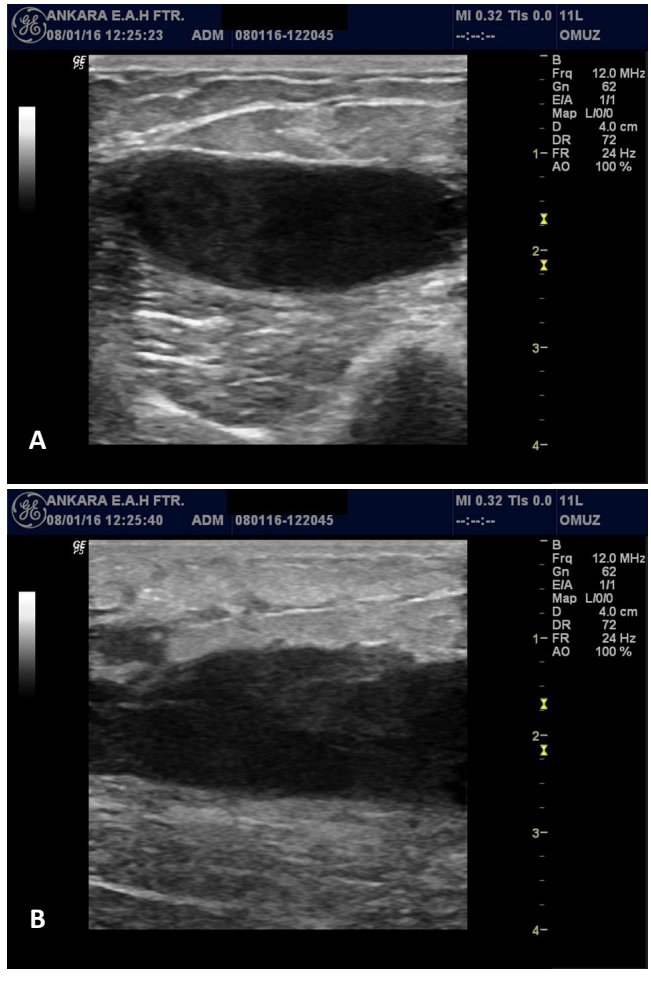

Figure 1. Liquid collection and synovial hypertrophy observed in the biceps fascia. **A)** Sagittal imaging, **B)** Axial imaging

Figure 2. A sample of aspirated fluid

than a monomicrobial infection (5,6). Polymicrobial NF is usually caused by enteric pathogens, whereas monomicrobial NF is usually due to skin flora. Yoshii et al. (7) reported a case of NF due to the injection of oral flora. Monomicrobial infection with *S. aureus* has been rarely reported as a cause of necrotising fasciitis. McHenry et al. (8) reported monomicrobial infections in 12 of 65 patients with NF. Only two of them had *S. aureus* infections. In another study, two of 6 patients with monobacterial infections had *S. aureus* infections among 83 patients with NF (9). *S. aureus* is a ubiquitous pathogen and one of the most common causes of severe community-associated infections of skin and soft tissue (10). It was also isolated from the oral cavity (11). In our case, *S. aureus* may be contamined from the the patient's skin or oral flora. Moreover it may be contamined from the oral flora of physician applying the injection procedure to the shoulder. So, we think that, it is important to use aseptic technique while injecting, as well as to use masks especially during periods when upper respiratory tract infections are increasing.

In our case, infection may have reached to bicipital fascia from injection site at the shoulder using fascial way. Stecco et al. (12) described that the pectoralis major fascia appeared to continue with the axillary, deltoid and brachial fascia. They reported that the fascia covering pectoralis major continued with the brachial fascia in two distinct ways: 1. the fascia overlying the clavicular part of pectoralis major had a thickening of collagen fibres extending in direction of the anterior brachial fascia; 2. the fascia covering the costal part continued with the axillary fascia and then with the medial brachial fascia.

US is the most practical and rapid method of obtaining images of the musculoskeletal system. It provides diagnostic benefits in daily clinical paractise because it is portable and relatively inexpensive (13). US offers many advantages for patients and physicians. It is well accepted by the patients because it is a noninvasive method with a quick scan time and without radiation. US also provide several advantages for clinicians. The ability to visualize needles and target structures in real time makes it an ideal tool for the guidance procedures used in diagnosis and management of musculoskeletal diseases. Its flexibility, availability, and low cost make it the best tool to guide interventional therapeutic procedures in musculoskeletal system lesions such as drainages of abscesses, bursitis, treatment of cystic lesions (ganglions, Baker's cysts), arthrocentesis, injection of substances in joints and soft tissues, and aspiration of calcific tendinitis (14).

The early diagnosis of musculoskeletal lesions using US can sometimes protect the patient from serious mortality. Testa et al. (15) reported a case of nonclostridial NF with extensive myonecrosis attributable to infiltrative procedure and detected early by bedside US in emergency department. They concluded that the bedside US may save time for the prompt management of life-threatening necrotising infections. Similarly with this report, in our case, the early diagnose of fasciitis was made possible through US. As shown in the images, blind aspiration of the fasciitis coludn't be possible due to synovial reaction. We able to get fluid sapmles thruough the US guided technique and thus, we able to make early detection of pathogen. In this way, we can be prevented the development of severe NF which may cause severe mortality.

In this article, we aimed to emphasize the importance of aseptic technique during injection and the use of US in the early diagnosis of soft tissue infections.

Ethics

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Authorship Contributions

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