CUMHURIYET DENTAL JOURNAL

ISSN: 1302-5805 e-ISSN: 2146-2852

Volume : 20 Issue : 2 2017

Cumhuriyet Üniversitesi

Diş Hekimliği Fakültesi Dergisi

Cumhuriyet Dental Journal

http://dergipark.gov.tr/cumudj

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ISSN 1302-5805 **e-ISSN** 2146-2852 Volume/20 – Number/2 2017

CUMHURİYET ÜNİVERSİTESİ

Diş Hekimliği Fakültesi

Dergisi

Diş Hekimliği Fakültesi

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Cumhuriyet Dental Journal

An official publication of the Faculty of Dentistry, Cumhuriyet University, Issues ara published 3 times a year.

Our Faculty Journal first went into press in 1998.

http://dergipark.gov.tr/cumudj

http://dergi.cumhuriyet.edu.tr/cumudj

ISSN 1302-5805

e-ISSN 2146-2852

Volume/20-Number/2-2017

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Should not exceed 300 words and should be presented under the following subheadings: Objectives, Materials and Methods; Results; Conclusions (For Reviews: Objectives; Data; Sources; Study selection; Conclusions). These subheadings should appear in the text of the summary. Provide a short, nonstructured, 1-paragraph abstract that briefly summarizes the problem encountered and treatment administered for clinical report.

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-discuss the results of the study.
-agreement with other studies should also be stated.
-identify the limitations of the present study, and suggest areas for future research.

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-concisely list conclusions that may be drawn from the research.

-do not simply restate the results.

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-References must be identified in the body of the article with superscript Arabic numerals.
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- include key for symbols or abbreviations used in Figures.

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ARAŞTIRMA / RESEARCH

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DERLEME / REVIEW

Cumhuriyet Dental Journal 2017; 20(2): 90-97
Doi: 10.7126/cumudj.345926 *RESEARCH ARTICLES*

DEMOGRAPHICS AND CHARACTERISTICS OF PATIENTS WITH TRAUMATIC BONE CYSTS: A RETROSPECTIVE REVIEW

Travmatik Kemik Kisti Bulunan Hastaların Demografik ve Karakteristik Özelliklerinin Değerlendirilmesi: Retrospektif Bir Çalışma

Yavuz Tolga KORKMAZ[1] Burak CEZAİRLİ[1] Turgay Peyami HOCAOĞLU[2]

Makale Kodu/Article Code ***:*** *155384*

Makale Gönderilme Tarihi ***:*** *02.12.2015*

Kabul Tarihi ***:*** *05.12.2015*

ABSTRACT

Objectives: The aim of this study was to evaluate the demographics and characteristics of the patients treated for traumatic bone cyst (TBC).

Materials and Methods: A retrospective review was conducted to determine the radiological, clinical and demographic characteristics of patients with TBC who were surgically treated over a 2-year period using data retrieved from computerized databases.

Results: The study sample consisted of 22 patients (24 lesions in total) with mean age of 22.9 years. All lesions were located in the mandible (16 in anterior mandible, 8 in posterior mandible) and diagnosed incidentally during routine dental examinations. There was no statistically significant difference between male and female patients in demographic characteristics. All patients were followed up for 6-18 months with uneventful healing.

Conclusions: TBCs should be kept in mind during examination of radiolucent lesions of the mandible particularly in younger patients. Along with the histopathological examination, clinical and radiological findings, symptoms of the patients, and surgeon's experience should be considered for a definitive diagnosis.

Keywords: Traumatic bone cysts, radiolucent lesion, mandible

ÖZ

Amaç: Bu çalışmanın amacı, travmatik kemik kisti (TKK) tanısıyla tedavi edilen hastaların demografik özelliklerini ve karakteristik bulgularını değerlendirmektir.

Gereç ve Yöntem: Çalışmamızda 2 yıllık süre içinde TKK tanısıyla cerrahi olarak tedavi edilen hastaların hasta takip dosyalarındaki klinik, radyolojik ve demografik kayıtları retrospektif olarak incelenmiş ve değerlendirilmiştir.

Bulgular: Bu çalışmaya, ortalama yaşları 22.9 olan 22 hasta (24 TKK) dahil edilmiştir. Çalışmaya dahil edilen hastalardaki lezyonların tümü mandibulada belirlenmiş (16'sı anterior mandibulada, 8'i posterior mandibulada) ve rutin dental muayene sırasında tespit edilmiştir. Hastaların cinsiyet dağılımında istatistiksel olarak anlamlı bir fark bulunamamıştır. Hastalar 6 ile 18 ay takip edilmiş ve sorunsuz bir iyileşme sağlanmıştır.

Sonuçlar: Mandibula yerleşimli radyolüsent lezyonların ayırıcı tanısında özellikle genç bireylerde TKK da değerlendirilmelidir. Ayırıcı tanıda histopatolojik inceleme ile birlikte hastanın semptomları, klinik ve radyografik bulguları ve cerrahın tecrübesi de göz önünde bulundurulmalıdır.

Anahtar kelimeler: Travmatik kemik kisti, radyolüsent lezyon, mandibula

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INTRODUCTION

The term “traumatic bone cyst” (TBC) was first used by Lucas in 1929 to describe cavities within the mandible that do not have a true epithelial lining. Since then, numerous synonyms have been used to refer to the same entity including solitary bone cyst, hemorrhagic bone cyst, extravasation cyst, progressive bone cavity, simple bone cyst and juvenile bone cyst. However, traumatic bone cyst is more extensively used in literature. The World Health Organization (WHO) defines TBC as a non-neoplastic osseous lesion because it demonstrates no epithelial lining, which differentiates this lesion from the true cysts. Thus, TBCs are included in the group of bone-related lesions, together with the aneurysmal bone cyst, ossifying fibroma, fibrous dysplasia and osseus dysplasia.[1,2]

Traumatic bone cysts slowly progress and usually there is no bone expansion; lesions are mostly detected during routine radiological examination. While facial deformity is not always present, occlusal radiography may show perforation of the buccal cortex in some cases.[1] Pain is reported in 10-30% of cases.[2, 3] Other symptoms including tooth sensitivity, paresthesia, fistulae, delayed eruption of permanent teeth, pathological fracture of the mandible and displacement of the mandibular canal inferiorly occur rarely and may suggest malignancy.[1, 4, 5] Usually, the adjacent teeth are vital and there is no mobility or displacement.[6] Although root resorption is reported in some studies, it is rarely seen.[7]

The lesion is mainly diagnosed in young patients most frequently during the second decade of life.[2] Men are affected somewhat more frequently than women. Apart from the jaws, large TBCs may also develop in an extremity and have the potential for further growth. On radiological examination, a traumatic bone cyst often appears as a unilocular radiolucent area with an irregular but well-defined outline. Characteristic for the traumatic bone cyst is the "scalloping effect" when extending between the roots of the teeth. Histological examination is often inconclusive; thus, histological data should be supported with an evaluation of characteristic clinical and radiological findings to reach a TBC diagnosis. Due to clinical and radiological similarities with TBC, central giant cell granuloma and aneurysmal bone cyst should be taken into account in the differential diagnosis.[8-10]

The majority of traumatic bone cysts are located in the mandibular body between the canine and the third molar or mandibular angle.[2, 11] The second most common sites include mandibular symphysis, ramus and condyle. Mandibular TBC has a much higher incidence (89%) compared to that of maxillary TBC (11%).[2]

TBC may be diagnosed when the surgeon encounters an empty, well-circumscribed cavity with no epithelial lining upon entering the cyst during a surgical procedure or biopsy. Curettage of the bone cavity gives a mixed material consisting of blood, bone fragments and connective tissue. There is no evidence for epithelial lining and microscopic examination frequently shows fibrin aggregates, erythrocytes and occasional giant cells adjacent to the bone.[6] Since material for histologic examination is often inadequate, it is difficult to reach a definitive TBC diagnosis.[1] The surgical exploration serves as both a diagnostic manoeuvre and as definitive therapy. Cryosurgery, bone grafting and autogenic blood injection could be use as alternative treatment options in some cases.[12] TBC recurrence is a rare event.[13]

This report presents the clinical, radiological and surgical findings of 22 patients diagnosed with TBC who were followed at our clinic.

MATERIALS AND METHODS

A retrospective review was conducted with the aim to evaluate clinical, radiological and surgical findings of TBC cases treated between January 2013 and January 2015 at the Faculties of Dentistry, Departments of Oral and Maxillofacial Surgery of Karadeniz Technical University and Cumhuriyet University. Age at admission, gender,

location and multiplicity of the lesion, imaging method used [Panoramic Radiography (PR) and Cone Beam Computed Tomography (CBCT)] and treatment procedures were reviewed for all patients by joint assessment of patient follow-up files and data recorded in the computerized system.

For this study, patients with clinical and radiological evidence of TBC were identified and among those, patients who were followed subsequent to confirmation of the diagnosis by surgery and histopathological examination were enrolled. Patients with TBC involving devitalized teeth or cystic epithelium and those with insufficient follow-up and inconsistent histopathology reports were excluded from the study. Patients were operated under local anesthesia using routine surgical approach. Only one vertical incision and marginal incision was performed for each lesion which was enough to expose the surgical site. Bone cavity was exposed using an appropriate flap removal procedure using a periosteal elevator followed by removal of a small part of the osseous barrier by a dental electric motor under irrigation to obtain an entrance into the bone cavity. This procedure serves as both a diagnostic manoeuvre and as definitive therapy for supplying blood accumulation in to the cavity. No futher bone removal was performed after exploring the bone cavity if the case was diagnosed as TBC. For cases suggesting a TBC, the cavity was curetted and curettage materials were sent for histopathological examination. The surgical site was closed with 3-0 silk sutures upon observing that the cavity was filled with blood spontaneously. Following the surgery, 1 g Amoxicillin + clavulanic acid twice daily, flurbiprofen 100 mg twice daily and a mouthwash containing benzydamine HCl and chlorhexidine gluconate twice daily (excluding the day of surgery) were prescribed and routine instructions were given to all patients; sutures were removed seven days after the operation.

RESULTS

Clinical, radiographic and demographic data of study patients are shown in Table 1.

Table 1. Summary of clinical, radiographic and demographic data of study patients

Patient no.	Gender	Age (y)	Location	Jaw	PR	CBCT	Bilateral TBC	Apical Resection
1	M	18	anterior	mandible	+			
2	F	23	anterior	mandible	+			
3	M	17	anterior	mandible	+			
4	F	16	anterior	mandible	+			
5	M	20	anterior	mandible	+			+
6	F	19	anterior	mandible	+			
7	M	36	anterior	mandible	+			
8	F	21	anterior	mandible	+			
9	F	19	anterior	mandible	+			
10	M	21	anterior, posterior	mandible	+	+	+	+
11	M	22	posterior	mandible	+			
12	F	19	anterior	mandible	+			
13	M	58	posterior	mandible	+			
14	F	32	anterior	mandible	+			
15	M	20	anterior	mandible	+			
16	M	18	posterior	mandible	+			
17	M	19	anterior	mandible	+			
18	F	32	anterior	mandible	+			
19	F	26	anterior	mandible	+			
20	M	12	posterior	mandible	+			
21	M	20	posterior	mandible	+	+	+	
22	M	16	posterior	mandible	+	+		

Abbreviations:
M, Male; F, Female; PR; Panoramic radiography, CBCT; Cone Beam Computed Tomography. TBC, Traumatic bone cyst

Of 22 patients enrolled in the study, 12 (45%) were male and 10 were female (%55). There was no substantial difference between sexes with respect to TBC prevalence. The mean age of patients was 22.9 years (23.7 years for males and 21.9 years, respectively). A total of 24 TBC cavities were found in 22 patients. Unilateral lesions were observed in 20 patients (Figure 1) and two patients had bilateral lesions (involving anterior and posterior mandible in a patient and posterior mandible in a patient) (Figure 2).

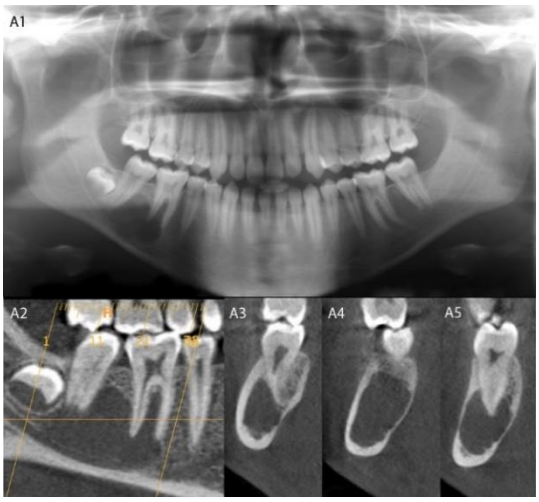

Figure 1. PR (A1) and CBCT (A2, A3, A4, A5) images of Patient No. 22 with a unilateral TBC

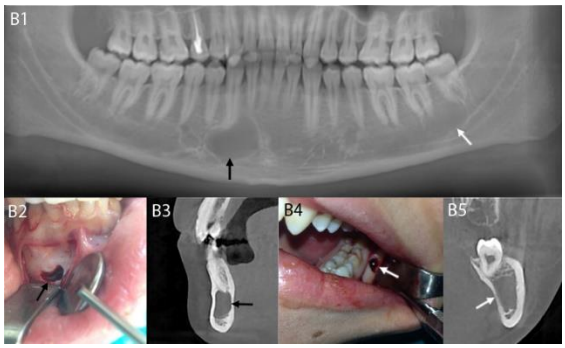

Figure 2. PR (B1), CBCT (B3, B5) and intraoperative (B2, B4) images of Patient No. 10 with bilateral TBCs

All lesions were located in the mandible including 16 in anterior mandible and 8 in posterior mandible. Apical resection treatment was performed in 2 patients due to root surface resorption possibly related to the lesion. Additional CBCT imaging was required only in 3 patients and panoramic radiographs were sufficient for other cases. All lesions found in patients included in the study were discovered incidentally routine clinical and radiological examinations. None of the lesions admitted to our clinic due to a specific complaint associated with lesion site. Patients were followed over a period ranging between 6 and 18 months (mean duration, 14 months). Healthy resolution was considered when patients showed restoration of bone structure with reformation of bone during follow-up (Figures 3 and 4).

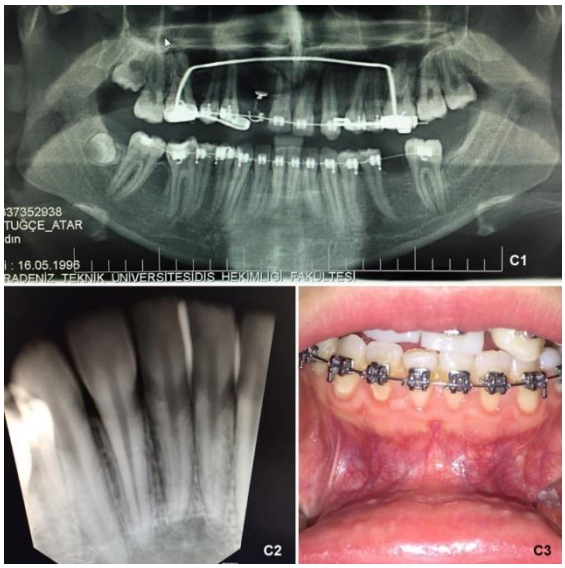

Figure 3. Preoperative periapical radiography (C2), postoperative PR (C1), and intraoral (C3) images of Patient No. 6 with a TBC

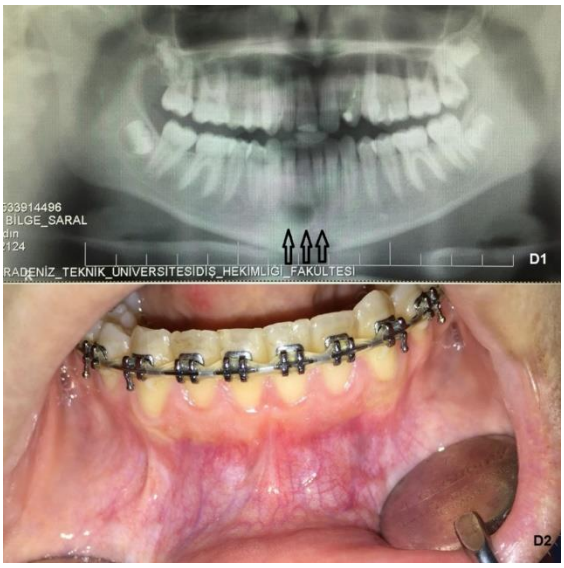

Figure 4. Preoperative PR (D1) and postoperative intraoral images of Patient No. 9 with a TBC (D2)

A second operation was performed only in one patient who showed no signs of bone healing and achieved full resolution after 12 months.

DISCUSSION

Etiology and pathogenesis of TBC have not been fully elucidated yet but a number of theories were suggested in literature.[14] Degeneration of bone tumors, altered calcium metabolism, low-grade infections, local alterations in bone growth, venous obstruction, intensified osteolysis, intramedullary bleeding, local ischemia or combinations of such factors are believed to be involved in the etiology. The most widely accepted theory suggests that a hematoma within the bone caused by any form of trauma including tooth extraction may have a pivotal role in the development of this lesion.[15] Following a trauma, resorption of the blood clot takes place in the presence of impaired healing and liquefactive necrosis. The surrounding bone is destroyed by enzymatic activity and as a result, the bone cavity enlarges, stimulated by poor venous drainage.[10, 16] Nevertheless, unusually large TBCs with an aggresive course have been rarely reported.[12]

While panoramic radiography is usually sufficient for diagnosing a traumatic bone cyst.

Although roots resorption is not a common finding in TBCs, CBCT is useful for assessment of the extent of destruction/resorption within the affected bone or teeth. However panoramic radiographs do not provide much information for differential diagnosis since PR does not allow examination of the cystic epithelium. Nevertheless, some researchers reported that dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) may be used for differential diagnosis of TBC to exclude other cysts.[17] In the present study, CBCT was required only for 3 patients to evaluate the presence of lesion-related root resorption in the teeth and PR imaging sufficed for other patients.

Traumatic bone cysts are commonly found in long bones such as proximal humerus and femur but maxillofacial bones may also be affected, albeit rarely (0.5-1.2%).[18] Among all cysts of the jaw, TBCs have a prevalence of 1%. When these cysts develop in the jaws, mandibular involvement is frequent with rare involvement of maxilla. Several reasons were suggested to explain this difference in the site of origin including differences between maxilla and mandible in the amount and quality of bone marrow and in the extent of vascularization or difficulties in detecting maxillary lesions by radiography due to maxillary sinus.[19, 20] Consistently, in our study, all lesions originated from the mandible and no lesion was found in the maxillary bone.

It is rare for a TBC to develop after 25 years of age because it will have been detected earlier during routine dental examinations.[21] Literature data show that TBCs are mainly diagnosed in young patients during the second decade of life but they may also occur at a later age. Availability of PR imaging in many centers, advances in oral and dental care services and increased awareness for this condition contribute to the detection of these lesions during routine examinations at an early stage. Also, some lesions may spontaneously heal over time and this is probably the reason why they are rarely found in older age groups as suggested by several reports.[8] Similarly, the mean age of our patients was 22.9 years and only 3 patients were older than 25 years of age (28, 36 and 58 years of age). While predominance of female gender was reported in literature, generally it is considered that TBCs occur in both sexes with comparable incidences.[2, 19] Consistently, there were 12 males and 10 males in our study (total n=22) with no significant differences between sexes. Multifocal TBCs were reported to be diagnosed during the second decade of life and this is consistent with our findings.[19]

Anterior mandible has been reported in literature as the most common site for TBC.[22] Consistent with literature data, the majority of lesions were located in the anterior mandible in the present study: 8 lesions (33%) in the posterior mandible and 16 (67%) in the anterior mandible.

Multifocal and bilateral TBC cases usually occur during the second decade of life at an incidence varying between 0% and 11%.[19, 23, 24] In the current study, two patients had multiple TBCs bilaterally (one 20-year old patient and one 21-year old patient) and this low incidence was consistent with literature. Insufficient venous circulation, trauma, impaired calcium metabolism, osteogenesis imperfecta and idiopathic thrombocytopenic purpura have all been implicated in the etiology of these multifocal lesions.[9] However, none of our patients showed evidence for these etiological factors.

Studies reported that expansion of the bone was rare in TBC cases.[8] Similarly, in the present study, there were no cases of bone expansion noticed during surgical procedure or preoperative CBCT examination.

Root resorption is frequently observed with orthodontic teeth movements, occlusal traumatism, periodontal disease, periapical granulomas, and re-implanted teeth, cyst or

neoplasms while the mechanisms of root resorption in association with cysts and neoplastic lesions are mostly unknown.[7] It is known that intracystic pressure or a high level of prostaglandin released by tumors may have effects on root resorption.[25] While the lack of cystic epithelium and cystic fluid in TBCs eliminates this possible pressure related cause. Immunohistochemical studies have shown that the receptor activator of nuclear factor κB (RANK), RANK ligament (RANKL), osteoprotegerin (OPG) proteins are involved in root resorption. Osteoclast (odontoclast) maturation and activity are regulated in vivo by RANK–RANKL and OPG levels of expression and mediators such as TNF-α, interleukin (IL)-1ß, IL-2ß, IL-6, and prostaglandins, can exert their effects through regulation of RANK, RANKL and OPG levels.[26] In the present study, TBCs were reported to cause resorption adjacent teeth[8] and apical resection was required only in 2 patients for devitalized teeth or teeth with excessive root resorption.

In order to decide on the final diagnosis, it is necessary to carry out an exploratory surgery, which at the same time is the main therapeutic procedure.[8] Following surgical exposition, the affected area is curetted and organization of a blood clot takes place, resulting in healing by the formation of new bone. While cochleation of the cavity stimulates the bleeding, injection of autogenic blood into the bony cavity could be preferred with large bony defects to speed up the healing period.[8, 27] Spontaneously healing may also occur in some cases.[8] Cryosurgery and bone grafting could be a treatment option in suitable cases.[12] In longstanding or large lesions treatment is by curettage of the cavity, which results in clot formation and complete bony infill.[28] Recurrences are very rare after appropriate surgical treatment (%1.7). Follow-up for 6 to 12 months is sufficient to assess healing and recurrence. Inadequate curettage was reported as the most common cause of recurrence.[15, 29] In our study, a second surgical treatment was performed for only 1 patient because no evidence for healing was observed within the bone cavity at 6 months of follow-up but full resolution of the lesion was achieved at 12 months.

Our findings are consistent with those reported in literature with respect to patient age, gender and location of the lesion. While routine CBCT examinations are not always considered to be necessary, they may sometimes be used to assess resorption in the adjacent teeth and to establish a treatment plan for root canal treatment and/or apical resection.

Care should be exercised during differential diagnosis of a traumatic bone cyst, since treatment procedure for TBC differs from those of radiologically similar cysts. In cases where the cyst arises around the apex of a tooth, radiological findings may resemble those of a radicular cyst; thus, a thorough evaluation of clinical findings such as vitality of the affected teeth is crucial. Thus, a correct diagnosis would avoid unnecessary apical resection procedures which are frequently undertaken for other cysts involving tooth roots.

In conclusion, although TBCs are infrequent, they should be considered in the differential diagnosis of radiolucent lesions located in the mandible particularly in younger patients.

In addition to histopathological examination, surgeon’s experience, patient’s symptoms, case history and radiological findings should also be taken into account for differential diagnosis.

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EFFECT OF LOCAL RIFAMYCIN APPLICATION ON EXPRESSION OF BMP-2 AND BONE REGENERATION

Yerel Rifamicin Uygulamasının Bmp-2 ve Kemik Rejenerasyon Açısından Etkisi

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Makale Kodu/Article Code *: 189624*
Makale Gönderilme Tarihi *: 17.05.2016*
Kabul Tarihi *: 27.05.2016*

ABSTRACT

Objectives: The aim of this study was to evaluate effect of local rifamycin application on BMP-2 expression and bone healing.

Materials and Methods: A standardized 5.0-mm- diameter critical size bone defect was created mandible angulus region. In the control group (8 rats) defects were left empty. In the Group 1 (n=8 rats) defect was irrigated with rifamycin solution and 25 mg rifamycin solution injected defect area at 1, 3, 7 days after surgery. In the group 2 (n=8 rats) defects were grafted with a gelatin sponge mixed 25 mg rifamycin solution. Rats were sacrificed at 21 days after surgery. Histological slides were prepared from defect site for both immunohistochemical analysis (bone morphogenetic protein-2 (BMP-2) antibody) and histomorphometric analysis. Data were analyzed using Mann Whitney U and Kruskall Wallis test.

Results: The average new bone formation, number of osteoblast and new vessel formation count were increased more in both of experimental groups in comparison with control group. Anti-BMP-2 labelling (Cell count) was increased more in both of experimental groups in comparison with control group.

Conclusion: Local rifamycin application has positive effects on BMP-2 expression and bone regeneration at critical sized bone defects.

Keywords: Rifamycin, critical sized bone defect, bone regeneration, bone morphogenetic protein – 2

ÖZ

Amaç: Bu çalışmanın amacı lokal rifamisin uygulamasının kemik iyileşmesi sırasında BMP–2 salınımı üzerine etkisinin değerlendirilmesidir.

Materyal ve method: Rat mandibula angulus bölgesinde standart olarak 5 mm çapında kritik boyutta kemik defektleri oluşturulmuştur. Kontrol grubunda (8 rat) defektlere herhangi bir uygulama yapılmamıştır. Birinci deney grubunda (8 rat) defekt bölgesi rifamisin solüsyonu ile irrige edildikten sonra, defekt bölgesine 1, 3 ve 7. günlerde 25 mg rifamisin solüsyonu enjekte edilmiştir. İkinci deney grubunda (8 rat) defekt bölgesi 25 mg rifamisin solüsyonu ile karıştırılmış gelatin sponge ile greftlenmiştir. Cerrahiden 21 gün sonra ratlar sakrifiye edilmiştir. Defekt bölgesinden hem immünhistokimyasal analiz (kemik morfogenetik protein –2 antibody) için hem de histomorfometrik analiz için histolojik kesitler hazırlanmıştır. Elde edilen verilerin analizi Mann Whitney U ve Kruskall Wallis testi kullanılarak yapılmıştır.

Bulgular: Deney grubunda kontrol grubuna göre ortalama yeni kemik formasyonu, osteoblast sayısı ve yeni damar oluşum sayısında artış olduğu görülmüştür. Her iki deney grubunda da anti–bmp–2 ile işaretlenmenin (hücre sayma) kontrol grubuna göre daha fazla olduğu görülmüştür.

Sonuç: Kritik boyutta kemik defektlerine lokal olarak rifamisin uygulamasının BMP–2 salınımı üzerine pozitif etkileri olduğu tespit edilmiştir.

Anahtar Kelimeler: Rifamisin, kritik boyutta kemik defekti, kemik rejenerasyonu, kemik morfogenetik protein – 2

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INTRODUCTION

Despite improvements in antibiotic therapy and surgical procedures, bone defect reconstruction, bone infection, and bone graft resorption, are still remaining as problems in oral and maxillofacial surgery. Bone reconstruction success depends on the size of defect, regeneration capability, stability, vascularization, and infection.

Infection is a crucial factor and can have hazardous effects on bone healing.[1,2] Bone resorption increases at lower pH levels, resulting in bone augmentation failures.[1,2] Infection inhibits cytokine release, resulting in compromised bone wound healing or bone graft resorption.[3] Local antibiotics are commonly used to treat bone infections such as osteomyelitis, or perimplantitis, to prevent initial infection risk, or, recently, as prophylactic treatment added to bone grafting materials empirically.[4] Bone graft vascularization and blood supply is poor; therefore, systemic use of antibiotics cannot reach adequate levels of antibacterial concentration. This dilemma can be resolved by local delivery of antibiotics. Locally administrated antibiotics may reach a twenty-fold higher concentration in graft site versus intravenous administration.[4]

The infected bone area is must provide a framework of both osteoinductive and osteoconductive materials, along with antibiotics.[5] An osteoconductive carrier system delivering antibiotics and osteoinductive agents locally would be an ideal and novel approach for treating infected bone defects.[6] Several osteoconductive bone substitutes and natural polymers are used as local antibiotic delivery vehicles. Collagen is widely used as a carrier material for drug delivery and provides a physical scaffold around the antibiotic, mechanically limiting fluid flow, or as a scaffold for bone engineering. Collagen can also stimulate the proliferation of osteoblasts and the production of collagenous callus tissue, thereby aiding the formation of new bone.[7]

The healing of bone defects involves in three mechanisms; osteogenesis, osteoconduction and osteoinduction.[8,9] Osteoinduction provide the biological stimulus along with signal pathways for the transformation and stimulation of stem cells into bone-producing cells during bone regeneration.[9,10] Bone morphogenetic Proteins (BMP) are members of the transforming growth-factor superfamily β that are known to regulate the differentiation and proliferation of several cells.[11] BMP-2 has the highest osteoinductive capacıty among BMPs.[12] Release of BMP-2 begins in the early stages of the bone healing process; recombinant BMP-2 is used to induce bone formation in reconstructive procedures.[11,13]

Rifamycins are semisynthetic bactericidal antibiotics, and that are effective against Gram positive and Gram negative bacteria. Rare allergic reactions and a few adverse effects may occur after local application of rifamycin.[14,15] Rifamycins are used for surgical site infection in orthopedic and maxillofacial surgeries and are well tolerated by bone tissue. Previous studies reported rifamycin may positively affect bone tissue, extraction socket healing, and osteomyelitis treatment.[16-19] In the literature, there is a little knowledge about the effect of antibiotics on BMP expression. Previously Ufuk *et al.*[20] reported rifamycin is a suitable solution for bone decontamination and may induce BMP-2 expression. We aimed to investigate the effect of local rifamycin application on BMP-2 expression and bone formation.

MATERIAL AND METHODS

All animal procedures were approved by the Institutional Animal Care ε Use Ethical Committee of Cumhuriyet University (permit no: 2011-248), and their care was in accordance with institution guidelines. Wistar albino rats (n=24) were used for this study. The rats were at

the adult stage and weighed approximately 300 g. The animals were kept in cages and fed a solid diet and water and libitum.

The 24 rats were divided into one control group (n = 8) and two experimental groups of 8 (group 1 and group 2). Standardized 5 mm diameter critical-size bone defects (CSDs) were created in the right mandible angulus. CSDs were left empty in control group. Defects in experimental group1 were irrigated with Rifamycin SV (Rifetem 250 mg, Ulagay, İstanbul, Turkey; Figure 2) and 25 mg Rifamycin SV was injected with an insulin injector at the defect area on the first, third, and seventh days after surgery. Defects in experimental group 2 were grafted with a gelatin sponge (Spongostan, hemostatic absorbable gelatin sponge, Ferrosan, Denmark) mixed with 25 mg Rifamycin Solution (Figure 1).

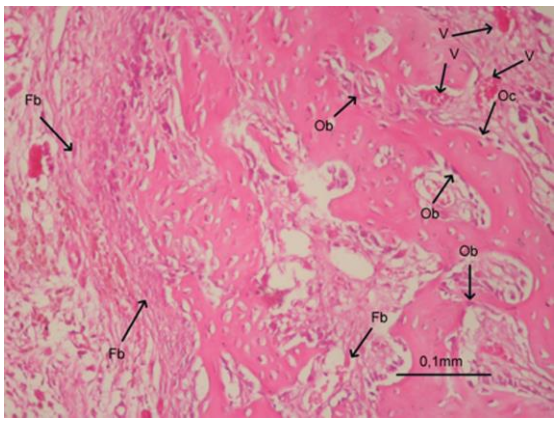

Figure 2. Histologic evaluation of defect area (Ob:osteoblast, Oc: osteoclast, Fb: fibroblast, V: vessel). Bar: 100µm. Haematoxylin-and-eosing staining.

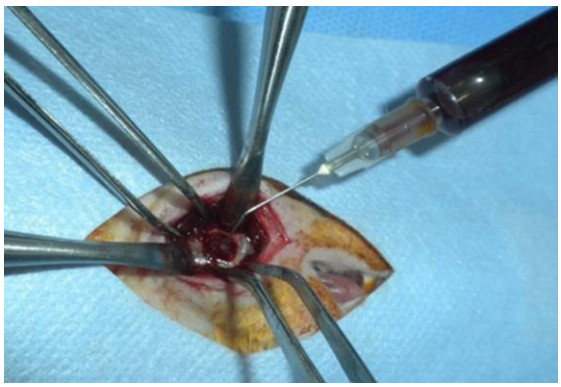

Figure 1. Irrigation of rifamycin solution.

Surgical Procedure

For all surgical operations, the rats were anesthetized with an intraperitoneal injection of 3 mg/kg Xylazine (Rompun 2%; Bayer, İstanbul, Turkey) and 90 mg/kg Ketamine HCl (Ketalar; Eczacıbaşı- Warner Lambert, İstanbul, Turkey). Defects were created on right the mandible angulus.

The skin of the mandible was shaved and disinfected with iodine. An incision was made inferior to the angle of the mandible extending to the mandibular bone and the periosteum of the mandible was ablated. A standardized 5 mm diameter defect was created using a surgical trephine with an internal diameter of 5 mm. Subcutaneous tissues were sutured with 5-0 Vicryl (Pegelak, poly-glycolide-co-lactide) [PGLA]; Doğsan, Trabzon, Turkey), while the skin flaps were closed using 5-0 nylon sutures (Ethicon, Edinburgh, UK) and allowed to heal by primary intent. All the animals received a subcutaneous antibiotic and analgesics: 25mg/ kg ceftraixone (Rocephine, Roche, Basel, Switzerland) and 4 mg/kg carprofen (Rimadyl, Pfizer, New York, NY, USA), respectively, for 3 days at every 24 hours, starting immediately after operation.

Histologic and Immunohistochemical Analysis

The rats were sacrificed on the 21st day after surgery with an overdose of sodium pentobarbital. The mandible bones were excised and separated into hemimandibles together with the surrounding tissue, and fixed in 10% buffered paraformaldehyde for 48 hours; they were then decalcified in ethylenediamine tetra-acetic acid (EDTA) solution. The tissue specimens were prepared in an autotechnicon, embedded in paraffin, and sectioned (5 µm) with a microtome. The sections were stained with haematoxylin-eosin. The tissue sections were examined and imaged by means of a Nikon Eclipse E400 light microscope and Nikon Coolpix 5000

digital camera. All photographs were then transferred into a PC environment and analyzed (Clemex Vision Lite 3.5 Image Analysis, Clemex Technologies, Longueuil, Quebec, Canada). The length was calibrated by comparing the photograph of the specimen with the photograph of the Nikon micrometer microscope slide, which was taken under the same magnification. An area of 0.4 mm^2 was designated using the Clemex Vision Lite 3.5 Image Analysis program, and osteoblasts, osteoclasts, and new bone areas were marked with the same Image Analysis program in a 0.4 mm^2 area. Damaged cells were not evaluated. The marked cells were counted automatically with the same image analysis program. The histological procedure was performed in a different department, pathologists were blinded to the animal group's information, and measurements were evaluated with the image analysis programme for reproducibility of procedure.

For immunohistochemical staining, the sections (5 µm) were stained with hematoxylin and eosin and monoclonal antibodies for analysis of BMP-2 expression (rhPro-BMP-2, clone: 253717, mouse monoclonal antibody, [R&D Systems, Inc]). Immunostained cells were evaluated in the same manner described above. First, a 0.4 mm^2 area was designated using the image analysis program; then positive-stained cells were then marked with the same image analysis program in a 0.4-mm^2 area. Damaged cells were not evaluated. The marked cells were counted automatically with the same image analysis program. The measurements were repeated 5 times, and then the average data were obtained.

The mean (SD) was calculated for each group. The data were analyzed using Kruskal Wallis analysis and Mann-Whitney U- test. Probabilities of less than .05 were accepted as significant.

Histometric Results

The histological specimens of all groups are shown in Figure 2-5.

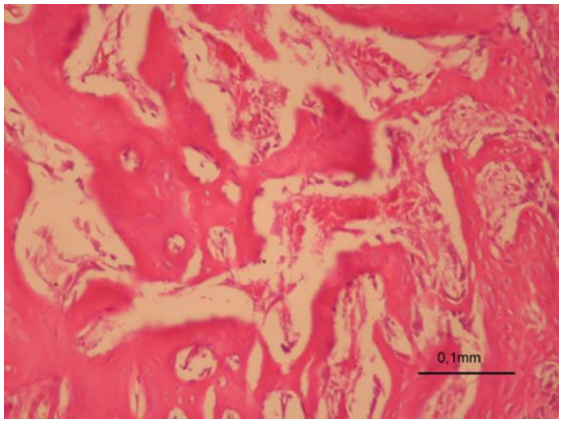

Figure 3. Histological evaluation of Control Group. Bar: 100µm. Haematoxylin-and-eosing staining.

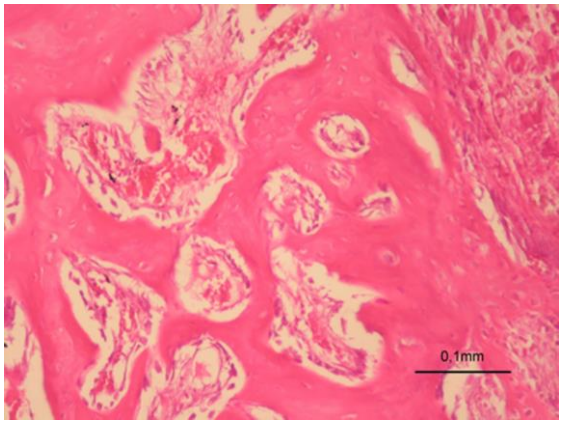

Figure 4. Histological evaluation of Group 1 (Rif injected). Bar: 100µm. Haematoxylin-and-eosing staining.

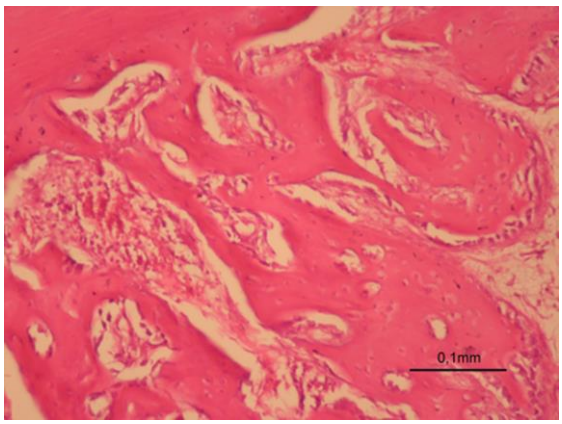

Figure 5. Histological evaluation of Group 2 (Rif mixed with gelatin sponge). Bar: 100µm. Haematoxylin-and-eosing staining.

New bone area, and osteoblast, osteoclast, fibroblast and new vessel counts were evaluated. New bone volume, as well as osteoblast, fibroblast and new vessel counts were higher for group1 and group 2 than the control Group ($p<0,05$). No statistically significant differences were found in terms of new bone area or osteoclast, fibroblast, and new vessel counts between groups 1 and 2 (Table 1).

Table 1. Mean (SD) histometric results of defect regions of selected 0.4 mm^2 area.

Variable	Control group Mean (SD)	Group 1 (R.I) Mean (SD)	Group 2 (G.S) Mean (SD)	P- value
New bone area (mm^2)	78583,71 (4834,92)	88789,09 (3643,30)	89287,44 (3413,64)	KW: 12,62 P: 0,001*
Osteoblast Count	15,20 (2,69)	17,30 (1,63)	19,40 (2,06)	KW: 4,75 P: 0,029 *
Osteoclast Count	1,10 (1,37)	1,10 (0,56)	0,70 (0,67)	KW: 0,69 P: 0,403
New Vessel Count	2,80 (0,78)	4,10 (0,73)	4,20 (0,78)	KW: 8,49 P: 0,004 *
Fibroblast Count	19,60 (2,67)	22,40 (2,87)	21,80 (2,65)	KW: 4,52 P: 0,033*

Data were analysed using Kruskall Wallis and Mann Witney U test. The level of significance was set at $P < 0.05$. * : $P < 0.05$. R.I: Rif injection and irrigation. G.S: Rif mixture with gelatin sponge.

Immunohistochemical Results

The immunohistochemical specimens of all groups are shown in figures 6-7. The BMP-2 counts in group 1 and group 2 were more statistically significantly higher when compared to the control Group ($p<0,05$). No statistically significant differences were seen in terms of the BMP-2 count between group 1 and group 2 (Table 2).

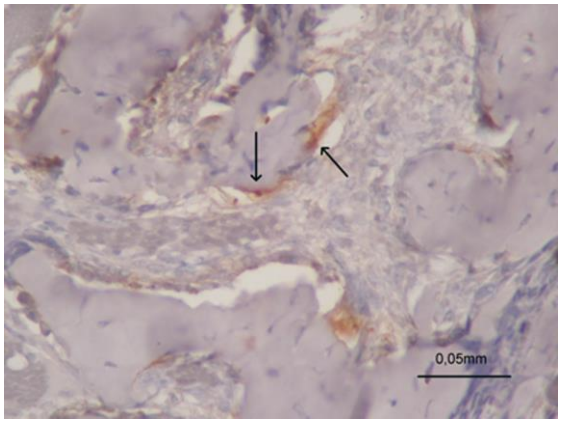

Figure 6. Immunohistochemical analysis of Control group defect area. The sections were stained with monoclonal anti-human Pro-BMP-2 antibody. Bar: 50µm.

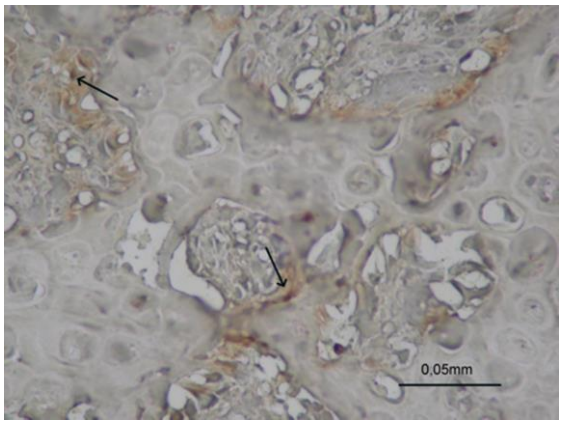

Figure 7. Immunohistochemical analysis of Experimental Group defect area. The sections were stained with monoclonal anti-human Pro-BMP-2 antibody. Bar: 50µm.

Table 2. Mean (SD) immunhistochemical measurements of defect regions of selected 0.4 mm^2 area.

Variable	Control group Mean (SD)	Group 1 (R.I) Mean (SD)	Group 2 (G.S) Mean (SD)	P- value
Anti-BMP-2 labelling (cell count)	4,00 (0,94)	6,40 (0,96)	6,40 (0,96)	KW: 12,27 P: 0,001*

Data were analysed using Kruskall Wallis and Mann Witney U test. The level of significance was set at $P < 0.05$. *: $P < 0.05$. R.I: Rif injection and irrigation. G.S: Rif mixture with gelatin sponge.

DISCUSSION

Local antibiotics were preferred for reducing risk of initial surgical infection, adverse systemic effects, systemic toxicity, and unnecessary high dose of antibiotic intake.[21] However dose-dependent and systemic administered rifamycin's possible cytotoxic effects on the cells were demonstrated in vitro studies.[22,23] On the other hand; previous studies and our study demonstrate higher osteoblast counts, enhanced bone formation[19], high tolerance by bone[17,18], and no histological damage.[16] Cytotoxicity may associated with dose, concentration, the type of antimicrobial and exposure time.[2] The selection of appropriate local antibiotics should consider antimicrobial effects, cytotoxicity, concentration and dosage factors. In our study the dose and concentration of rifamycin were selected with the guidance of Ferhan *et al.*[16], and Sivollella *et al*.[18] The antimicrobial effect of rifamycin was demonstrated by the same studies.[16,18]

There is little information available regarding the relationship between antibiotic

delivery and tissue regeneration. Recently, local delivery growth factors or antibiotics delivered from an implanted biomaterial have been used as novel approaches to stimulate bone regeneration areas of infected bone or compromised bone healing. Our methods present to overcome this situation basically and at lower cost.

In addition to rifamycins antibacterial use, their other anti-inflammatory effects have been shown in previous studies. Rifamycins are used for the treatment of rheumatoid arthritis[24] or chronic arthritis by direct intra-articular injection.[25] Anti-inflammatory and immunomodulatory effects have been shown to inhibit cytokine and chemokine synthesis by Rosetta *et al*.[24] The advantages of our study are positive effects on bone formation and, stimulating effects on BMP-2 release with other beneficial properties of rifamycin. Doxicyclin, gentamycin, and rifamycin have shown enhanced bone formation.[19,26,27] On the other hand controversial study reported antibiotics can inhibit bone formation.[28] Negative results may be related to dosage and concentrations.

New vessel formation was enhanced in our rifamycin groups. BMPs can stimulate vascular endothelial growth factor (VEGF) expression and promote angiogenesis.[29,30] However a previous study reported there was no significant differentiation in VEGF expression between control and experimental groups.[20] Further study is required regarding rifamycin's effects on angiogenesis and VEGF expression.

We investigated rifamycin induced BMP-2 expression. The mechanism remains unclear. This effect may be a pleotropic effect the same as statins. We thought that increased bone formation was related to BMP-2 release. Muthukuru *et al.*[31] reported doxycyclin was a stronger inducer of alkaline phosphatase expression but combined with BMP-2, counteracted the ınduction of osteogenic mediators. Wübbenhorst *et al*.[32] investigated whether tetracycline had a positive effect on inducible BMP-2 expression. Liu *et al*.[33] reported doxycyclin induced Smad 1C expression and indirect effect on BMP's influence. Smads are a group of intracellular effectors of the pathway of BMP and expressed by BMP.[33] Unfortunately there is not enough information about the mechanisms of rifamycin's effect on BMP expression. Our study may be a pioneer study for the pleotropic effect of rifamycin on BMP expression.

Our results demonstrate higher osteoblast counts at the collagen delivery system. This result may be related to the osteoconductive properties of collagen. BMP-2 expression and new bone area were not different between group 1 and group 2. Carvalho *et al*.[17] used rifamycin for the treatment of fibrinolytic alveolitis and observed that only rifamycin irrigation had better bone formation than mixed rifamycin and gelfoam. Kaya *et al.*[19] reported rifampin mixed with allogenic bone grafts could have a negative effect on bone formation compared to rifamycin-only irrigation and mixed with other graft types. In our study bone defects were enclosed by surrounding tissues; however, in clinical applications, a collagen delivery system may be useful in open bone defects.

We conclude that the simple application and beneficial effects of rifamycin used for extraction socket preservation, sinus bone augmentations, bone infections, or mouthwash following third-molar surgery. This study may be a guiding light of the pleotrophic effect (BMP-2 expression) or pathway of rifamycin induced BMP-2 expression. We conclude that rifamycin is the best local antibiotic which clinicians add bone grafts safely.

Acknowledgements

This work was supported by the Scientific Research Project Fund of Cumhuriyet University under Project number DİŞ-108.

Ethical approval

İlker ÖZEÇ, Asistant Professor, DDS, Phd, Medical Ethics Committee of Medical Faculty, University of Cumhuriyet. Reference No: B. 248 05-05-2011.

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Cumhuriyet Dental Journal 2017; 20(2): 113-121
Doi: 10.7126/cumudj.345944

A THREE-DIMENSIONAL EVALUATION OF THE EFFECTS OF DIFFERENT INCISOR INTRUSION MECHANICS TO THE PERMANENT MAXILLARY FIRST MOLAR TEETH BY USING CONE BEAM COMPUTED TOMOGRAPHY (CBCT)

Farklı Kesici İntrüzyon Mekaniklerinin Daimi Üst Birinci Molar Dişlere Etkilerinin Üç Boyutlu Olarak Değerlendirilmesi

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Makale Kodu/Article Code ***: 318138***

Makale Gönderilme Tarihi ***: 01.06.2017***

Kabul Tarihi ***: 13.06.2017***

ABSTRACT

Objective: The present study aims to evaluate the impacts of the upper incisor teeth intrusion in deepbite patients by two different techniques to the permanent maxillary first molar tooth using the three-dimensional cephalometric analysis in the individuals.

Materials and Methods: The population of this study consists of 34 patients with >4 mm overbite and a ≥2 mm gummy smile during post-pubertal period. Patients who underwent intrusion of upper incisor teeth were randomized to receive Connecticut intrusion arch (CTA) or miniscrew anchorage intrusion system (MAIS) to compare the impacts on permanent maxillary first molar teeth. Cone Beam Computed Tomography (CBCT) data obtained before (T1) and after (T2) intrusion were evaluated through three-dimensional (3D) cephalometric analysis. Intragroup assessment of treatment-related variables were performed via "t-test in dependent samples" and intergroup comparisons were assessed by "t-test in independent samples".

Results: In patients who underwent intrusion of upper incisors, permanent maxillary first molar teeth became deviated distally (1.48 mm/7.63 degree) only in CTA group, a statistically significant difference was found between two groups ($p<0.05$). The distance between resistance centers of maxillary first molar teeth was only increased in CTA group (0.31 mm), which also statistically differed from MAIS group.

Conclusion: CTA and MAIS techniques resulted in similar intrusive effects overall at the end of the treatment. While MAIS is recommended when anchorage from posterior region is not desired in patients with deep overbite, we believe that CTA may serve a suitable treatment alternative where miniscrew technique could not be performed.

Keywords: Deep overbite, intrusion, miniscrew, three-dimensional cephalometric analysis

ÖZ

Amaç: Bu çalışmanın amacı, derin örtülü kapanışa sahip bireylerde üst kesici dişlerin farklı tekniklerle intrüzyonunun daimi üst 1. Molar dişe etkilerinin 3 boyutlu sefalometrik analiz ile karşılaştırılmasıdır.

Materyal ve Metot: Araştırmamıza, postpubertal dönemde, overbite'ı >4mm. ve dişeti gülümsemesi ≥2 mm. olan toplam 34 hasta dahil edilmiştir. Hastalar rastgele bir şekilde Connecticut intrüzyon arkı (CTA) ile minivida ankrajlı intrüzyon sistemi (MAİS) gruplarına ayrılarak üst kesici dişlerin intrüzyonu gerçekleştirilen bireylerde üst 1. büyük azılarda ortaya çıkan etkileri değerlendirilmiştir. İntrüzyondan önce (T1) ve sonra (T2) alınan konik ışınlı bilgisayarlı tomografi (KIBT) verileri 3 boyutlu (3D) sefalometrik analizle incelenmiştir. Tedaviye bağlı değişenlerin grup içi değerlendirilmesinde "Bağımlı örneklerde t-testi"; gruplar arasındaki karşılaştırılmasında "Bağımsız örneklerde t-testi" uygulanmıştır.

Bulgular: Üst kesici dişlerin intrüzyonu gerçekleştirilen hastalarda, üst 1. molar dişler yalnızca CTA grubundaki bireylerde distale devrilmiş (1.48 mm/7.63 derece) ve bu durum gruplar arasında istatistiksel olarak önemli bulunmuştur ($p<0.05$). Üst birinci molar dişin direnç merkezleri arası mesafe yalnızca CTA grubunda artarken (0.31 mm), gruplar arasında oluşan değişim istatistiksel olarak önemli çıkmıştır.

Sonuç: Tedavi sonunda CTA veya MAİS teknikleri genel olarak benzer intrüziv etkiler oluşturmuşlardır. Özellikle derin örtülü kapanışa sahip bireylerde posterior bölgeden ankraj alınmak istenmediğinde MAİS prosedürünün kullanılmasını önerilirken, minivida uygulamasının yapılamayacağı bireylerde ise CTA uygulamasının başarılı bir alternatif olacağını düşünmekteyiz.

Anahtar Kelimeler: Derin örtülü kapanış, intrüzyon, minivida, üç boyutlu sefalometrik analiz.

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INTRODUCTION

Deep anterior overbite is a common orthodontic problem and also can be seen with many malocclusion types.[1-3] According to clinical and radiological examinations, excessive overbite can be treated either extruding the posterior buccal segments, intrusion of maxillary and mandibular anterior teeth or both.[3-7] Decision of treatment depends on miscellaneous factors like an optimal incisor position, incisor display, smile line, upper lip length, and vertical dimension.[5-7] For example, maxillary incisor intrusion is recommended for the patients with normal vertical dimension and gummy smiles[8] with over-eruption of incisors that produces anterior deep bites in non-growing patients.[2, 9-13] Traditionally, incisor intrusion performed by anterior bite plate[6], functional appliances[14, 15], j-hook headgears[16], reverse curved arches[17], step-up/step-down bends[10], 2x4 appliances like a utility arches[18] or 3-piece intrusion arches.[4]

In addition to this, extrusion of posterior teeth, retroclination of molars and labial tipping of anterior teeth is generally outcome of these techniques.[3, 8, 19-22] Clockwise rotation of mandibula forced by lifting of molar teeth within alveolar sockets is an unfavorable feature that increases risk of relapse in adults.[4, 23, 24]

To eliminate above-mentioned negative aspects of intrusion of incisor teeth, treatment of deep overbite by miniscrew supported bone anchorage has been introduced during the last quarter-century.[25, 35, 48] Miniscrews has numerous advantages such as allowing for placement in many intraoral regions; low cost; immediate loading opportunity, and simple placing and removing procedure compared with conventional dental implants.[49, 50] Impacts of various techniques used for intrusion of incisor teeth on skeletal and dentoalveolar structures have been comprehensively evaluated through utilization of cephalometric analyses.[4, 5, 8, 16, 19, 21, 25-30] However, to our knowledge, there is no study investigating their effects on posterior teeth by three-dimensional cephalometric analysis.

The aim of this study was to investigate the impacts of intrusion of each upper incisor tooth of patients with deep overbite, by either Connecticut intrusion arch (CTA) or miniscrew anchored intrusion system (MAIS), on permanent first molar teeth, by using three-dimensional cephalometric analysis.

MATERIAL AND METHODS

This study was approved by the Medical Scientific Ethics Committee of Atatürk University. Informed consent form was obtained from the patients and parents. Subjects that had been referred to Atatürk University Dentistry Faculty Orthodontics Department for treatment were enrolled to the study.

Inclusion criteria to this prospective clinical study were supra-positioning of upper incisor teeth according to occlusal plane, increased overbite (>4 mm), increased gingival display on posed smile (≥2 mm), increased incisor display at rest (≥3 mm), post-pubertal period, and good periodontal health condition. Patients were excluded from the study if following criteria were present: orthodontic treatment history, any dental abnormality in upper incisor region (malformation, supernumerary tooth, etc.), root abnormality of incisor teeth as detected by radiological examinations (resorption, dilaceration, alveolar crest resorption, and presence of impacted canine teeth extending to upper incisor region).

A total of 36 patients were randomly assigned to two different intrusion technique groups. Group 1 consisted of 18 patients (14 females and 4 males) who underwent maxillary incisor intrusion by Connecticut intrusion arch (CTA), and Group 2 consisted of 18 patients (13 females and 5 males) who underwent maxillary incisor intrusion by miniscrew anchorage (MAIS). Two subjects were further excluded from the study due to lack of oral

hygiene, yielding a total of 34 patients who completed the study.

No other orthodontic treatment was applied before intrusion of all the patients were completed.

In CTA group, a molar band was placed on upper first molar teeth before leveling, and passive transpalatal arch (TPA) was applied to increase anchorage. For avoiding incisor protrusion during intrusion, 0.017x0.025-inch long-form CTA was cinched back from molar band, and it was bilaterally tied anteriorly by ligature wires to hooks distal to lateral incisors. A total of 80 g force was applied onto maxillary incisors, 40 g in average for each tooth. Force calibration was performed by either increasing or decreasing CTA-exclusive V-bends through intraoral dynamometer (Dentaurum, Ispringen, Germany) at every three weeks. Intrusion of maxillary incisor teeth was terminated in both active intrusion groups when amount of resting displayed incisor reached at esthetic margin or incisal surfaces of incisor teeth were intruded till the level of occlusal plane.[13, 31]

In MAIS group, after radiological screening, miniscrews were inserted into the alveolar bone between roots of lateral and canine teeth. Miniscrews, we used in our study were 1.5 mm in diameter and 6 mm in length (Absoanchor, Dentos, Daegu, South Korea). One week after insertion, closed coil springs (G&H 9F NiTi Feather Light Close Coil Spring, Indiana, USA) were placed between miniscrews and anchor twists distal to lateral teeth. Afterwards, 80 g force was applied, 40 g in average for each tooth with a follow-up interval of three weeks.

CBCT records of patients were obtained through Cone Beam Volumetric Computed Tomography (NewTom 3G, Verona, Italy) device in Department of Oral and Maxillofacial Radiology of Ataturk University Faculty of Dentistry. Irradiation parameters of the device were 110 kVp with an effective dose of 60 μSv (2007 IRCP) per adult.

Computed tomography data of 34 patients, which had been acquired just before (T1) and after (T2) intrusion, were analyzed in a three-dimensional cephalometric method via Simplant Pro O&O (Materialise, Leuven, Belgium). In this software, position of the head was calibrated in a way that Frankfurt horizontal plane was parallel to the ground in sagittal section, lower orbital borders were at the same level in coronal section, and median palatine suture was perpendicular to the ground in axial section in 3D model. All cephalometric assessments were performed by the same investigator (FK). Pal 3D cephalometric analysis, developed by Ilhan M. Dagsuyu, was utilized in this study.

Skeletal landmarks regarding 3D cephalometric analysis were indicated at Table 1.

Table 1: Skeletal landmarks regarding 3D cephalometric analysis.

Skeletal landmark	**Definition**
OrR-OrL	Deepest external point of infraorbital border (double points; right and left)
Mid-Orbital	Midpoint of OrR and OrL points
(PoR-PoL	Most superior midpoint of external acoustic meatus (double points; right and left)
PtR-PtL	Most inferior midpoint of foramen rotundum it reaches on pterygomaxillary fossa (double points; right and left)
CP	Center point; midpoint of right and left pterygoid points
FSR-FSL	Geometrical center of foramen spinosum (double points; right and left)
ELSA:	Midpoint of right and left foramen spinosum points
ANSR-ANSL	Most anterior and apical point of hard palate at the sagittal plane (double points; right and left)
ANS	Midpoint of ANSR and ANSL
PNS	Most posterior and apical point of hard palate at the sagittal plane
IFR-IFL	Most external right and left point of incisive foramen (double points; right and left)
Incisive foramen	Midpoint of IFR-IFL points
U1Cr	Apical point of alveolar crest that is mesial to upper central teeth
UR2Cr-UL2Cr	Apical point of alveolar crest that is between upper lateral and canine teeth (double points; right and left)
Dental Landmarks	
UR1-UL1	Midpoint of incisive border of upper central incisor tooth (double points; right and left)
MoR-Mol	Apical point of mesiobuccal tubercle of upper maxillary first molar tooth (double points; right and left)
ApUR1-ApUL1	Apex of upper central incisor tooth (double points; right and left)
ApUR6-ApUL6	Mesiobuccal apex of upper first molar tooth (double points; right and left)
TriUR6-TriUL6	Midpoint of trifurcation of upper first molar tooth (double points; right and left)
Ur1ResCrestal-UL1ResCrestal	Point at the proximal 1/3 of the distance that extends from alveolar crest within the tooth on the long axis of upper incisor teeth to ApUR1 point (double points; right and left)
Reference Planes Used in Current Study	
FH	Frankfort horizontal plane. Horizontal reference plane passing through right (PoR) and left (PoL) porion and MidOrbital points
PP	Horizontal palatine reference plane passing through ANSR, ANSL, and PNS points
VPP	Vertical palatine reference plane that is perpendicular to palatine plane that passes through ANSR and ANSL points and crosses it at ANS

Landmarks and reference lines and planes that were used in the study were based on the published studies in the literature.[32-34] 3D

cephalometric measurements used in current study indicated at Table 2.

Table 2: 3D cephalometric measurements used in current study.

Measurement	Definition
U1RCrPPOrt:	Mean of the perpendicular distance that extends from both UR1ResCrestal and UL1ResCrestal points to PP reference plane
U1RCrVPPOrt:	Mean of the perpendicular distance that extends from both UR1ResCrestal and UL1ResCrestal points to VPP reference plane
MorMol	Shortest distance between MoR and MoL points
TriUR6TriUL6	Shortest distance between TriUR6 and TriUL6 points
MoPP	Mean of the perpendicular distance that extends from both MoR and MoL points to PP reference plane
MoVPP:	Mean of the perpendicular distance that extends from both MoR and MoL points to VPP reference plane
TriU6PPOrt:	Mean of the perpendicular distance that extends from both TriUR6 and TriUL6 points to PP reference plane
TriU6VPPOrt:	Mean of the perpendicular distance that extends from both TriUR6 and TriUL6 points to VPP reference plane
U6AngleOrt:	Mean of the angles formed between PP reference plane and both UR6Axis and UL6Axis lines

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics (version 20.0.0 New York, USA) The Kolmogorov-Smirnov test was used to evaluate normality; independent-sample T test and Mann-Whitney U test were used to analyze the relationships between parameters in two time-points (T1, T2). All statistical analyses were performed at the 5% significance level.

All measurements were repeated in 15 randomly selected samples after 10 months by the same investigator (FK). The Houston error analysis[35] was used to examine the differences between T1 and T2 time-points (Houston analysis reports that all measurements are between 0.9710 and 0.9986 coefficients). All landmarks and measurements were found highly repeatable.

RESULTS

Ages, total duration of intrusion, and mean values (and standard deviations) of the subjects in CTA and MAIS groups before the intrusion were shown in Table 3. While pre-treatment age statistically differed between treatment groups ($p<0.05$), duration of intrusion of upper incisors did not show a significant difference ($p>0.05$).

Table 3: Comparison of chronological ages and duration of intrusion of upper incisors in CTA and MAIS groups.

	CTA		MAIS	
Parameter	Mean	St. Deviation	Mean (St. Dev.)	Test
Chronological age (months)	191.88	11.62	200.17 (11.09)	S*
Duration of intrusion of upper incisors	3.64	0.82	3.36 (1.25)	NS

*p<0.05, S: significant; NS: non-significant

3D Cephalometric Analysis Findings

Intergroup Baseline Characteristics

Baseline characteristics of the groups with comparisons were indicated in Table 4. Study groups did not statistically differ in terms of any pre-treatment parameters.

Table 4: Comparison of baseline characteristics by groups.

	CTA		MAIS	
Parameter	Mean	St. Deviation	Mean (St. Dev.)	Test
MoVPP	28.72	2.31	30.60 (4.19)	NS
MoPP	22.78	2.57	23.26 (2.00)	NS
TriU6VPPOrt	31.39	1.68	32.85 (3.42)	NS
TriU6PPOrt	11.32	2.48	12.06 (1.86)	NS
U6AngleOrt	90.42	4.51	90.11 (4.52)	NS
MorMol	50.29	3.33	50.74 (2.41)	NS
TriUR6TriUL6	44.13	2.75	45.56 (2.39)	NS

*p<0.05, S: significant; NS: non-significant

Comparison of Intragroup Parameters Before and After Intrusion

CTA Group

CTA group showed significant alterations from baseline (T1 to T2) in MoVPP, U6Angleort, MorMol, TriUR6, and TriUL6 parameters ($p<0.05$). All other values were found similar (Table 5).

Table 5: Assessment of parameters before and after treatment in CTA group.

	Before Treatment		After Treatment	
Parameter	Mean	St. Deviation	Mean (St. Dev.)	Test
MoVPP	28.72	2.31	30.20 (2.26)	S *
MoPP	22.78	2.57	22.96 (2.43)	NS
TriU6VPPOrt	31.39	1.68	31.27 (1.44)	NS
TriU6PPOrt	11.32	2.48	11.49 (2.47)	NS
U6AngleOrt	90.42	4.51	82.78 (4.66)	S *
MorMol	50.29	3.33	50.94 (2.84)	S *
TriUR6TriUL6	44.13	2.75	44.44 (2.66)	S *

*p<0.05, S: significant; NS: non-significant

MAIS Group

MAIS group did not show any statistically significant difference in terms of any parameter from baseline (T1) to study end (T2), as demonstrated in Table 6.

Table 6: Assessment of parameters before and after treatment in MAIS group.

	Before Treatment		After Treatment	
Parameter	Mean	St. Deviation	Mean (St. Dev.)	Test
MoVPP	30.60	4.19	30.60 (4.27)	NS
MoPP	23.26	2.00	23.20 (2.08)	NS
TriU6VPPOrt	32.85	3.42	32.93 (3.50)	NS
TriU6PPOrt	12.06	1.86	11.96 (1.83)	NS
U6AngleOrt	90.11	4.52	90.29 (4.45)	NS
MorMol	50.74	2.41	51.02 (2.63)	NS
TriUR6TriUL6	45.56	2.39	45.52 (2.42)	NS

NS: non-significant

Comparison of Intergroup Parameters Before and After Intrusion

Comparison of changes from T1 to T2 between study groups were presented in Table 7. While U6AngleOrt was found as elevated in MAIS group, it was decreased in CTA group, where the difference was statistically significant.

Table 7: Intergroup comparison of mean changes after incisor intrusion in CTA and MAIS groups.

	CTA Group		MAIS Group	
Parameter	Mean	St. Deviation	Mean (St. Dev.)	Test
Duration	3.64	0.83	3.36 (1.25)	NS
MoVPP	1.48	.55	.00 (.47)	S *
MoPP	.18	.60	-.06 (.41)	NS
TriU6VPPOrt	-.12	.67	.08 (.41)	NS
TriU6PPOrt	.17	.44	-.09 (.41)	NS
U6AngleOrt ♦	-7.63	2.71	.18 (1.06)	S *
MorMol ♦	.65	1.14	.28 (.59)	NS
TriUR6TriUL6	.31	.50	-.03 (.47)	S *

*p<0.05, S: significant; NS: non-significant
♦ non-normally distributed parameter where Mann-Whitney-U test was performed.

Despite being significantly increased in CTA group, the distance (MoVPP) between crown of upper first molar teeth (MoR, MoL) and vertical palatal reference plane (VPP) did not alter in MAIS group. This intergroup difference was also found as statistically significant.

While the distance between resistance centers of upper first molar teeth was statistically lengthened in CTA group, it was non-significantly shortened in MAIS group. The difference between the groups was also found as statistically significant. No other parameters were statistically significant between study groups.

Intrusion was achieved on the resistance center of upper central incisor teeth in CTA and MAIS groups (intrusion: CTA/MAIS: 1.46/1.78 mm), where no difference was detected for the amount of intrusion.

DISCUSSION

Management of deep overbite consists of three principal approaches, namely extrusion of upper/lower posterior teeth, intrusion of upper/lower incisors, and combination of intrusion and extrusion.[4, 15, 23, 24] In addition, orthognathic surgery may also be preferred for extreme cases.[24, 36]

Intrusion performed with either CTA or MAIS has distinctive impacts on upper first molar teeth. Since no anchorage was performed from posterior teeth in MAIS group, no significant alteration was detected in crown or resistance center of molar teeth for either sagittal or vertical direction. Therefore, anchorage was preserved in MAIS group in our study. This is consistent with other studies regarding performance of incisor intrusion via miniscrew anchorage.[5, 8, 16, 37-39]

In CTA group, while 1.48 mm distal displacement of upper first molar teeth crown was found as statistically significant ($p<0.001$), its vertical displacement was not significant ($p>0.05$). This is consistent with the finding of Nanda who reported distal bending moment of CTA on the crown of molar tooth during creating the intrusion force.[7] Also consistent with our results, Senisik et al. reported distal advancement of the crown after incisor intrusion by CTA.[30]

Absence of extrusion in upper first molar teeth in CTA may originate from the ability of sufficient anchorage of opposite occlusal

forces against low vertical extrusion forces.[3, 40] Moreover, TPA application that increases anchorage may also prevent extrusion. It was reported that extrusion of molar teeth after intrusion of incisors may cause relapses particularly in adults.[23] This is because extrusion of posterior teeth in adults may affect position of the condylar head by rendering clockwise rotation at lower jaw, which in turn, may influence temporomandibular joint and muscles. On the contrary, temporomandibular joint and its surrounding tissues that had not been adapted may lead to relapses after intrusion treatment by successful remodeling.[9] Therefore, absence of extrusion in our study may imply a more stable property of our intrusion treatment. Our study is consistent with the vertical effects on molar teeth that was reported by the authors performing intrusion of upper incisor teeth via the other intrusion arch.[3]

Our study is not consistent with the findings of Senisik and Turkkahraman who reported 0.80 mm and 0.92 mm extrusion for the crown of upper first molar teeth after intrusion of upper incisor through CTA.[30] This may arise from lack of either intrusion in lower jaw or preventive measures improving anchorage (TPA, headgear) by the investigators.[30]

We found no significant alteration of resistance center of upper first molar teeth in either sagittal or vertical direction after intrusion of incisor teeth in CTA group. This was inconsistent with those CTA-intrusion studies reporting mesial advancing of molar resistance center by 0.30 mm at anteroposterior axis.[30] This dissimilarity may be attributed to absence of anchorage-improving measures at upper jaw or observation of more protrusion in incisors.

In our study, a distal deviation of 7.63^0 at the long axis of maxillary first molar in CTA group was statistically significant. It was reported that anchorage from molar teeth by intra-arch intrusion techniques might lead to distal deviation of molar teeth.[19] Our finding was in line with those of other authors performing intrusion through CTA.[29, 30] On the other hand, there was no significant change in MAIS group. In fact, studies where miniscrew anchorage incisor intrusion was performed reported no significant alteration in the angle created by the molar tooth and palatal plane, consistent with our findings.[8, 16, 39]

Evaluation of Transversal Direction Alterations

Expansion of the distances between each crowns and resistance centers of respective upper first molar teeth by 0.65 mm and 0.31 mm, respectively in CTA group was not clinically important, albeit being statistically significant. These increments were parallel to that of Van Steenbergen et al. reporting increased width between molar teeth after only anchoring from upper first molar tooth by segmental arch for the intrusion of upper incisor teeth.[19]

In MAIS group, the distances between each crowns and resistance centers of corresponding molar teeth were not significantly altered. In fact, this is expected since no procedure was done posteriorly. This is consistent with Senisik's finding that showed unaltered distance between crowns of molar teeth after miniscrew anchored intrusion of incisor teeth.[37] On the contrary, Upadhyay et al., in their study where they closed extraction gaps and performed miniscrew anchored incisor intrusion, reported a 1.83 mm reduction in the distance between crowns of upper first molar teeth.[39] We attribute this discrepancy to the differences of investigators in mechanics and therapeutic strategies they used.

Statistical Comparison of Observed Alterations Between Groups

Mean differences of changes of resistance centers at sagittal and vertical planes showed

no differences between groups, which is consistent with those of Senisik & Turkkahraman and Polat-Ozsoy et al. using miniscrew anchored intrusion system alone or with utility arch, respectively.[30, 8]

While upper first molar tooth was deviated distally in CTA group, this was not observed in MAIS group. Yielding a significant difference between groups, this finding is parallel to the reports of Senisik & Turkkahraman and other authors, utilizing CTA and miniscrew anchorage intrusion systems.[30, 8]

In terms of alteration between groups at transverse plane, the distance between resistance centers of upper first molar teeth was increased by about 0.3 mm in CTA group, whereas it did not change in MAIS group, which was statistically significant. This may be explained by CTA's anchorage from upper first molar and by the possibility that TPA which we used as anchorage-improving measure might be prepared as slightly active during laboratory phase.

Our findings could be accepted as similar to the findings published in the literature overall. We suggest that the differences in intrusion values may result from the variety of techniques used, vector properties of the intrusion force (intensity, direction, application point), total duration of therapy, and diversity of age groups.

CONCLUSIONS

While the crown of upper first molar teeth were displaced to distal and buccal direction in CTA group, there was no displacement in MAIS group.

While upper first molar crown tipped and displaced distally, there was no alteration in MAIS group.

Though transpalatal arch was used in CTA group, crowns of upper first molar crown were displaced to buccal direction.

We recommend in favor of using MAIS procedure when anchoring from posterior region is not desired especially in patients with deep overbite and CTA may serve as an effective alternative where miniscrew technique could not be performed. Use of MAIS may offer advantages when no impact on posterior relation is desired in incisor intrusion.

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Cumhuriyet Dental Journal 2017; 20(2): 106-112
Doi: 10.7126/cumudj.345941 *RESEARCH ARTICLES*

A BRIEF RADIOGRAPHIC REPORT FROM TWO COMMON ODONTOGENIC CYSTS IN JAWS WITH FOLLICULAR RADIOLUCENT APPEARANCE

Çenelerdeki Foliküler Radyolüsent Görünümlü İki Ortak Odontojenik Kistten Kısa Bir Radyografik Rapor

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Makale Kodu/Article Code *: 163098*
Makale Gönderilme Tarihi *: 27.12. 2015*
Kabul Tarihi *: 15.01.2016*

ABSTRACT

Objectives: Pericoronal radiolucencies are common pathologic findings in regular dental checkups. Since dentigerous cyst is the most common pathologic pericoronal radiolucency and as odontogenic keratocyst (OKC) is a common cyst also and an aggressive lesion with high recurrence, radiographic features of these lesions were discussed in this study using panoramic radiography and cone beam computed tomography.

Materials and Methods: In this cross-sectional case series study, radiographs from 56 patients who were referred to a private maxillofacial radiology center or dentistry faculty in Mashhad/Iran from 2008 to 2013 in which radiolucent pericoronal lesion was observed in jaws with histopathologic results of dentigerous cyst or OKC were separately examined by two maxillofacial radiologists. Both observers were unaware of pathology results. Lesions were assessed based on their location, periphery, and impaction on the surrounding structures. Then, obtained data were analyzed using descriptive tables.

Results: 56 lesions were identified in 56 patients. There were 20 odontogenic keratocyst and 36 dentigerous cysts. The majority of dentigerous cysts and OKCs occurred in the posterior mandible and showed a well corticated border. External root resorption was higher in OKC cases. In addition, displacement tendency of surrounding structures (other than tooth) such as nasal floor, mandibular canal, buccal and lingual cortex (in the form of expansion) as well as destruction of cortex, nasal floor or sinus walls was higher in OKC than in dentigerous cyst.

Conclusion: Except of tooth displacement other parameters related to the effect on surrounding structures in this study showed higher frequency in OKC than dentigerous cyst.

Key words: odontogenic cyst, panoramic radiography, cone beam computed tomography, odontogenic keratocyst, dentigerous cyst

ÖZ

Amaç: Perikoronal radyolüsensiler rutin dişhekimliği muayenelerinde yaygın patolojik bulgulardır. Dentigeröz kist en yaygın patolojik perikoronal radyolüsensi ve aynı zamanda odontojenik keratokist (OKC) yaygın ve yüksek nüks gösteren agresif bir lezon olduğundan dolayı, bu lezyonların radyografik özellikleri panoramik radyografi ve konik ışınlı bilgisayarlı tomografi kullanılarak tartışıldı.

Gereç ve Yöntem: Bu kesitsel vaka serisi çalışmasında 2008-2013 yılları arasında Mashhad / İran'da özel maksillofasiyal radyoloji merkezine veya dişhekimliği fakültesine sevk edilen 56 hastanın radyolüsent perikoronal lezyonunun dentigeröz kist veya OKC'nin histopatolojik sonuçları ile çenelerde görüldüğü radyografileri iki maksillofasiyal radyolog tarafından ayrı ayrı incelendi. Her iki gözlemci de patoloji sonuçlarının farkında değildi. Lezyonlar, yerleri, çevresi ve çevreleyen yapılar üzerindeki etkisine göre değerlendirildi. Elde edilen veriler tanımlayıcı tablolar kullanılarak analiz edildi.

Bulgular: 56 hastada 56 lezyon tespit edildi. 20 OKC ve 36 dentigeröz kist vardı. Dentigeröz kistlerin ve OKC'lerin çoğunluğu posterior mandibulada ortaya çıkmış ve iyi kortekslenmiş bir sınır göstermiştir. OKC olgularında dış kök rezorpsiyonu daha yüksekti. Ek olarak, burun zemini, mandibuler kanal, bukal ve lingual korteks (genişleme şeklinde) gibi çevreleyen yapıların (diş hariç) yer değiştirme eğilimleri, korteks, burun zemini veya sinüs duvarlarının tahrip edilmesi, OKC'de dentigerous kistten daha yüksekti.

Sonuçlar: Bu çalışmada, diş yer değiştirmesinin haricinde, çevre yapılara etkisi ile ilgili diğer parametreler OKC'de dentigeröz kistten daha yüksek dağılım gösterdi.

Anahtar Kelimeler: odontojenik kist, panoramik radyografi, konik ışınlı bilgisayarlı tomografi, odontojenik keratokist, dentigeröz kist

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INTRODUCTION

Dental follicles associated with impacted teeth are remnants of tissues that participated in teeth formation and remain attached to the teeth. Despite the physiological role of dental follicle in growth, histopathologic changes in different stages of growth can lead to development of odontogenic cysts and tumors.[1] Pericoronal radiolucencies are common pathologic findings in regular dental practice. These lesions are typically observed as a normal or slightly enlarged follicle in radiographies, which sometimes show larger lesions needing appropriate intervention and histopathological interpretation.[2] Pericoronal space larger than 2.5 mm in intraoral radiography or larger than 3 mm in panoramic radiography should be considered as suspicious.[3] Several pathological processes may be radiographically manifested as radiolucencies associated with impacted tooth. These lesions can grow significantly and have the potential of pathological displacement.[4] Pericoronal radiolucencies are a diagnostic dilemma because of multiple differential diagnoses and different treatments. Therefore, differentiation of these lesions from each otheris essential to avoid unnecessary medical intervention.[5] Cases commonly causing pericoronal radiolucencies include hyperplastic follicular space, dentigerous cyst, odontogenic keratocyst, mural (unicystic) ameloblastoma, adenomatoid odontogenic tumor, calcified odontogenic cyst and ameloblastic fibroma.[6,7]

Since dentigerous cyst is the most common pathologic pericoronal radiolucency and OKC is a common and aggressive lesion with high recurrence that can present with an appearance similar to dentigerous cyst, in this study radiographic features of these lesions were investigated using panoramic radiography and cone beam computed tomography (CBCT).

MATERIALS AND METHODS

In this cross-sectional case-series study, images from 56 patients that showed a pericoronal and unilocular radiolucent lesions in panoramic (Planmeca Model 2007 ProlineXC instrument made in Finland) or CBCT (using Planmeca Model 2009 ProMax3D device made in Finland with 320- and 160-micron resolution) images with histopathology results of dentigerous cyst or OKC in a private maxillofacial radiology center and Mashhad Dental School were calculated from 2008 to 2013. The images have been prescribed as a diagnostic workup. This study was approved by the ethical committee of Mashhad University of Medical Sciences (Mashhad/Iran) regarding ethical and methodological issues.

Two qualified radiologists reviewed the images separately. Observers had no knowledge of the histopathological results. In case they did not concur, a third opinion was sought through consultation with another expert who was also blind to pathology reports.

The radiographic features of these lesions, including the involved site, border of lesion, and effect on surrounding structures were recorded in a checklist in this study.

Location

The lesions could be situated in the anterior (incisor-canine) and posterior (pre-molar/ molar/ ramus (mandible) / tuberosity (maxilla) of the mouth, or even extend from anterior to posterior.

Border

The periphery of the lesions included three categories; well-corticated, well-sclerotic and well non-corticated.

Effect on surrounding structures

A range of findings were also studied including root resorption, tooth displacement, cortical perforation, mandibular canal displacement due to mandibular lesions and maxillary sinus and

nasal walls displacement owing to maxillary lesions.

Finally, frequency distribution tables and charts were used to describe the data. It should be noted that evaluation of findings such as destruction of buccal and lingual cortex, nasal or sinus walls is only possible through sectional images such as CBCT and CT, and in this study, these findings were assessed only in patients with CBCT images. Cases associated with effect on mandibular canal, nasal or sinus walls were calculated from among cases in the respective jaw in close proximity to these structures, and frequency of external root resorption and displacement of adjacent teeth were also checked and calculated from among the cases adjacent to teeth.

RESULTS

56 lesions in 56 patients were studied. 36 cases of dentigerous cyst and 20 cases of odontogenic keratocyst were observed.

Most patients with dentigerous cyst and OKC were in the second decade of their life (41.17% and 42.85%, respectively). 52.94% of patients with dentigerous cyst were male and 47.06% were female, while OKC showed the same frequency in men and women. All 56 patients in the study had a panoramic radiography image.

In this study, CBCT was used as additional imaging technique in 40% of dentigerous cyst lesions in maxilla and 8% in mandible, which was 50% and 25% in relation to OKC, respectively.

Frequency distribution of lesions according to location

The results of our study on dentigerous cyst location showed that a total of 72% of the lesions occurred in the mandible, and almost 85% of lesions tended to occur in the posterior mandible. In maxilla, the lesions had a relatively higher anterior tendency. OKC was found in mandible in 60% of cases. Mandibular lesions had a higher posterior tendency but the majority of lesions in maxilla had antero-posterior extension. (Table 1)

Table 1. Frequency distribution of lesions based on the involved site

	Location	Anterior	Posterior	anterior to posterior	Total
Dentigerous cyst	Mandible	1 (3.8%)	22 (84.6%)	3 (11.5%)	26 (72.2%)
	Maxilla	4 (40.0%)	3 (30.0%)	3 (30.0%)	10 (27.7%)
	Total	5 (13.9%)	25 (69.4%)	6 (16.7%)	36 (100%)
OKC	Mandible	3 (25.0%)	8 (66.7%)	1 (8.3%)	12 (60.00%)
	Maxilla	1 (12.5%)	1 (12.5%)	6 (75.0%)	8 (40.0%)
	Total	4 (20.0%)	9 (45.0%)	7 (35.0%)	20 (100.0%)

Frequency distribution of lesions according to border:

The majority of dentigerous cysts both in mandible (73.1%) and maxilla (50%) had well-corticated borders. Most cases of OKC had also well-corticated borders in the mandible (83.3%) and maxilla (87.5%). Only four dentigerous cysts had well-sclerotic margin, two in mandible and two in maxilla. (Table 2)

Table 2. Frequency distribution of lesions based on the border

	Border / Location	Well-Corticated	Well-Non Corticated	Well-Sclerotic	Total
ntigerous cyst	Mandible	19 (73.1%)	5 (19.2%)	2 (7.7%)	26 (72.2%)
	Maxilla	5 (50.0%)	3 (30.0%)	2 (20.0%)	10 (27.7%)
	Sum	24 (66.7%)	8 (22.2%)	4 (11.1%)	36 (100.0%)
OKC	Mandible	10 (83.3%)	2 (16.7%)	0 (0%)	12 (60.0%)
	Maxilla	7 (87.5%)	1 (12.5%)	0 (0%)	8 (40.0%)
	Total	17 (85.0%)	3 (15.0%)	0 (0%)	20 (100.0%)

Frequency distribution of lesions according to their effect on surrounding structures

The results of our study showed that external root resorption was more frequent inOKC. Furthermore, displacement tendency of surrounding structures (other than teeth) such as nasal walls, mandibular canal or buccal and lingual cortex (in the form of expansion) as well as destruction of cortex, nasal or sinus walls was

higher in OKC than dentigerous cyst. (Table 3) (Fig.1, 2 and 3)

Table 3. Frequency distribution of lesions based on the effect on surrounding structures

	Dentigerous cyst			*OKC*		
The effect on surrounding structures	***Positive cases number***	***All of studied cases ****	***Frequency percent***	***Positive cases number***	***All of studied cases ****	***Frequency percent***
External root resorption	15	31	48.38	11	20	55
Displacement of adjacent teeth	23	31	74.19	9	20	45
Destruction of the cortex	5	8	62.5	7	7	100
Displacement of the nasal or sinus walls	7	10	70	7	7	100
Destruction of the nasal or sinus walls	0	4	0	2	4	50
Displacement of the mandibular canal	8	21	38.09	9	12	75
Cortical expansion	23	36	63.88	16	20	80

*Based on the methods and material

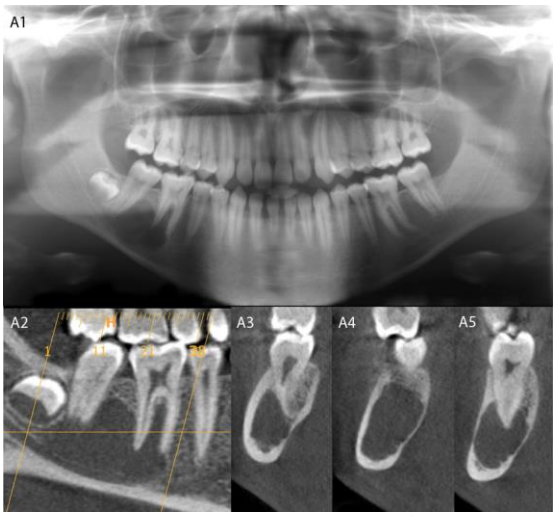

Fig.1. An OKC in maxilla. A:coronal view of CBCT shows two impacted teeth inside of lesion.B&C: axial views in two different levels show buccal cortical expansion (C) and erosion (B).

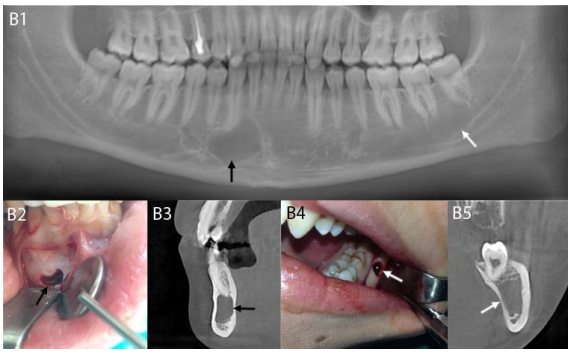

Fig.2. A dentigerous cyst in maxilla. A & B: axial and sagittal scans of CBCT do not show any cortical expansion in maxillary sinus.

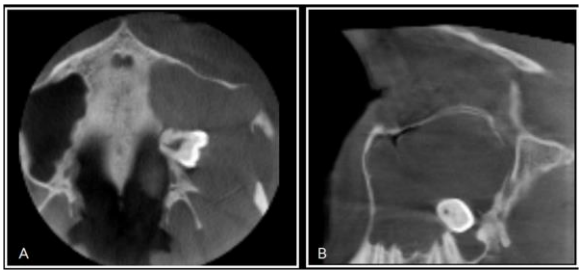

Fig.3. A large OKC in left maxilla. A&B: axial and sagittal scans of CBCT: Note to the molar tooth position and erosion in the lateral wall of maxillary sinus.

DISCUSSION

Dentigerous cyst is the most common pathologic pericoronal radiolucency. It is the most prevalent odontogenic cyst after radicular cyst. Dentigerous cyst is associated with crown of impacted or developing teeth. Third mandibular molars, maxillary canines, mandibular premolars and third maxillary molars are the most common teeth affected with these lesions. These cysts vary in size from less than 2 cm to the extent that causes expanded jaws. Expanded jaw may cause deformity of the affected area. Although a cyst may slowly grow and cause thinning of cortical bone plates, it rarely destroys these plates. Dentigerous cysts cause resorption of adjacent teeth roots in 55% of cases.[7] Dentigerous cysts are frequently discovered whenradiographs are taken to investigate a failure of tootheruption, a missing tooth or malalignment. There is no painor discomfort associated with the cyst unless it becomessecondarily infected.[7,8]

Odontogenic keratocyst (OKC) accounts for 5 to 11% of cysts in jaws. This lesion is observed in mandible in 65% of cases and occur usually in the second and third decades of life. It is relatively more prevalent in men (56.9%).[6] Posterior body of mandible (90% occur posterior to the canines) and ramus (50%) are the most common sites of OKC. The epicenter is located superior to the inferior alveolar nerve canal. This

cyst sometimes has pericoronal position and is indistinguishable from dentigerous cyst. OKC has often a cortical border unless it is secondarily infected. The cyst may be round or oval or it may have a scalloped outline (a series of contiguous arcs). The most common internal structure is radiolucent. In some cases, the inner wall is curved and may give a multilacular appearance to the lesion. An important characteristic of the OKC is its propensity to grow along the internal aspect of the jaws, causing minimal expansion of the cortical plates. Relatively low expansion of these cysts causes delayed diagnosis of them, and sometimes these cysts reach a large size before being detected. OKC can cause tooth displacement and resorption but to a slightly lesser degree than dentigerous cysts.[7]

Since there has been no comprehensive study to assess radiographic features of pericoronal radiolucencies and previous studies have been limited to a few cases reports, it seemed necessary to conduct a study for appropriate description of radiographic features of these lesions.

In this study, radiographic features of two common cases of pericoronal radiolucencies, including dentigerous cyst and OKC, were examined.

In the present study, out of 56 patients evaluated, there were 29 men (52%) and 27 women (48%). Although there has been no explanation to interpret these findings, comparison of results suggests that similar to many odontogenic cysts and tumors, the incidence of these lesions is slightly higher in men.[9,10] However, in our study and Zhu in 2014[11], OKC showed an equal prevalence among men and women, which was contrary to results of Gonzalez[12] in which 51% of involved cases were males.

Both of dentigerous cysts and OKCs occurred most commonly in the second decade of life in this study. According to Tsukamoto *et al.* in 2001[13], odontogenic keratocyst occur with a peak in the second and third decades of life. In the study of Gonzalez in 2008[12] and Kornafel in 2014[9], stated that age peak of OKC occurs at the third decade of life. Moreover, according to the study of Imanimoghadam *et al.*[14], there was a higher prevalence of dentigerous cyst in the second and OKC in the third decade of life. Perhaps this is related to odontogenesis process because it is the source of activity and subsequent differentiation of dental development.

Out of 36 dentigerous cysts studied, 26 (72.2%) were in the mandible and 10 (27.7%) were in the maxilla. Posterior mandible was the most common site of involvement for dentigerous cyst and OKC. According to Imanimoghadam *et al.* in 2007[14], the most common sites of dentigerous cyst and OKC were anterior maxillary and posterior mandibular, respectively, which was contrary to our results with respect to dentigerous cyst. In the study of Gonzalez-Alva *et al.*[12] in a Japanese population, similar results were obtained with ours. In this study, 70.5% of OKC were in mandible and 16.4% in the maxilla. In the study of Habibi *et al.* in 2007[15] in which 83 cases of OKC were retrospectively reviewed over 10 years, 67.5% of lesions were in mandible and 32.5% were in maxilla. In the study of Sharifian *et al.* in 2011[16], dentigerous cyst showed a higher tendency to maxilla. In our study, well-corticated margin was the most common external border in both dentigerous cyst and OKC. Results of other studies have demonstrated an obvious margin for pericoronal lesions, especially dentigerous cyst and odontogenic keratocyst.[10,14] The most common border of cysts is well-corticated margin according to reference texts. Secondary infection at the site of lesion causes external border likely to disappear or thicken.[6] In this study, four infected dentigerous cysts had sclerotic borders.

Imanimoghadam *et al.*[14] examined 41 panoramic radiography images including 26

cases of dentigerous cyst and 15 odontogenic keratocyst, in which 30.77% of dentigerous cysts and 67/6% of OKCs caused root displacement of adjacent teeth. In addition, 34.62% of dentigerous cysts and 20% of OKCs caused root resorption of adjacent teeth. In our study, root resorption of adjacent teeth showed a higher prevalence in both lesions. In the study of Habibi *et al.*[15], OKC-associated expansion was 45%, which was 65%in the study of MacDonald[10], and was lower compared to our study (80%). Higher prevalence of expansion associated with OKC in our study could be due to the fact that the majority of OKCs in our study occurred in ramus, which is usually associated with more expansion in this area. In our study, root resorption showed a higher prevalence in OKC compared to dentigerous cyst, which may be due to small size of the majority of dentigerous cysts in this study. This also justifies lower rate of expansion in this cyst compared to OKC, which was contrary to what was expected.

CONCLUSIONS

One of the most differential diagnoses to make is between a dentigerous cyst and OKC. When in a pericoronal position, an OKC may be indistinguishable from a dentigerous cyst. According to our results this lesion is likely to be an OKC if was seen destruction of buccal and lingual cortex and nasal and sinus walls.

Acknowledgments: This study was made possible by the generous support rendered by the Vice Chancellor for Research of Mashhad University of Medical Sciences, in the form of grant no 920335 for which the authors are very grateful.

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Conflict of interest: There are no conflicts of interest or financial ties to disclose.

*Cumhuriyet Dental Journal 2017; 20(2):*72-76
Doi: 10.7126/cumudj.345891 *CLINICAL REPORTS*

KISSING MOLARS: REPORTS OF THREE CASES INVOLVING SUPERNUMERARY TOOTH, DENTIGEROUS CYST AND FIBRO-OSSEOUS LESION

Kissing Molarlar: Süpernümere Diş, Dentigeröz Kist ve Fibro-Osseöz Lezyon ile İlişkili Üç Vaka

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Makale Kodu/Article Code *: 168775*

Makale Gönderilme Tarihi *: 18.01.2016*

Kabul Tarihi *: 19.01.2016*

ABSTRACT

Objectives: The term “kissing molars” refers to a rare entity in which impacted mandibular second and third molars have contacting occlusal surfaces in a single follicular space and roots are pointed in opposite directions. The aim of this article is to describe three cases of mandibular kissing molars assessed through cone-beam computerized tomography and their management.

Materials and Methods: In one case the condition was associated with a supernumerary teeth. In the other case the kissing molars were associated with a dentigerous cyst, which disarticulated the two impacted teeth. The third case of kissing molars involved a fibro-osseous lesion in the anterior mandibular region.

Result: We are in the opinion that kissing molar cases should be further examined for other lesions of the jaws.

Keywords: Kissing Molars, Supernumerary Teeth, Dentigerous Cyst, Fibrooseous Lesion

ÖZ

Amaç: "Kissing molarlar" terimi, gömülü mandibuler ikinci ve üçüncü molar dişlerin, tek bir foliküler alan içerisinde oklüzal yüzeylerinin temas halinde olduğu ve köklerin ters yönlere doğru işaret ettiği nadir bir durumu tanımlamaktadır. Bu makalenin amacı konik ışınlı bilgisayarlı tomografi ile değerlendirilmiş üç ayrı kissing molar vakasını tanımlamak ve tedavilerini paylaşmaktır.

Materyal ve Metotlar: Vakaların biri süpernümere diş ile ilişkiliyken ikinci vaka iki dişin oklüzal yüzeylerinde ayrılmaya sebep olan bir dentigeröz kist ile ilişkiliydi. Üçüncü vakada ise mandibuler anterior bölgede bulunan bir fibro-osseoz lezyon mevcuttu. Sonuç: Kissing molar vakalarının çenelerde bulunabilecek diğer lezyonlar için de incelenmesi gerektiği fikrindeyiz.

Anahtar Kelimeler: Kissing molar, Süpernümere diş, Dentigeröz kist, Fibroosseöz lezyon

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INTRODUCTION

The extremely rare entity "kissing molars" (KM) was initially described by Van Hoof in 1973 as the existence of two mandibular impacted molars with contacting occlusal surfaces, surrounded by a single dental follicle and up to date very few cases have been reported in the literature.[1] Although knowledge about the etiopathogenesis, clinical features, diagnostic and therapeutic options are reported to be limited[1], KM has been linked to mucopolysaccharidoses (MPS), which results from a quantitative, or qualitative deficiency of lysosomal enzymes required to break down glycosaminoglycans.[2] KM has also been reported to be associated with hyperplastic dental follicles and dentigerous cysts in the jaws[2,3]. In this study we describe three cases of KM and their link to pathological lesions of the jaws and discuss the management. Due to the retrospective nature of this study, it was granted an exemption in writing by Ankara University Faculty of Dentistry, Ethics Committee.

MATERIALS AND METHODS

Case-1: A 38-year-old female patient admitted to our clinic for routine examination. During clinical examination an orthopantomograph was obtained and KM consisting of a third and forth molar in the left mandible were revealed. The occlusal surfaces of the two KM were in relation (Figure-1). Patient was informed about the condition and surgery was chosen as the treatment option. A cone-beam computerized tomography (CBCT) was obtained for further evaluation (Figure-1). Surgery was performed under general anesthesia, via transalveolar approach. Routine impacted third molar incision was used and bone was removed using a round bur. Crowns and roots of the impacted teeth were separated. Operation was completed uneventfully. Patient had no complaints of postoperative inferior alveolar nerve complications.

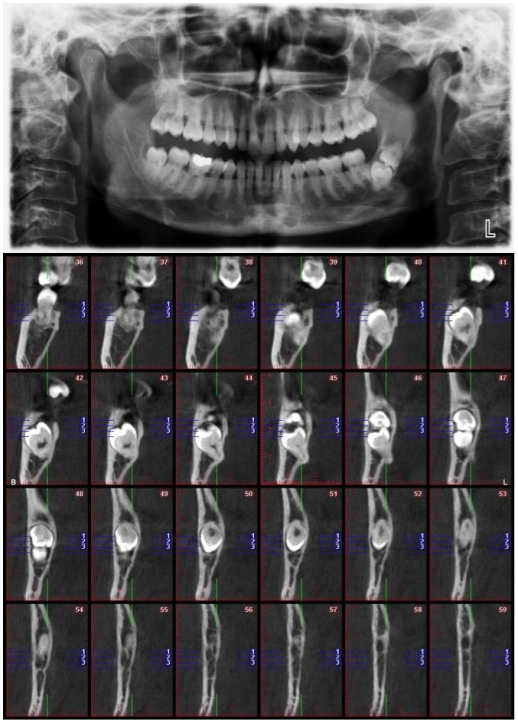

Figure 1. Orthopantomograph and CBCT of the patient revealing kissing molars.

Case-2: A 27-year-old male patient admitted to our clinic with complaint of pain and swelling in the left mandibular molar region. An orthopantomograph was obtained and KM associated with a radiolucent and well-defined lesion was observed (Figure-2). The occlusal surfaces of the KM were reasonably separated. Patient was informed and fine needle biopsy was obtained. Histopathological evaluation of the material revealed a dentigerous cyst. Patient's approval was received for surgery and a CBCT was obtained before surgery for further evaluation (Figure-2). Surgery was performed under general anesthesia, via transalveolar approach. Routine impacted

third molar incision was used and bone was removed using a round bur. Crowns and roots of the impacted teeth were separated. Cyst was enucleated using dental currettes. Operation was completed uneventfully. Patient had no complaints of postoperative inferior alveolar nerve complications. The excisional biopsy material was examined histopathologically and the initial diagnosis of dentigerous cyst was confirmed.

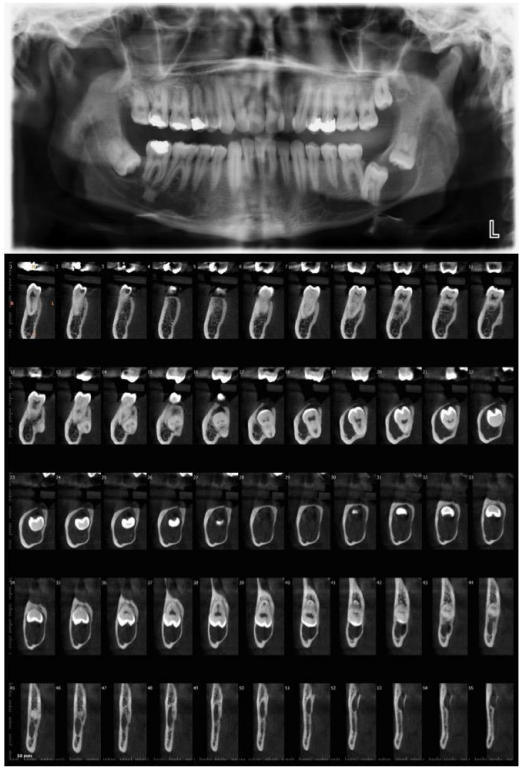

Figure 2. Orthopantomograph and CBCT of the patient revealing kissing molars and dentigerous cyst.

Case-3: A 22-year-old female patient admitted to our clinic for routine examination. Although patient had no history of extraction, right mandibular second and third molars were absent. An orthopantomograph was obtained and the KM in the right mandible were revealed (Figure-3). A lesion in the right canine-incisor area was also observed. A CBCT examination was made and the KM (Figure-3) and the lesion in the anterior mandible (Figure-4) were further examined before surgery.

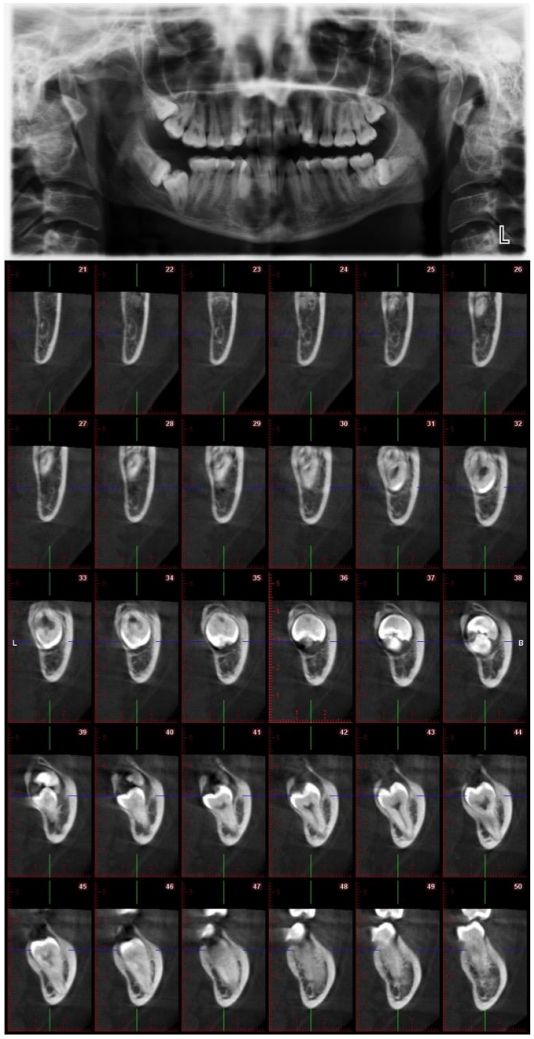

Figure 3. Orthopantomograph and CBCT of the patient revealing kissing molars and fibro-osseous lesion.

Patient was informed about the conditions and surgery was chosen as the treatment option for the KM and biopsy was planned for the lesion in the anterior mandible. Surgery was performed under general anesthesia, via transalveolar approach. Routine impacted third molar incision was used for the KM and bone was removed using a round bur. Crowns and roots of the

impacted teeth were separated. Operation was completed uneventfully. In order to obtain biopsy material a semilunar incision was used. A round window, which dental currettes can enter, was prepared with a very small round bur and the window was taken as biopsy material. Bone chips were obtained from inside the window with dental currettes. Materials were sent to biopsy. Patient had no complaints of postoperative inferior alveolar nerve complications. The incisional biopsy material was examined histopathologically and diagnosed as a fibro-osseous lesion.

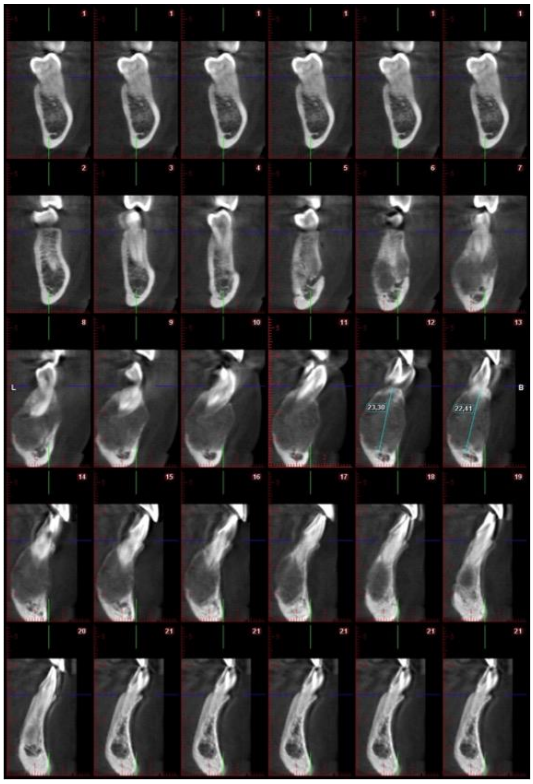

Figure 4. CBCT examination of the fibro-osseous lesion.

DISCUSSION

In 1973, Van Hoof gave a description of a rare condition “kissing molars”, which are permanent molars with their occlusal surfaces contacting each other in a single follicular space, with roots pointing in opposite directions.[1] Although factors influencing tooth impaction is not yet fully understood, it has been hypothesized that, resorption of bone can result in bone loss along mesial root of the impacted third molar and cause movement and tipping, also presence of a fourth molar can be a predisposing factor.[4] KM is also observed to occur in patients diagnosed with MPS and related disorders.[5]

Maintenance of KM can be associated to complications such as decreased mandibular bone tissue and increased risk of mandibular fracture, root resorption of adjacent teeth, pericoronitis, local pain and cystic changes.[6] Gonzalez-Perez *et al*.[1] conducted a MEDLINE search based on the topic KM, and came across twenty-two cases of KM which symptoms and associated signs were evaluated. In six patients dentigerous cysts were present and confirmed histopathologically. One patient had symptoms of pericoronitis. The most frequent signs and symptoms were pain and swelling on the ipsilateral side of the mandible or TMJ. Five asymptomatic KM cases were reported. None of the reports involved a fibro-osseous lesion or any other lesions in the related jaw.

In literature removal of lower impacted teeth is associated with significant postoperative morbidity including alveolitis, jaw fracture and sensorineural impairment of the inferior alveolar nerve, displacement of the tooth or tooth root into the adjacent anatomical spaces and localized osteomyelitis.[5, 7] Gülses *et al*.[8] reported 9 cases treated surgically in which 3 of the patients had mild paraesthesia of the lower lip after surgery. The condition resolved 3-6 months after surgery. None of the stated

complications were observed in our cases.

RESULTS

Presence of KM can be associated with pathologies such as hyperplastic dental follicles and dentigerous cysts. We are in the opinion that jaws involving KM can be further examined for lesions of the jaws such as in our case fibro-osseous lesions.

Acknowledgements: None

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*Cumhuriyet Dental Journal 2017; 20(2):*77-84
Doi: 10.7126/cumudj.345911 *CLINICAL REPORTS*

PYOGENIC GRANULOMA: A CASE REPORT

Piyojenik Granüloma: Olgu Raporu

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Makale Kodu/Article Code ***:*** *148349*

Makale Gönderilme Tarihi ***:*** *23.10.2015*

Kabul Tarihi ***:*** *06.11.2015*

ABSTRACT

Purpose: The purpose of this study is to evaluate the treatment and pursuit process of the pyogenic granuloma which has seen on 12 years old girl patient.

Case Presentation: Pyogenic granuloma is a lesion which classified in vascular tumors which constitute 30-60% of all the reactive lesions of gingival tissue. Trauma, infections of capillary wall, hormonal factors, foreign materials, hypertension and poor oral hygiene are accused for development of pyogenic granuloma. It may occur at all age groups and in both sexs. In the oral cavity, pyogenic granuloma lesions are most frequently encountered on the gingiva. Definitive diagnosis can only be made by histopathologic examination of biopsied tissue. The treatment of this lesion is surgical excision. If surgical excision removal is incomplete, the lesion has got the risk of the recurrence.

A twelve years old female child applied to our clinic with complaints like on anterior palatinal region gingival bleeding and swollen condition on the same region. As beginning treatment oral hygiene education was given to the patient and plaque and calculus were removed. Under local anesthesia the lesion was taken with excisional biopsy for doing distinctive diagnosis from the other pathological lesions which can be seen in oral cavity. In addition frenectomy surgery in the maxilla and ridge augmentation operation in the mandible is performed to provide more comfortable oral hygiene. Three-month follow-up results of the patients were found to occur again in the region of the palatal gingival enlargement. As a result, it was decided to re-operations performed gingivectomy. Patient after operations carried out gingivectomy was called to the appointment checked and encourage oral hygiene.

Conclusion: After taking of the excisional biopsy, the diagnose was made shaping “The Pyogenic Granuloma” to the mass which was examined histopathologically under the light microscope. In the control inspection, there was not any recurrence clinically.

Keywords: Pyogenic granuloma, excisional biopsy.

ÖZ

Amaç: Bu çalışmanın amacı, 12 yaşında bir kız çocuğunda görülen piyojenik granülomanın tedavisinin ve 12 aylık takip sürecinin değerlendirilmesidir.

Olgu bildirimi: Piyojenik granüloma vasküler tümörler içinde yer alan bir lezyondur. Gingivadaki bütün reaktif lezyonların %30-60’ını teşkil eder. Piyojenik granülomanın gelişmesinde travma, damar duvarı enfeksiyonları, hormonal faktörler, yabancı cisimler, hipertansiyon ve zayıf oral hijyenin etken olduğu bildirilmektedir. Tüm yaş gruplarında ve her iki cinste de görülebilir. Oral kavite de piyojenik granüloma lezyonlarına en sık gingivada rastlanır. Kesin tanı sadece biyopsisi alınan dokunun histopatolojik incelenmesi ile konulabilir. Bu lezyonun tedavisi cerrahi eksizyondur. Yeterli bir cerrahi eksizyon yapılmazsa lezyonun tekrarlama riski vardır.

12 yaşındaki kız çocuğu üst çene anterior palatinal bölgesindeki dişetinde şişlik ve bu bölgede kanama şikayetleri ile kliniğimize başvurdu. Başlangıç tedavisi olarak hastaya oral hijyen eğitimi verildi, plak ve diş taşları uzaklaştırıldı. Lezyonun ağızda görülebilecek diğer patolojik oluşumlardan ayırıcı tanısını yapabilmek için lokal anestezi altında bisturi yardımıyla eksizyonel biyopsi ile alındı. Maksillada ek olarak frenektomi operasyonu, mandibulada ise oral hijyen koşullarını daha rahat sağlaması için vestibül derinleştirme operasyonu yapılmıştır. Hastanın üç aylık takibi sonucu palatinal bölgedeki dişeti büyümelerinin tekrar oluştuğu görülmüştür. Bunun sonucunda tekrar gingivektomi operasyonu gerçekleştirilmesine karar verilmiştir. Gerçekleştirilen gingivektomi operasyonu sonrasında hasta oral hijyen konusunda teşvik edilip kontrol randevularına çağrıldı.

Sonuç: Eksizyonel biyopsi sonrasında ışık mikroskobu altında histopatolojik olarak incelenen kitleye “Piyojenik Granüloma” tanısı konuldu. Kontrol muayenesinde klinik olarak herhangi bir nüks mevcut değildi.

Anahtar Kelimeler: piyojenik granüloma, eksizyonel biyopsi

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INTRODUCTION

Pyogenic Granuloma is a benign, non-neoplastic and mucocutaneous lesion that occurs in skin and mucous membranes. [1,2] The first case of pyogenic granuloma was described in English literature by Hullien in 1884.[3] In fact, the name "pyogenic granuloma" is a wrong description because no infection is present, no histological representation of granuloma as well. However it was thought to be an horse-borne mycotic infection, it has been found to be caused by inflammatory changes in benign oral tumors.[4,5,6]

When clinically examined, it appears as soft, smooth, lobulated, exophytic, red papule with or without pedicle.[7,8] In second and third decades of the life it is encountered more frequently (3/2) in females than in males.[9,10] It is generally believed that sex hormones in females play an important role in pathogenesis of the lesion.[11] Low-degree local irritation, traumatic injury, hormonal factors and use of various drugs play roles in.[12] Apart from that, local irritants such as dental calculi, foreign materials and poor oral hygiene are among triggering factors.[7,13] In a study in which cases of 293 pyogenic granuloma was investigated, Gordon-Nunez et.al[14] has stated that of the lesions; 83% on gums, 5.3% on lips and on tongue with same percentage, 4.2% on hard palate, 0.8% on buccal mucosa and 0.4% on floor of the mouth were found. Any radiological finding is present in granuloma. The definitive diagnosis is achieved by histological examination.[15] Differential diagnosis is made with peripheral giant-cell granuloma, metastatic cancer, hemangioma, hyperplastic growth of gums, Kaposi sarcoma, angiosarcoma and non-hodgkin lymphoma.[16] Treatment methods are surgical excision, electrocauterization, sclerotherapy, curettage, cryotherapy, laser application and keeping local irritants apart.[17,18,19,20]

Aim of this study is to evaluate the treatment and follow-up period of pyogenic granuloma found in a 12 year-old girl.

CASE REPORT

12 year-old female patient was admitted to department of pedodontics of Cumhuriyet University Faculty of Dentistry with complaints of swelling and bleeding in upper jaw palatine region. No extra-oral finding was detected in patient that was learned to be systemically healthy. Also, it has been learned that the swelling in palatine region was getting bigger during last month and she had complaint of bleeding during chewing. Lymphadenopathy, sign of infection or pain was not present.

In intraoral examination of the patient, swollen, pedunculated, partially ulcerated, hyperemic-looking and faint pink-colored mass with diameter of 13mm was determined in supero-anterior palatine region.

In addition, oral hygiene of the patients is not proper and gums are hyperemic and edematous (Figure 1-2).

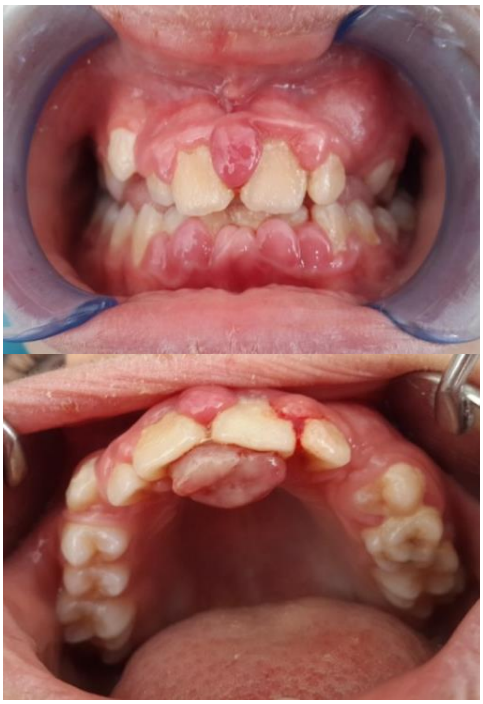

Figure 1. Initial clinical apperance

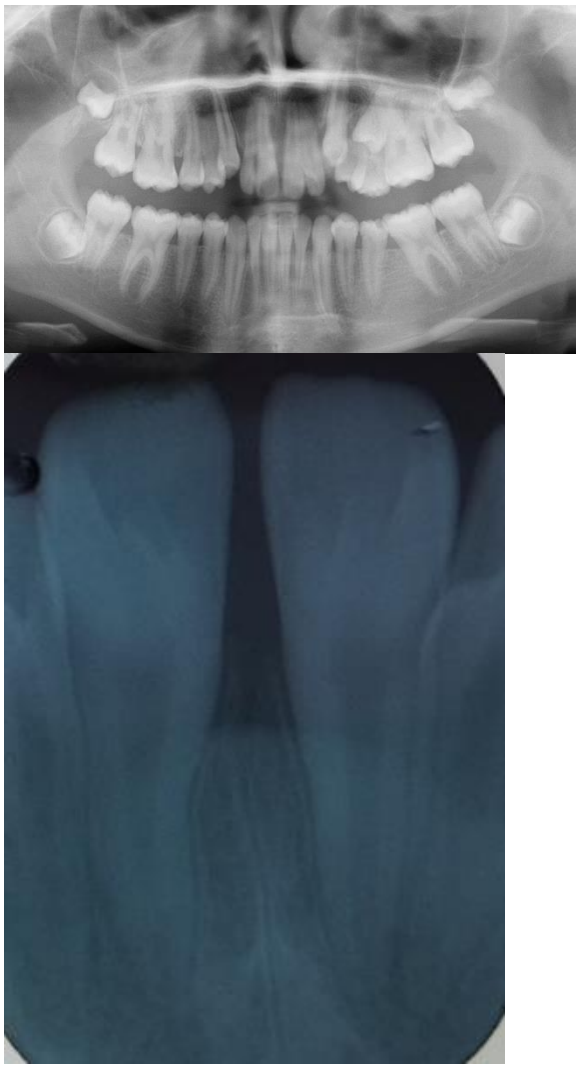

Figure 2. Initial radiographic appearance

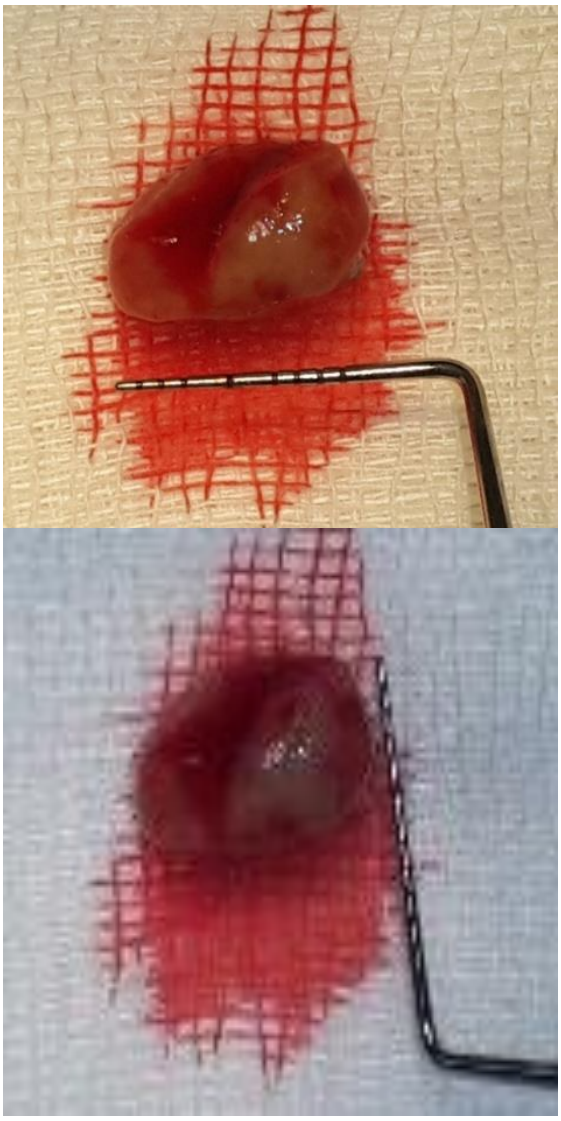

Figure 3. Excised mass

Periodontal and surgical approach

For an initial treatment, especially dental plaques and calculi were scaled and oral hygiene education was told in order to achieve oral hygiene and to decrease gingival inflammation. After optimal oral hygienic conditions were achieved, the lesion located in supero-anterior region was removed with excisional surgery. Periodontal pat was inserted and kept for a week. Oral hygiene education was told to the patient. The patient was prescribed mouthwash containing 0.12% clorhexidine, an analgesic containing paracetamol and an antibiotic of amoxicillin type following the surgery (Figure 3).

Histopathological Evaluation

The excised tissue sample was sent within 10% formalin solution for pathological examination. Biopsy sample was buried into paraffin after routine follow-up procedures and slices with thickness of 0,5µ was obtained by using microtome and examined under light microscope with Hematoxylin-eosin staining. In slices, a soft tissue demonstrating ulcerated-looking surface, proliferative vascular structures in stroma and dense mixture of cellular inflammatory infiltration sparsely have been determined (Figure 4-5).

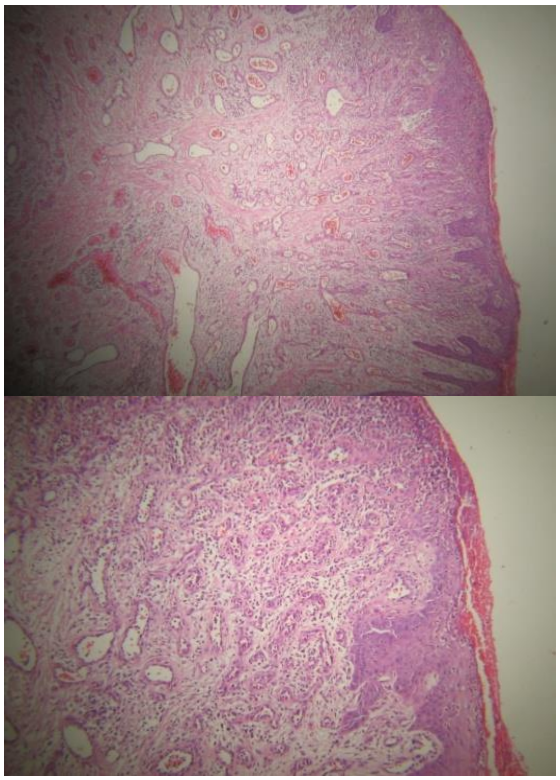

Figure 4. Histopathologic examination a:4x10 magnification, b:10x10 magnification

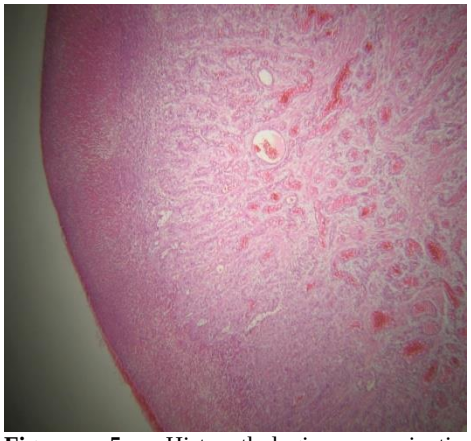

Figure 5. Histopathologic examination 4x10 Magnification

Stratified squamous epithelium is destroyed, ulcerated surface containing intraepithelial PMNL necrotic debris and peripheral blood elements, and intensive inflammatory granulation tissue have been determined. However in stroma, intensive capillary proliferation, increased vascularity, congestion in vessels (engorgement and blood pooling) are present.

On the surface squamous epithelium is completely destroyed and the surface is completely ulcerated. Collection of fibrin exudate has been intensively observed on ulcerated ground and more deeply intensive inflammatory granulation tissue and capillary proliferation have been observed. As a result of histopathological evaluation, the lesion was reported as pyogenic granuloma. One week after the operation, gingivectomy with internal bevel incision was performed in order to eliminate gingival swellings and tissue irregularities, granulation tissues are completely cleared. And additionally frenectomy in maxilla and vestibular enlargement operations in mandibula have been performed in order to patient to provide oral hygienic conditions more easily (Figure 6).

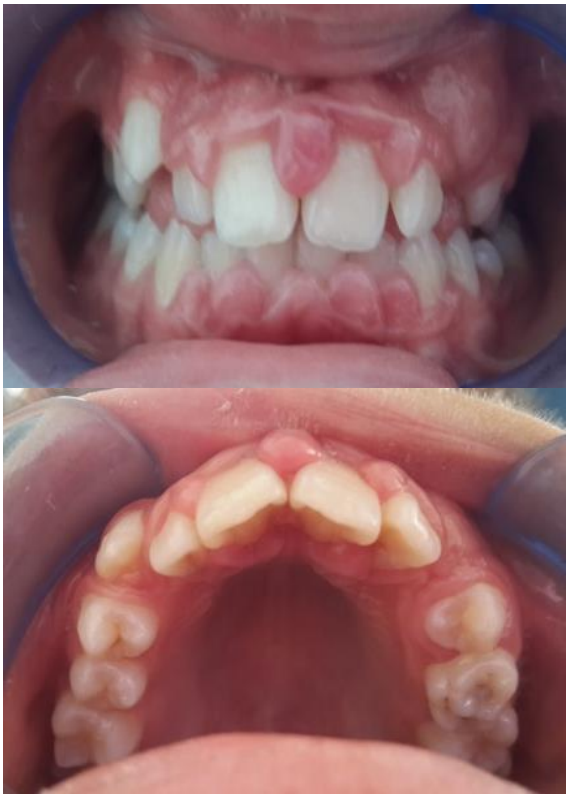

Figure 6. Clinical appearance after 1 week from the operation

As a result of patient three-month controls, gingivectomy has been decided again for recurred inflammatory swellings in gums. One week after gingivectomy, patient was called for a control and informed again about oral hygiene and control sessions will be on going (Figure 7).

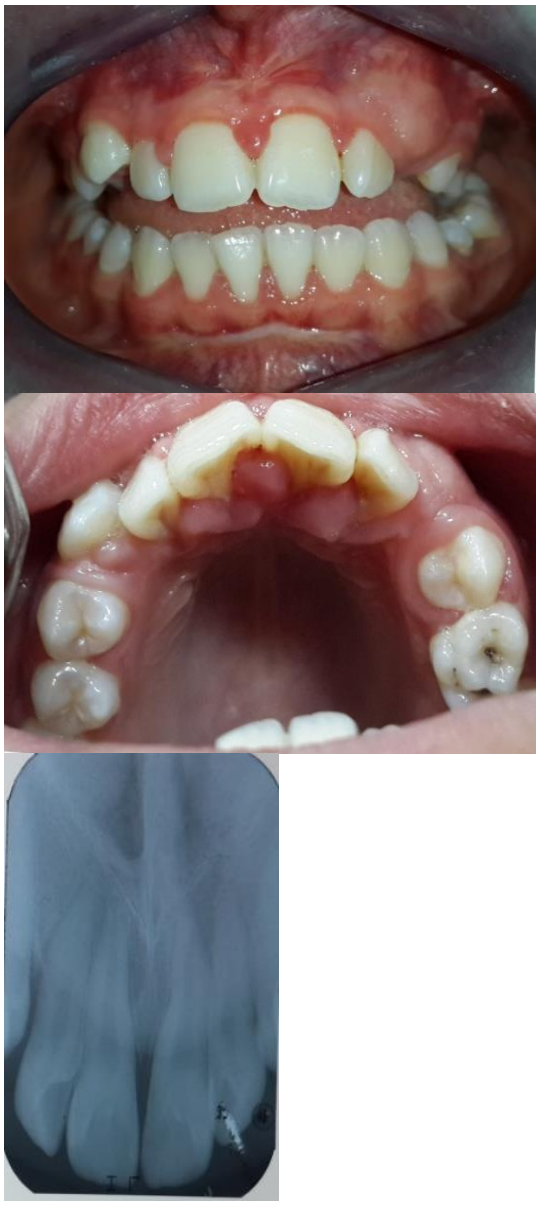

Figure 7. Clinical and radiological appearance 3 months after operation

In control appointment of the patient after 1 year, it was observed that gingival tissues were healthy with a successful recovery and there was no recurrence (Figure 8).

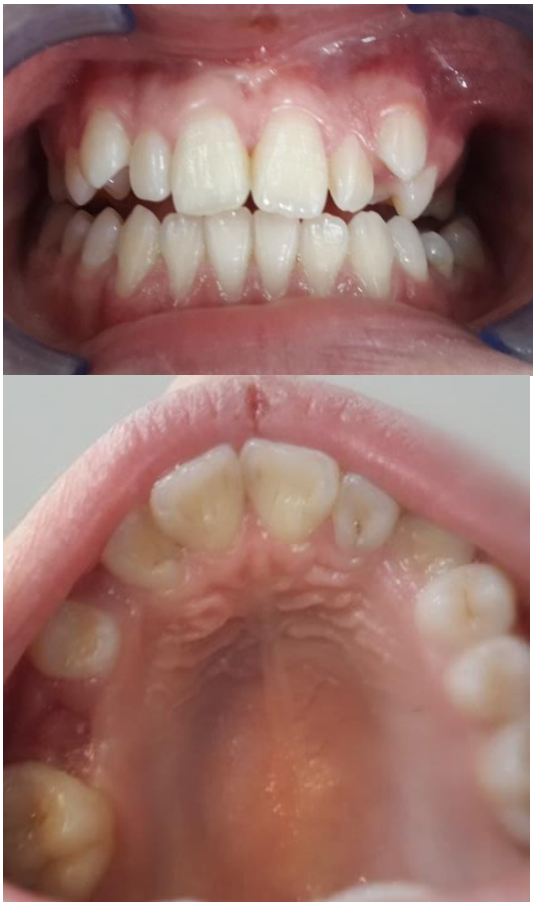

Figure 8. Clinical appearance of patient after 1 year

DISCUSSION

Oral Pyogenic Granuloma is a mucosal vascular hyperplasia affecting the tissues.[21] It occurs due to respond of connective tissue to a minor injury or irritation.[22] Irritating factors may be dental calculi, poor oral hygiene, nonspecific infections, excessive restorations and buccal biting. Due to irritation the underlying fibrovascular connective tissue becomes hyperplastic and granulation tissue that cause formation of Pyogenic Granuloma is formed.[23] Due to high vascularity in the lesion, even a slight trauma can cause severe hemorrhages. Presence of obvious capillaries in newly-formed pyogenic granulomas and hyperplastic granulation tissue increases the probability of bleeding. Lesions tend to be more collagenized as the duration of stay increases.[7] Colours of the lesions depend on duration of stay in mouth. Whereas newly-formed lesions vary from red to purple, lesions with long-term stay have pinkish coloration. In

our case, swollen, pedunculated, partially ulcerated, hyperemic-looking and faint pink-colored mass with diameter of 13mm was determined in supero-anterior palatine region. Also it was learned that the patient had poor oral hygiene, and her gums were hyperemic and edematous.

Pyogenic Granuloma is commonly encountered in second decade of life. Especially in females due to effects of hormones on vessels it is the most commonly encountered in teenagers and young adults.[24,25] Our case is a 12 year-old teenager female patient. We think that hormonal status during puberty plays important role in etiology.

Lesions are encountered more frequently in maxilla than in mandibula and anterior parts are more commonly affected than posterior parts. However pyogenic granuloma in oral cavity occurs most commonly in gingiva (in 75% of the cases) it may occur in lips, tongue, oral mucosa, hard palate, too.[7,26] In our case, lesion has occurred in maxillary palatine region.

Incidence of Pyogenic Granuloma among all reactive oral lesions is found to be between 26.8-32%. Clinically, these lesions are formed as a single nodule or unpedunculated papule and with smooth or lobulated surfaces. Their dimensions can vary from some mm to some cm. Clinical progression is slow, asymptomatic and painless but it can also show a rapid progression.[24,27] In our case, it has been learned that the swelling in palatine region has got bigger within last 1 month and he has had complaint of bleeding during chewing.

Histologically, the lesion demonstrates high vascular proliferation that resembles that of granulation tissue.[7] Lesion contains smaller and larger blood vessels divided by a fibrotic septa. Definitive diagnosis concerning Pyogenic Granuloma is achieved by presence of polymorphic and chronic inflammatory cells along the edematous stroma and microcyst formation.[28] In our case, in slices, a soft tissue demonstrating ulcerated-looking surface, proliferative vascular structures in stroma and dense mixture of cellular inflammatory infiltration sparsely have been determined. Stratified squamous epithelium is destroyed, ulcerated surface containing intraepithelial PMNL necrotic debris and peripheral blood elements, and intensive inflammatory granulation tissue has been determined. However in stroma, intensive capillary proliferation, increased vascularity, congestion in vessels (engorgement and blood pooling) are present. Intense inflammatory infiltration is found around the vessels.

Treatment of Pyogenic Granuloma is based on removal of the lesion. Treatment of Pyogenic Granuloma depends on size and location of the lesion. Excisional biopsy is the treatment option in majority of cases but other treatment options can be thought. Larger lesions are treated with incisional biopsy in order to prevent deformity. Neodymium: Yttrium Aluminum: Garnet (Nd: YAG) laser surgery can be used in excision of the lesion due to its superior coagulative ability and its ability to cause less intraoperative bleeding. Other alternative treatment options are cryotherapy, electrocauterization, pulsed dye laser and chemical agents.[29, 30] In our case, The lesion was removed with excisional surgery. Gingivectomy was performed in order to eliminate gingival tissue irregularities, and frenectomy in maxilla and vestibular enlargement operations in mandibula have been performed in order to patient to provide oral hygienic conditions more easily.

Because of being an encapsulated lesion the pyogenic granulomas can reoccur if they are not completely removed. Whereas in excision involving the floor of the lesion and the periosteum and in removal performed with full-thickness flaps recurrence does not occur, in application of electrocauterization in which lesion is removed superficially, this ratio is

43.5%.[31] When pulsed dye laser is used this ratio is 9%.[32] Whereas in electrocauterization following excision that performed on 128 cases by Paglia and Kohen recurrence is not encountered, in the study in which Lee et.al. used CO_2 laser 2 unsuccessful cases were reported.[33,34] In the study conducted by Krisnapillai et.al recurrence was reported in 14.88% of 215 cases.[35] During postoperative period patient follow-up should be performed precisely and all dental calculi in area of the lesion and neighboring areas against possibility of recurrence, possibility of recurrence should be tried to be decreased by removal of periosteum and bone in the localized area and educating the patient about oral hygiene. In our case, in the control after 3 months, recurrence found in palatine region was removed by gingivectomy again.

In conclusion, it should be remembered that pyogenic granulomas can develop rapidly and possibility of recurrence following the treatment is present. It shouldn't be forgotten that even after an effective treatment following the definitive clinical and histological diagnosis of Pyogenic Granuloma recurrence may occur and the patient should be followed-up.

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*Cumhuriyet Dental Journal 2017; 20(2):*85-89
Doi: 0.7126/cumudj.345921

COMBINED TREATMENT OF A LARGE AGGRESSIVE CENTRAL GIANT CELL GRANULOMA (CASE REPORT)

Büyük Agresif Bir Santral Dev Hücreli Granuloma Birlikte Tedavisi (Olgu Sunumu)

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Belgin GULSUN[3]

Makale Kodu/Article Code ***:*** *177750*
Makale Gönderilme Tarihi ***:*** *14.02.2016*
Kabul Tarihi ***:*** *18.02.2016*

ABSTRACT

Central giant cell tumours are rare, accounting for less than 7% of all jaw tumours. These tumours are usually observed in women, occur most often in the mandible, and are more common in the second decade of life. Treatment consists of local removal, partial resection, or total resection. In this case, a 32-year-old female patient presented in our clinic with pain in the anterior mandible. No cervical lymphadenopathy was detected upon physical examination. No ulceration was observed during the intraoral examination, but sensitivity was found in the vestibular area. However, no sensation loss in any teeth or in the lips was detected. This case report presents a 32-year-old female patient with central giant cell tumour causing extensive bone loss in the mandible base that was treated with partial resection.

Keywords: Giant cell granuloma, Mandible, Resection

ÖZ

Santral Dev Hücreli tümörler; çene tümörleri içinde kadınlarda ve mandibulada sık görülürler ve yaşamın ikinci dekatında daha sıktırlar. Tedavisinde lokal küretaj, parsiyel rezeksiyon ve total rezeksiyon uygulanabilir. Bu vaka raporunda 32 yaşında kadın hasta anterior mandibulada ağrı şikayetiyle kliniğimize başvurdu. Fiziksel muayenede servikal lenfadenopati saptanmadı. İntraorol muayenede ülserasyon saptanmadı fakat vestibül bölgede hassasiyet mevcuttu. Bununla beraber dişlerde ve dudakta his kaybı yoktu. Bu vaka raporunda 32 yaşında kadın hastada mandibula basisinde yaygın kemik kaybına neden olan santral dev hücreli tümörün parsiyel rezeksiyon ile tedavisi sunuldu.

Anahtar Kelimeler: Dev Hücreli Granüloma, Mandibula, Rezeksiyon

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INTRODUCTION

Granulomas are benign aggressive jaw tumours that are rare and occur mostly in the mandible of women under 30 years of age.[1] This tumour type was first identified by Jaffe. Although the aetiology is not completely known, granulomas are thought to occur due to trauma, infection, and heredity.[2] Granulomas have a unilocular or multilocular radiolucent appearance, and curettage is most commonly used for treatment. However, partial resection or total resection is needed in delayed cases.[3] The treatment by partial resection of a central giant cell tumour (CGCT) in the mandible is presented in this case report.

CASE REPORT

A 32-year-old female patient presented to our clinic with pain in the gonion, but no intraoral or extraoral panicula or lymphadenopathy (LAP). Sensitivity was observed in the gonion. Upon intraoral examination, the central mandible teeth were found to have been previously extracted, but no infections were present in the teeth. A multilocular wide radiolucent lesion, which had caused full bone loss in the mandible base, was identified in a panoramic radiography and tomographic image (Figure 1, 2).

Figure 1. Panoramic film before operation

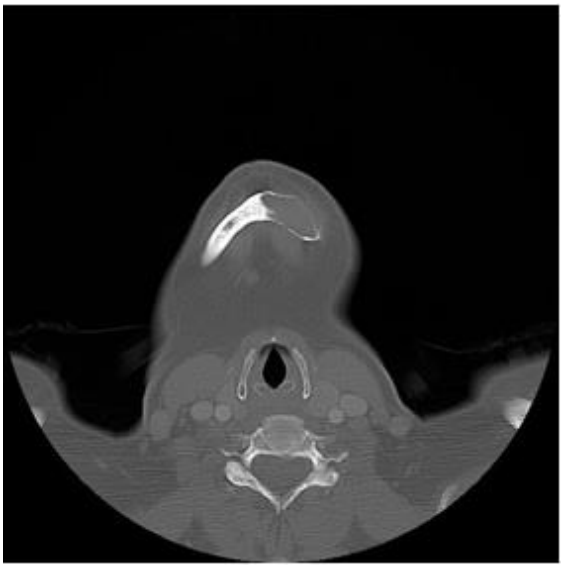

Figure 2. BT imaging before operation

The decision to operate was made. Due to the lesion's position and the estimated need for reconstruction, the lesion was resected using an extraoral approach (Figure 3).

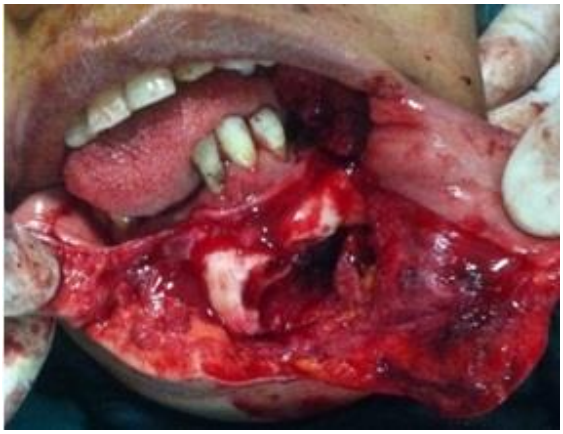

Figure 3. Extraoral approach to tumour

The missing mandible section was reconstructed with plaque at the base (Figure 4), and the surgical incision was closed with primary sutures (Figure 5).

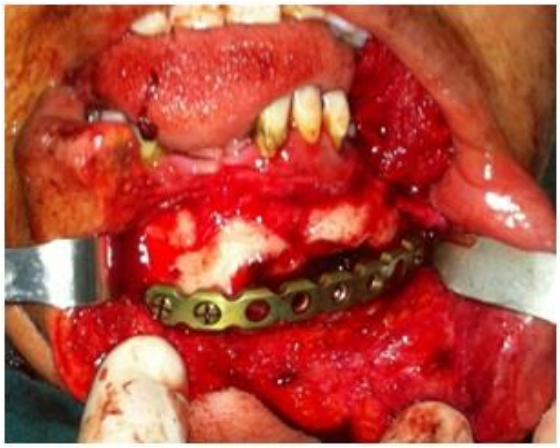

Figure 4. Placement of reconstruction plaque

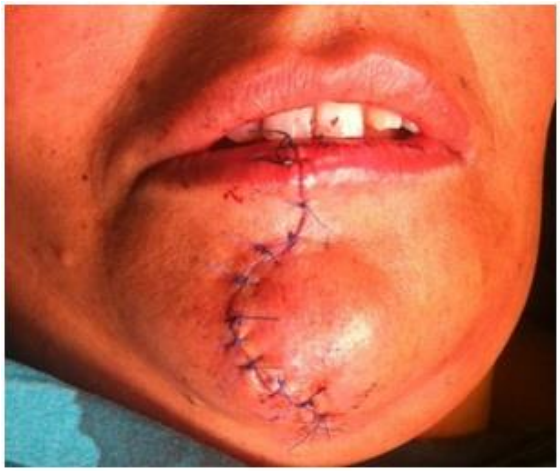

Figure 5. Extraoral suturing

The post-operative images revealed that the plaque position resulted in continuity in the mandible base (Figure 6).

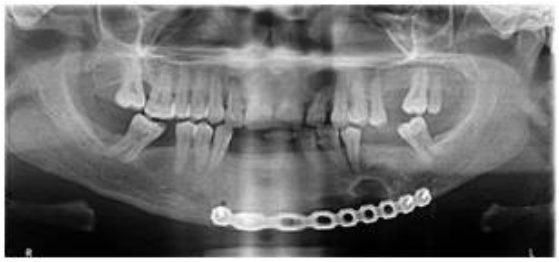

Figure 6. Panoramic film taken after operation

The patient was given post-operation antibiotics and analgesics. The obtained tumour material was delivered to the department of pathology for histopathologic examination (Figure 7).

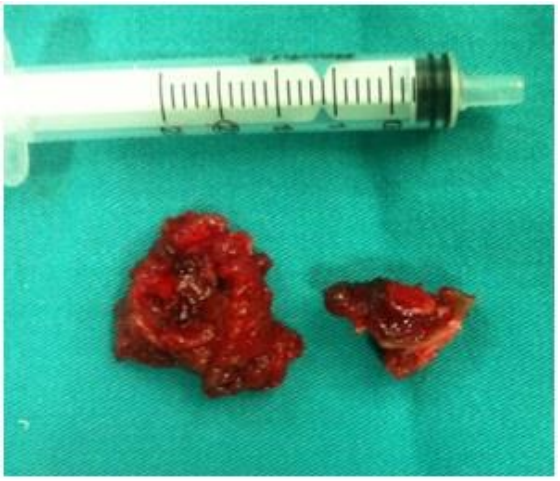

Figure 7. Removed tumour material

Histopathologic examination identified the tumour as CGCT (Figure 8).

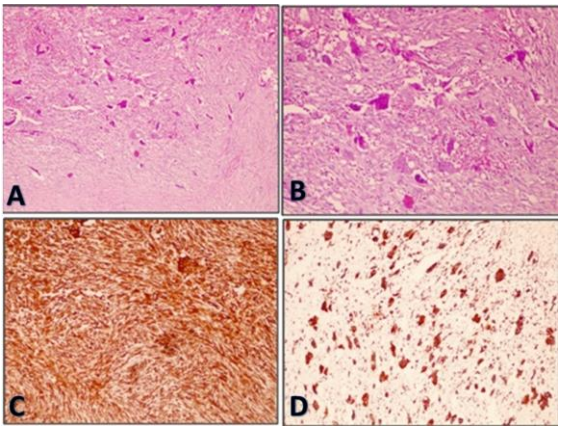

Figure 8A (H&E× 10) and 8B (H&E×20). In addition to many osteoclast-like multinuclear giant cells, haemorrhagic vascular areas, and occasional mitotic activity are observed by H.E. staining.
Figure 8C (Vimentin×20) and 8D (CD 68). The specific appearance of giant cells and mesenchymal structure are shown on immunohistochemical examination with vimentin staining.

After 1,5 year following there was no recurrence and healing was perfect on panoramic graphy (Figure 9).

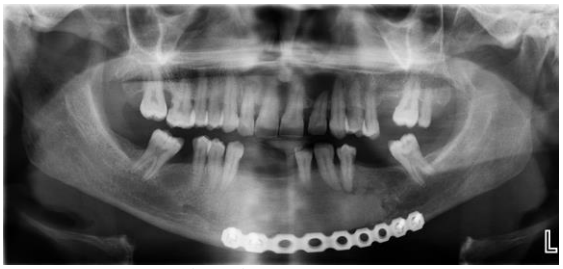

Figure 9. Panoramic film after 1,5 year

Intraoraol and extraoral viev after 1,5 year was natural and there was no esthetic problems (Figure 10,11).

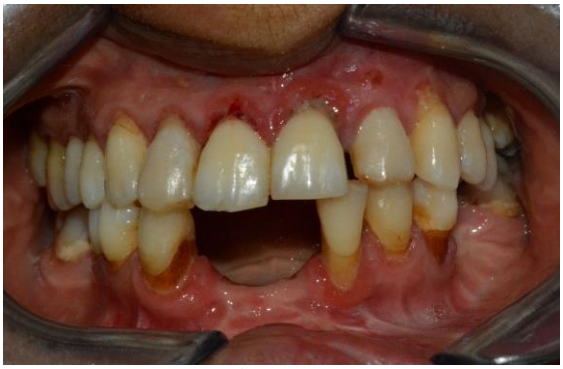

Figure 10. Intraoral view after 1,5 year

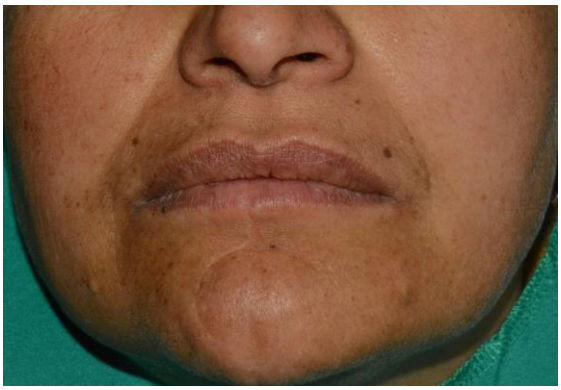

Figure 11. Extraoral view after 1,5 year

DISCUSSION

There are two clinical types of giant cell granulomas. Central giant cell granulomas are generally observed in the mandible, and rarely in the maxilla; they usually occur between 10 and 20 years of age. Giant cell tumours can also be found in the ethmoid, sphenoid, and temporal bones.[4,5] The peripheral type of giant cell tumour is mostly observed in women under 30 years of age. Called giant cell epulis, it is observed on the gingiva, as distinct from CGCT.[6] Despite the difference in their locations, there are no histopathologic differences between these tumour types. The detection of giant cells during histopathologic examination is definitive for the diagnosis.[2] Giant cells are generally observed in the mandible and first molar.[7] In this case, the cells were observed in the anterior mandible, which is different from previously reported cases.

CGCTs usually reveal themselves as painless paniculas.[4] In this case, no panicula was present, and the patient's primary complaint was pain. Pain can be related to tumour size and location relative to nerves. The general approach to CGCT treatment is curettage, and this approach has a reported success rate of 80%.[7, 8] However, curettage may not be sufficient in cases of delayed diagnosis, and partial or total resection can be implemented in such cases.[9] Some researchers have suggested corticosteroid injection into the lesion to minimise tumour volume before surgery.[10] In this case, the patient recuperated after partial resection and later reconstruction.

CONCLUSION

A surgical approach generally results in successful CGCT treatment, and a surgical method can be modified based on patient need and the size, localisation, and recurrence likeliness of a tumour.

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*Cumhuriyet Dental Journal 2017; 20(2):*122-131
Doi: 10.7126/cumudj.345953 *REVIEWS*

BISPHOSPHONATE INDUCED OSTEONECROSIS OF THE JAWS AND CURRENT THERAPIES

Çenelerin Bisfosfonata Bağlı Osteonekrozu ve Güncel Tedaviler

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Makale Kodu/Article Code ***:*** *191062*

Makale Gönderilme Tarihi ***:*** *01.06.2016*

Kabul Tarihi ***:*** *06.06.2016*

ABSTRACT

Bisphosphonates are pharmacological agents which are the potent inhibitors of osteoclastic activity. Nowadays, bisphosphonates are used to treat a variety of bone disease or related complications such as metastatic or osteolytic bone disease, hypercalcemia of malignant origin and osteoporosis. Although, bisphosphonates are significantly reduces the skeletal complications of these diseases, they are inevitably cause a specific osteonecrosis characterized by treatment resistant exposed necrotic bone, especially seen in the jaw bones where the bone turnover is high. Currently there is no definitive treatment for this complication induced by the use of bisphosphonates. The search for new treatments methods to prevent the complications that cause patients to become a victim of the economic and social aspects of this situation is still ongoing.

This review is intended to provide information about the chemical structure of bisphosphonates, their mechanisms of action and current diagnosis/treatment methods of the osteonecrosis.

Keywords: Bisphosphonates, malignant diseases, complications, osteonecrosis, treatment options.

ÖZ

Bifosfonatlar osteoklastik aktivitenin güçlü inhibitörü olan farmakolojik ajanlardır. Günümüzde osteolitik kemik hastalığı, malignite kaynaklı hiperkalsemi, metastatik kemik hastalıkları ve osteoporoz gibi birçok farklı kemik hastalığı veya ilişkili komplikasyonun tedavisinde kullanılmaktadırlar. Bifosfonatlar bu hastalıkların iskeletsel komplikasyonlarını önemli ölçüde azaltsa da özellikle yüksek kemik döngüsünün görüldüğü çene kemiklerinde tedaviye dirençli, ekspoze nekrotik kemik ile karakterize özgün bir osteonekroza neden olmaktadırlar. Bifosfonat kullanımına bağlı olarak gelişen bu komplikasyonun henüz kesin bir tedavisi bulunmamaktadır. Hastaların, bu durumun ekonomik ve sosyal yönleri nedeniyle mağdur olmasına sebep olan bu komplikasyonla mücadele etmek için yeni tedavi arayışları hala sürmektedir. Bu derlemede bifosfonatların kimyasal yapıları, etki mekanizmaları ve osteonekrozun güncel tanı /tedavi yöntemleri hakkında bilgi verilmesi amaçlanmıştır.

Anahtar Kelimeler: Bifosfonatlar, malign hastalıklar, komplikasyonlar, osteonekroz, tedavi seçenekleri.

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INTRODUCTION

Bisphosphonates (BPs) are the synthetic analogs of pyrophosphates which are the endogenous regulator of bone mineralization.[1] This pharmacological agents having a strong inhibitory effect on osteoclastic activity were first produced in Germany in the mid-19th century and used in industrial areas, in the prevention of kidney stone formation, in the content of the toothpaste and in obtaining bony gamma graphs in the past.[1-3] However, with changes in the molecular structure the effectiveness of the drug have increased.[2] BPs are currently used in the treatment of many diseases such as breast, prostate and lung cancers associated with bone metastasis, osteogenesis imperfecta, osteoporosis, paget's disease, fibrous dysplasia and multiple myeloma.[4-6]

Chemical structure of the bisphosphonate is similar to inorganic pyrophosphate and the "bis" prefix refers to two phosphonate groups attached to a common carbon atom.[7] Unlike the pyrophosphates, carbon atom is located in the center of bisphosphonates. This difference in molecular structure prevents the hydrolysis of BPs in acidic environments and increases the accumulation of bisphosphonates in the hard and soft tissues.[8] Biological activity of BPs determined by the peripheral chains. According to the nitrogen content in the peripheral chains BPs can be divided into 2 pharmacologic classes as; non-nitrogen-containing (alkaline bisphosphonates) and nitrogen-containing (aminobisphosphonates) BPs (Table 1).[9] Non-nitrogen-containing bisphosphonates are the group of bisphosphonates which have the lowest activity and show their antiresorptive effects by transforming into toxic analog of adenosine triphosphate (ATP) and inducing apoptosis.[10, 11] Antiresorptive activity of nitrogen-containing bisphosphonates involves inhibition of mevalonate pathway which is important for osteoblast function in multiple steps.[2, 10] Mevalonate pathway inhibition results with the failure of prenylation and inability of the Ras, Rho and Rac proteins that regulates the cytoskeleton organization and cell survival to be activated. Thus, intracellular vesicular transport in osteoclasts deteriorate and the resorption process is suppressed.[8, 12, 13]

Table 1. Classification of Bisphosphonates

Drug	Brand name	Generation/Type	Mechanism of action	Route	Indication	Potency
Etidronate	Didronel Disfosfen	1st/NNC	Induces osteoclastic apoptosis	PO, IV	OP, PGD, HMO	1
Clodronate	Bonefos Loron	1st/NNC	Induces osteoclastic apoptosis	PO, IV	OP, PGD	10
Tilduronate	Skelid Tildren	1st/NNC	Induces osteoclastic apoptosis	PO	PGD	10
Neridronate	Nerixia	2nd/ NC-A	Inhibits mevalonate pathway	IM, IV	OI, PGD,CRPS-I	100
Pamidronate	Aredia	2nd/ NC-A	Inhibits mevalonate pathway	IV	OP, HMO, PGD	100
Olpadronate	-	2nd/ NC-A	Inhibits mevalonate pathway	-	EP	500
Alendronate	Fosamax	2nd/ NC-A	Inhibits mevalonate pathway	PO	OP, PG	500
Ibandronate	Boniva	2nd/NC-A	Inhibits mevalonate pathway	PO, IV	OP	1000
Risedronate	Actonel Acral	3rd/NC-H	Inhibits FPS/stabilize conformational changes	PO, IV	OP, PGD, MM, HCM	2000
Zoledronate	Zometa Aclasta	3rd/NC-H	Inhibits FPS/stabilize conformational changes	IV	MM, HCM, MC	10000

NNC: Non nitrogen containing, **NC-A**: Nitrogen containing-alkyl, **NC-H**: Nitrogen containing-heterocyclic, **PO**: Peroral, **IV**: Intravenous, **IM:** Intramuscular**, OP**: Osteoporosis, **PGD**: Paget's disease, **HMO**: Hipercalcemia of malignant origin, **OI**: Osteogenesis imperfecta, **CRPS-I**: Complex regional pain syndrome type I, **MM**: Multiple myeloma, **MC**: Metastatic cancer, **EP**: Experimental purpose, **FPS**: farnesyl pyrophosphate synthase

BPs have particular affinity for hydroxyapatite crystals in areas with high bone turnover. When the bisphosphonate bound to the bone, it can remain stable for approximately 10 years without undergoing hydrolysis.[11, 13, 14] BPs show their activity by inhibiting the development and function of the osteoclasts in molecular, cellular and tissue levels.[15-17] BPs also triggers apoptosis of tumor cells, retarding tumor metastasis by anti-angiogenic properties and inhibit wound healing as well.[18-20]

BPs are drugs with low bioavailability. About 1 % of the drug from the gastrointestinal tract in oral administration and 50 % of the drug in intravenous administration is bound to the bone.[21] Mostly related to the gastrointestinal tract, BPs can cause side effects such as renal toxicity, acute renal failure and hypocalcemia.[6] Also, ocular side effects[22], osteonecrosis[23], esophageal cancer[24], atrial fibrillation[25] and

hepatitis[26] are among the other reported side effects of BPs.

Bisphosphonate-induced osteonecrosis of the jaws (BIONJ) is one of the most serious side effect of bisphosphonates which has defined as the exposed necrotic bone observed in the maxilla and /or mandible at least 8 weeks in the patients who receiving or had been exposed to a bisphosphonate and not had radiation therapy to the craniofacial region.[27] Similar findings of osteonecrosis were first seen in the match factory workers and named as 'Phossy Jaw' in 1899. Convertion of the phosphorus to the potent BPs such as pamidronate and alendronate by the chemical reactions in the body considered as the possible cause of this endemic osteonecrosis. It is reported that although phosphorus vapor in high temperature has a simple chemistry, it converted to the simple BPs when passed through the lungs and combine with the H_2O and either CO_2 and tetrahydrofolate. This simple BPs may have also circulated and combine with either ammonia or any of common amino acids in the respiratory tract such as lysine to produce more potent form of BPs.[28]

BIONJ is observed in the jaw bones rather than the other bones in the skeletal system. It is considered that the main reasons of this are the effect of tooth movement created in the periodontium and the high turn-over in the jawbones.[29] Dixon[30] investigate the remodeling rates in different regions and detected that more remodeling occurs in the alveolar crest than in tibia, in inferior border of mandible and bone in mandibular canal level; 10 times, 5 times and 3-5 times respectively. On the other hand, the microbial environment in the oral cavity, continuous relationship with the environment, susceptibility to trauma and vascularization were all shown among other factors increasing the risk of jaw bone osteonecrosis.[31, 32] In a review conducted by Hughes *et al*.[33] in 1962, it is claimed that the ideal environment for the osteonecrosis originate from the microorganisms and chemicals such as phosphorus together were the jaw bones.

Risk factors that play role in the occurrence of osteonecrosis in the jaw bones due to use of bisphosphonates has been classified as drug related, local and systemic risk factors[34] (Figure 1).

***Osteonecrosis**

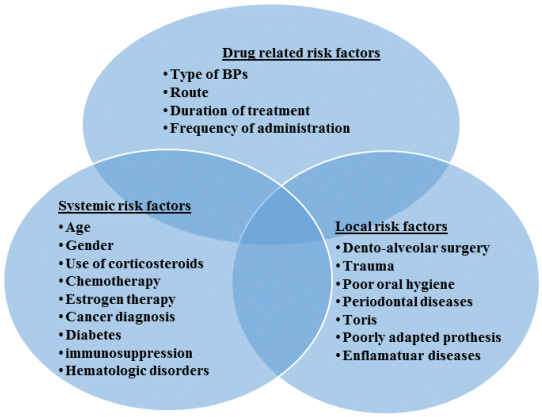

Figure 1. Risk Factors of BIONJ

In 2005 Marx *et al.*[35], in their study to evaluate inducing factors of the 119 cases of osteonecrosis, they found that osteonecrosis reported to occur in 25,2% of the cases spontaneously, 37, 8 % of the cases after tooth extraction, 28,6% of the cases associated with periodontal disease, 11,2% of the cases after periodontal surgery and 0,8% of the cases after apical resection.

The serological biochemical marker used to determine development risk of the osteonecrosis is the serum C-Terminal Telopeptide (CTX) value. CTX is the terminal cross-linked telopeptide of type I collagen which occurs in the cases of increased bone turnover, resulting the fragmentation of the type I collagen by osteoclasts.[36] However, in recent years the radiological detection of periodontal ligament expansion considered more sensitive than serum CTX in predicting the development BIONJ.[37] Also, in a case-control study conducted by Kim *et al*.[36] it is reported that the CTX values did not differ between the patients in the groups of BIONJ and non-BIONJ.

Clinically, BIONJ is characterized by the painful or painless inflammation, purulent drainage, fistula formation and osteolysis or pathologic fractures that may be associated with necrotic bone.[38, 39] Radiologically, non-specific findings of the osteonecrosis were seen in the initial phase but in the late stages sequestration, thickening of lamina dura/alveolar crest and multiple sclerotic areas can observed.[40] In radiological evaluation of BIONJ conventional radiography (panoramic radiography), bone scintigraphy, computed tomography (CT) and magnetic resonance imaging (MRI) are used.[41-44] Panoramic radiography is useful in the overall evaluation of the jawbones. However, it is not possible to detect changes in bone density depends on osteonecrosis otherwise the bone mineral loss exceed the rate of 30-50 %. Also panoramic radiographs is inadequate to determine the boundaries of necrotic and healthy bones.[45, 46] Technetium-99m-methylene diphosphate bone scintigraphy assessments made by CT was confirmed to be superior to magnetic resonance methods in the diagnosis of osteonecrosis. However, the low resolution of the scintigraphic images and not being able to distinguish malignant lesions with inflammatory stages are the most important disadvantages.[43, 45] CT method is useful to determine the spread of osteonecrosis in both cortical and trabecular bone, its borders and to determine its relationship with neighboring anatomical tissue.[47] MRI techniques is gives detailed information on the presence of more soft tissue involvement.[45, 48]

Prevention and Treatment Strategies in BIONJ

In 2009 American Association of Oral and Maxillofacial Surgeons (AAOMS) has been revised the prevention and treatment strategies (Table 2-3) for patients about to begin bisphosphonate therapy, asymptomatic patients using bisphosphonates and the patients have osteonecrosis.[34]

Table 2: Prevention and treatment strategies in BIONJ

Patients about to begin bisphosphonate therapy	Asymptomatic patients who are undergoing bisphosphonate therapy		Patients with osteonecrosis
	IV	PO	
- A comprehensive oral examination - Elimination of dental pathologies -Providing optimal periodontal/oral health and patient education -Evaluation of prosthesis -Providing the time required for mucosal healing (14-21 days) or adequate healing of the bone after dento-alveolar surgery. -Medical oncologists consultation (for IV BPs)	-Providing optimal oral hygiene -Avoiding traumatic procedures. -Avoiding placement of dental implants - Non-restorable teeth may be kept in the mouth by endodontic treatment of the remaining roots	*-In the patients who have taken oral BPs less than three years:* No alteration or delay in the planned surgery with regular recall *-In the patients who have taking oral BPs less than three years and combined with corticosteroids:* Drug holiday (3 month), providing the time required for osseous healing and utilization of bone turnover marker levels suggested if systemic conditions permit. *-In patients who have received oral bisphosphonates alone or prednisone or other steroid medication more than three years:* Drug holiday (3 month), providing the time required for osseous healing and utilization of bone turnover marker levels suggested if systemic conditions permit.	-Aim: Eliminate pain, control infection, prevent progression of osteonecrosis -Surgical treatment should be delayed (if possible) -Surgery perform in the patients with stage 3 disease or patients with well-defined sequestrum -Areas of necrotic bone that are a constant source of soft tissue irritation should be removed or re-contoured -Elective dentoalveolar surgical procedures should avoided -Symptomatic patients with stage 3 disease may require resection and immediate reconstruction

Table 3: BIONJ Staging and Management

BIONJ Staging and Management (2009 AAOMS) - Current Therapies			
Stage	**Treatment**	**Treatments in Literature**	**Success Rates**
Risk category: Absence of exposed necrotic bone in asymptomatic patients treated with IV and oral BPs	*Not require treatment *Patient education	-	-
*Stage 0:*Non-specific symptoms or clinical and radiological findings existing without clinical symptoms of exposed necrotic bone	*Management of local factors *Providing oral hygiene *Medical treatment, including the use of antibiotic and analgesic	-	-
Stage 1: The presence of exposed necrotic bone in asymptomatic patients with no evidence of infection	*Antibacterial mouth rinses *Patient education for continuing bisphosphonate treatment *No surgical treatment is indicated	***A)****Pharmacological therapy* ***B)****Pharmacological + surgical therapy* ***C)****Pharmacological + surgical+ platelet rich plasma + laser phototherapy* ***D)****Laser assisted surgery* ***E)****Conventional surgery* ***F)****Antibiotic therapy of minor debridement surgery + HBO* ***G)****Local Debridement or Resective Intervention* ***H)****Resection of necrotic tissues, irrigation with antibiotics, application of L-PRF* ***I)****Surgical treatment with PRP*	**A)**1/3[60], **B)**3/4[60], **C)**1/2[60], **D)**1/1[61], **E)**2/7[61], **F)**3/3[62], **G)**65/108[63] **H)**7/7[64], **I)**1/1[65]
Stage 2: Pain and the clinical signs of infection in patients with exposed necrotic bone	*Symptomatic treatment with oral antibiotics *Antibacterial mouth rinses *Superficial debridement to reduce soft tissue irritation *Pain management	***A)****LLLT applications during the postoperative period in addition to medical and surgical treatment* ***B)****pharmacological + surgical therapy* ***C)****pharmacological plus surgical + platelet rich plasma + laser phototherapy* ***D)****laser assisted surgery* ***E)****Conventional surgery* ***F)****Ultrasonic (piezo) surgery + antibiotics* ***G)****Antibiotic therapy of minor debridement surgery + HBO* ***H)****Local Debridement or Resective Intervention* ***I)****Conservative treatment* ***J)****Resection of all infected tissues, intensive irrigation with antibiotics, application of L-PRF* ***K)****Teriparatide treatment* ***L)****Surgical treatment with PRP* ***M)****Fluorescence-Guided Bone Resection* ***N)****Surgical treatment with ErCrYSGG-laser* ***O)****Segmental mandibulectomy + reconstruction with fibula free flap*	**A)**9/9[66], **B)**0/1[60], **C)**8/9[60], **D)**7/9[63], **E)**3/4[61], **F)**3/4[67], **G)**5/5[62], **H)**127/203[**I)**5/8[68], **J)**18/21[64], **K)**14/15[69], **L)**26/26[65], **M)**13/15[70] **N)**5/5[71], **O)**1/2[72]
Stage 3: Pain, infection and exposed necrotic bone being associated with at least one of the following; *Necrotic bone extending to the lower limit of the alveolar bone that causes pathological fractures *Extraoral fistula, nasal or oroantral communication *Osteolysis extending to the lower limit mandible or sinus	*Antibacterial mouth rinses *Antibiotic therapy and pain control *Surgical debridement/ resection to prevent long-term pain and infection	***A)****LLLT applications during the postoperative period in addition to medical and surgical treatment* ***B)****Resection of necrotic bone followed by PRGF* ***C)****Pharmacological + surgical + platelet rich plasma + laser phototherapy* ***D)****Ultrasonic (piezo) surgery and antibiotics* ***E)****Conventional surgery + pharmacotherapy* ***F)****Combined hyperbaric oxygen (HBO) therapy* ***G)****The use of pedicled buccal fat pad combined with sequestrectomy* ***H)****Local Debridement or Resective Intervention* ***I)*** *Resection of all infected tissues, intensive irrigation with antibiotics, application of L-PRF* ***J)****Surgical resection and immediate osseous microvascular reconstruction* ***K)****Doxycycline fluorescence-guided Er:YAG laser ablation combined with Nd:YAG /diode laser* ***L)****Segmental mandibulectomy and reconstruction with fibula free flap*	**A)**2/2[66], **B)**1/1[73], **C)**3/3[60], **D)**3/4[67], **E)**12/24[74], **F)**4/4[62] **G)**3/3[75], **H)**17/37[63], **I)**18/21[64], **J)**7/7[65], **K)**1/1[76], **L)**4/6[72]

In patients with osteonecrosis conservative treatment, minor surgery, invasive surgery and non-surgical approaches are the current management options.

Conservative Approach

The goal of treatment is controlling pain and secondary infections by preventing the expansion of the necrotic bone to improve the patient's quality of life. Conservative approach is generally indicated for patients in Stage 0, 1 or 2 (Figure 2, 3) stages of BIONJ.[34]

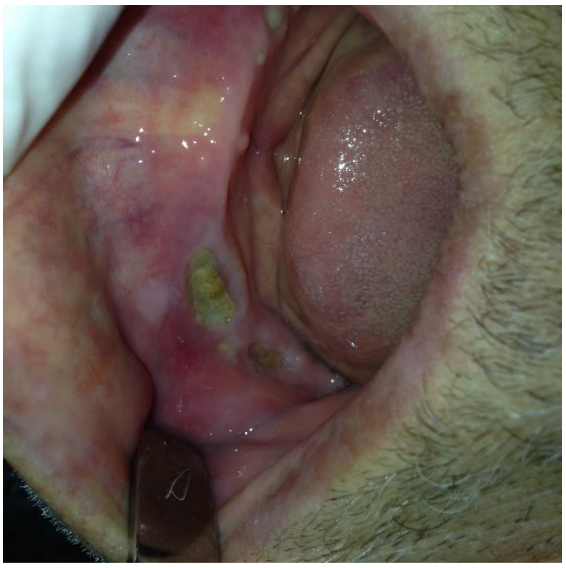

Figure 2. Clinical view of a BIONJ case

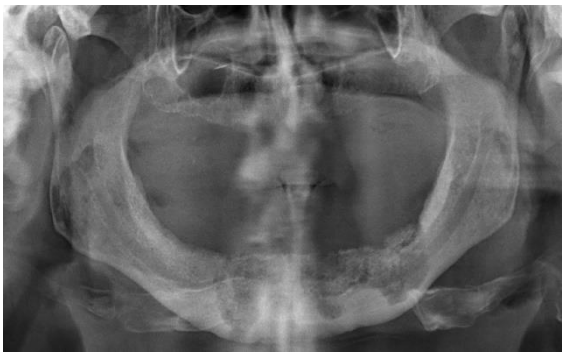

Figure 3. Panoramic view of a BIONJ case

In cases of BIONJ with symptoms of acute infection general approach is palliation of symptoms with antimicrobial chemotherapy. Penicillin or second generation cephalosporins, chlorhexidine rinses, and regular irrigation in the region is the basis of conservative treatment.[49]

Minor Surgery

Minor surgery is appropriate for patients with well limited bone sequestrum. Two different minimally invasive surgical procedures can be defined. Preventive surgery is aimed eliminate concurrent causes further worsen the patient's quality of life and palliative surgery aims to eliminate or alleviate the symptoms and osteonecrotic bone.[50]

Invasive surgery

Stage 3 symptomatic patients may require resection and emergent reconstruction using reconstruction plate or obturator. Candidates for surgery are the patients with Stage III lesions involves painful exposed bone and adjacent soft tissues, acute infection which cannot be treated with oral or IV antibiotics and extra-oral fistula or the cases of pathologic fracture.[51, 52]

Non-Surgical Alternative Treatment Methods in BIONJ

Cellular Mediators

As the Osteonecrosis is a biological degradation it is expected to be useful in treating BIONJ with growth factors. Cellular mediators play an important role in healing of bone and soft tissue defects.[53] Platelet concentrate reported in the literature as a treatment option for osteonecrosis.[54]

Low Level Laser Therapy

Low-level laser therapy (LLLT) is used in the case of BIONJ for supporting the antimicrobial chemotherapy.[55] The result of the study of Stubinger *et al.*[55] performed on 9 patients using Er-YAG laser reported to show uncomplicated postoperative recovery.

Ozone Therapy

Ozone therapy for the management of BIONJ has first reported in the literature in 2006. Rather than being a fundamental treatment, ozone therapy is used as a supportive treatment before and after surgical treatment to improve the patient's quality of life. 90 % improvement has been reported when the use of as ozone as a support to surgery and antibiotic therapy.[56]

Hyperbaric Oxygen Therapy

It is known that the effects of hyperbaric oxygen therapy (HBO) angiogenic. Although, for the treatment of osteoradionecrosis HBO is considered as a definitive treatment option, the effect HBO in the treatment and prevention of the jaw osteonecrosis has not proven yet.[57]

Hormone Treatment

In the treatment of osteonecrosis it is recommended to use of parathyroid hormone (PTH) to increase regeneration of bone. Increased PTH antagonized the effect of bisphosphonate by enhancing the tubular reabsorption of calcium in the bones and by stimulating the adrenal glands for producing 1,25-dihydroxyvitamin D.[58]

Today, there is an obvious trend towards surgical treatment in patients with a diagnosis of BIONJ. In a systematic review which aimed to investigate the efficacy of different therapeutic approaches for BIONJ, It has been shown according to research results that, regardless of the stage of the disease, a major operation or a comprehensive laser-assisted surgery which have recovery rate, respectively, 84 % and 85 % have better results compared with the conservative surgery with an average recovery rate of 75 %. Also, recovery rates for the non-surgical treatments for the combination therapy with the use of LLLT and HBO with antibiotic were found 30% and 52% respectively and recovery rate was found to be 36 % with antibiotic alone.[59]

In conclusion there is no definitive treatment for this drug specific bone necrosis yet. The search for new treatments to combat this condition is still ongoing.

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* Presented in 23th International Scientific Congress of Turkish Association of Oral and Maxillofacial Surgery, May 26 – 30, 2016 Xanadu Island, Bodrum, TURKEY

Cumhuriyet Dental Journal 2017; 20(2): 132-144
Doi: 10.7126/cumudj.345960 *REVIEWS*

ACID TOLERANCE RESPONCE OF CARIOGENIC MICROORGANISMS AND MALOLACTIC FERMENTATION

Karyojen Mikroorganizmaların Asit Tolerans Yetenekleri ve Malolaktik Fermantasyon

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Makale Kodu/Article Code ***:*** *204370*

Makale Gönderilme Tarihi ***:*** *15.10.2016*

Kabul Tarihi ***:*** *13.01.2017*

ABSTRACT

Dental caries is an infectious disease which occurs by the metabolism of bacteria acids released to dental enviroment which results hard tissue resolutions. Becouse of the oxygen-free structures of mature plaques complex and deep layers,cariojenic bacteries which have the ability of fermentation come forward. Strong acids like lactic acid, formic acid and pürivat deminish ph of the plaque and the acidty of the plaque causes demineralization of enamel during caries evolution. Existed plaque acidification is not only causes losing minerals from enamel but also threats microorganisms living in the biofilm of the plaque. So most of the microorganisms can't survive under the ph value of 2.5. The ability of bacteria to survive in this acidic environment depends on the acid tolerance responses they have. Protection against acidity is possible by the production of glicoses,lactic acid and ATP(Adenosine triphosphate) by bacteries. Malolactic fermentation is the most important system that provides these productions in acidic environment. In order to better understand the anti-caries treatment protocols used in current preventive dental practice, the role of bacteria in the fermentation process needs to be known. İn this review we examined: chemical reactions of fermentation, which acids has been occured by the result of these reactions, ph changes in dental plaque, acidojenic and aciduric properties of bacteries which realise fermentation, how can microorganisms survive in acidic environment, what are the advantages propable inhibition of acid tolerance responces for guest. So we tried to attract attention to the anti-cariojenic strategies such as flour, chitosan, α-mangostin and gene studies which are used in the inhibition of acid tolerance systems of bacteria.

Key words: Acidojenic&acidüric, dental plaque, fermentation, glycolyse, S.mutans

ÖZ

Diş çürüğü karyojen bakterilerin metabolizmaları sonucu ortama saldıkları asitler nedeniyle diş sert dokularında mineral çözünmesi sonucu oluşan bir çeşit enfeksiyon hastalığıdır. Olgunlaşmış plağın komplike ve derin tabakalardaki oksijensiz yapıdan dolayı çürük oluşumunda fermantasyon yapabilme yeteneği olan bakteriler ön plana çıkmaktadır. Fermantasyon sonucu açığa çıkan laktik asit, formik asit ve pirüvik asit gibi güçlü asitler, plak pH'sını düşürür ve oluşan plak asiditesi çürük gelişimi süresince minenin demineralizasyonuna yol açar. Oluşan plak asidifikasyonu sadece minenin mineral kaybına neden olmakla kalmaz aynı zamanda plak biyofilminin içerisinde yaşayan mikroorganizmalar için de tehlike oluşturur. Yani çoğu mikroorganizmalar, ölümcül pH değerleri olan pH 2.5 ve altında hayatlarını sürdüremezler. Bakterilerin bu asidik ortamda hayatta kalabilmeleri, sahip oldukları asit tolerans cevaplarına bağlıdır. Bakterilerin bu asiditeye karşı koyması glikoliz, laktik asit üretimi ve ATP(Adenozin trifosfat) üretimi sayesinde olur. Malolaktik fermantasyon ise asidik ortamda bu üretimleri sağlayan en önemli sistemdir. Güncel koruyucu diş hekimliği uygulamalarında kullanılan çürük önleyici tedavi protokollerinin daha iyi anlaşılması için bakterilerin fermantasyon sürecindeki rollerinin bilinmesi gerekmektedir. Bu derlemede fermantasyon sürecinin kimyasal tepkimelerini, bu tepkimeler sonucu hangi asitlerin oluştuğunu, dental plaktaki pH değişikliklerini, fermantasyonu gerçekleştiren bakterilerin asidojenik&asidürik özelliklerini ve özellikle oluşan asidik ortamda mikroorganizmaların hayatlarını nasıl sürdürebildiklerini ayrıca asit tolerans cevabının muhtemel inhibisyonunun konak için ne tür avantajlar oluşturabileceği incelenmiştir. Böylece bakterilerin asit tolerans sistemlerinin inhibisyonunda kullanılan flor, çitosan, α-mangostin ve gen çalışmaları gibi antikaryojenik stratejilere dikkat çekilmeye çalışılmıştır.

Anahtar kelimeler: Asidürik&asidojenik, dental plak, fermantasyon, glikoliz, S. mutans

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GİRİŞ

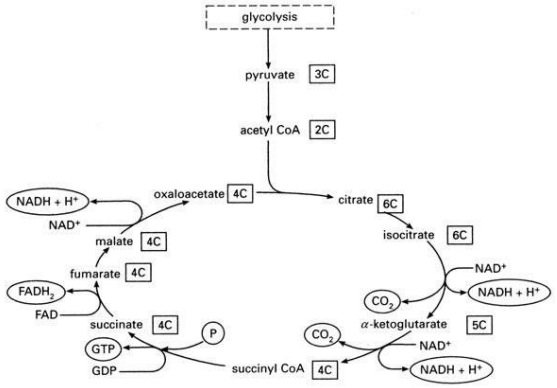
Diş çürüğünün oluşumunda fermantasyon sürecinin rolü zaten bilinmektedir.[1-4]

Fermantasyon, biyokimyada oksijensiz ortamda gerçekleşen enerji tepkimelerini tanımlarken, gıda sanayisinde mikroorganizmaların oksijen varlığında yaptığı yıkım reaksiyonlarını da kapsar (sirke fermantasyonu gibi). Biyoteknolojide ise fermantasyon, büyük tanklarda büyütülen bakterilere yaptırılan her türlü üretim (proteinler dahil) olarak tanımlanır. Ancak diş hekimliği bilimi ve dental plak açısından değerlendirecek olursak; fermantasyon, mikroorganizmaların oksijensiz ortamda yaşamlarını devam ettirebilmeleri için gerekli olan enerjiyi elde ettikleri kimyasal reaksiyonlar zinciridir. Esasında fermantasyon olayı bir bakıma mikroorganizmaların hayat mücadelesi demektir. Tüm canlılar gibi bakteriler de yaşamsal faaliyetlerini devam ettirebilmeleri için ATP'ye ihtiyaç duyarlar. [5-8]

Plak Fermantasyon Süreci

Enerji (ATP) üretimi sırasında meydana gelen reaksiyonlarda glikoz (veya benzeri diğer moleküller) hidrojenlerini teker teker kaybederek daha basit organik moleküllere dönüşürler. Bu reaksiyonlar glikoliz, krebs döngüsü ve oksijensiz ortamlarda fermantasyon olarak gerçekleşmektedir. Bu reaksiyonlardan glikoliz sonucunda pirüvat, pürivatın krebs döngüsüne girmesi sonucunda malik asit oluşur. Oksijen bulunmayan ortamlarda ise pirüvat ve/veya malik asit spesifik bakteriler tarafından fermantasyon ile laktik asite dönüştürülebilir. Daha önce de bahsedildiği gibi bu reaksiyonlar, yaşamsal faaliyetlerin devamı için gerekli ATP elde edilmesi için gerçekleştirilmektedir.

Birinci evre olan glikoliz hücre sitoplazmasında meydana gelir. (Şekil-1) Bu sırada substrat düzeyinde enzimler yardımıyla ATP sentezi gerçekleşir. Glikoliz sonucu oluşan pirüvat, krebs devrine katılmak için mitokondriye geçer.



Şekil 1. Glikozun krebs devrini de içeren yıkım döngüsü.

Krebs devri ve elektron taşıma sistemi(ETS) mitokondride gerçekleşen olaylardır. Glikoliz ve krebs devri sonucu oluşan $NADH_2$ (nikotinamit adenin dinükleotit)'lerden ETS sırasında ATP sentezlenir. Bu ATP elde edilme şekline de oksidatif fosforilasyon adı verilir. Tabiki bakterilerin ribozom harici gelişmiş organelleri bulunmadığından dolayı krebs döngüsü, ETS ve solunum tepkimeleri mezozom denilen mitokondrial yapılar içerisinde ve hücre çeperinde gerçekleşir. Pirüvat oluşumundan sonra tepkimeler ortamda oksijen bulunup bulunmamasına göre ikiye ayrılır.

-Oksijenli solunum

-Oksijensiz solunum

Oksijen bulunması halinde oksijenli solunum olarak devam eder ki bu aşamalar ise krebs döngüsü ve sonrasında elektron taşıma sistemidir. Krebs döngüsündeki tepkimelerde H^+ iyonları, NAD tarafından yakalanır ve $NADH_2$ sentezlenir. Daha sonra ise glikoliz ve krebs döngüsünde ortaya çıkan H^+ iyonları NAD molekülleri tarafından elektron taşıma sistemine aktarılarak ATP sentezi gerçekleşmiş olur. Ve bu şekilde oksijenli solunumla 34 net ATP üretimi gerçekleşmiş olur. Oksijensiz solunumda ise iki çeşit son ürün reaksiyonu gerçekleşir;

- Laktik asit fermantasyonu

- Etil alkol fermantasyonu

Fermantasyonun son adımı (pirüvatın fermantasyon ürünlerine dönüşmesi) enerji üretmese dahi, bu süreç anaerobik bir hücre için önemlidir. Çünkü bu süreç glikozun pirüvata dönüşmesi sırasında harcanan NAD^{+}'ların yenilenmesini sağlar. Bu da glikolizin devamı için gereklidir. Örneğin alkol fermantasyonunda pirüvattan oluşan asetaldehit, NADH ve H^{+} tarafından etanola dönüşür ve hücreden dışarı atılır.[9]

Son ürün reaksiyonlarının amacı indirgenen NAD'ı yükseltgemek, pirüvik asit birikimini önlemek ve glikoliz reaksiyonlarının tekrarını sağlamaktır.

-Laktik asit fermantasyonu: Laktat dehidrogenaz enzimi sayesinde pirüvat'dan sonra $NADH_2$ lerin H^{+} iyonlarını tutularak laktik asit oluşur. Ve bu oluşan laktik asit aktif diş çürüklerinde en yüksek oranda görülen asit olarak karşımıza çıkar.

Sonuç olarak oksijenli ve oksijensiz solunumlarda gerçekleşen son ürün reaksiyonlarından ve krebs döngüsünden bir takım organik asitler oluşur. Bu asitler dental plak formasyonunun irreversible aşamasında ortaya çıkmaya başlar. S.mutans'ın glikoliz ve fermantasyon sonucu oluşturduğu asitlerin bir kısmı güçlü ve yıkıcı yani demineralizasyon etkisi gösteren asitlerdir ki bunlar: laktik asit, malik asit**,** formik asit ve pirüvattır. Diğer oluşan asitler ise daha zayıftır ve tamponlanabilme kapasiteleri yüksektir. Bu asitler ise asetik asit, propionik asit, bütirik asit ve karbonik asitlerdir. Bunlara ilave olarak dental plaktaki diğer mikroorganizmaların fermantasyonu sonucu bir takım başka asitler de oluşur. Bunlar ise sükkinat, valerat ve kaproattır.[10-12]

Plak yapısındaki mikroorganizmaların çoğunluğunu asit üreten (asidojenik) mikroorganizmalar oluşturmasına rağmen, tüm mikroorganizmaların asit üretim oranları aynı değildir. Optimal şartlar altında bazı bakteriler diğerlerinden daha fazla asit üretebilirler. Mesela streptokokların asit üretimi aktinomiçeslerden daha hızlıdır. Yine aynı grup içerisinde de asit üretim oranı farklılığı vardır. S.mutans ve S.sabrinusun asit üretim oranları S.mitis, S.gordonii, S.sangius, S.oralis, S.intermedius, S.anginosus, S.vestibülaris, S.constellatus'a göre belirgin bir şekilde daha fazladır.[13]

Besin maddelerinin mevcudiyeti ve miktarına göre S.mutans, glikolitik yolla elde ettiği asit üretim modellerini değiştirebilir. Örneğin, küçük miktarlarda sukroz varlığında, glikoz ve fruktozdan türetilen major ürünler pirüvat, asetat ve formatken daha yüksek ve artırılmış oranlarda sukroz varlığında ürünler daha çok laktat ve daha az seviyede pirüvat olur.[10]

Organik asitlerin çeşitliliğinden dolayı değişik konsantrasyonlardaki asitlerin etkilerini dikkate almamız önem arz eder.

Mesela düşük karyojenik çevrede oluşan ve sınırlı fermantasyon kapasitesi olan dental plağın, primer ürünü asetattır daha az oranlarda propionat ve bütirattır. Bu zayıf asitler plak pH değişikliklerinde tamponlanabilirler. Aksine yüksek koryojenik çevrede oluşan ürünler ise yüksek oranlarda laktat, formate ve pirüvattır. Bu güçlü asitler ise minenin demineralizasyonuna çok daha fazla neden olurlar.

1994 yılında S.Hojo ve *ark.*[14] çekilmiş dişler üzerinde yaptığı bir çalışmada, çürük dentindeki asit profilleri ve pH değerlerini saptamışlardır. Aktif dentin çürüklerinde pH 4.9 ± 0.2 iken laktat dominant asit olarak bulunmuştur. İlerlemesi durmuş dentin çürüklerinde ise pH 5.7 olarak tespit edilmiştir. Bu da aktif çürüklere göre daha yüksek bir değerdir. Bununla birlikte ilerlemesi durmuş lezyonlardaki dominant asitler ise asetat ve propionat'dır. (Tablo 1)

Tablo 1. Çürük dentindeki asit profilleri

	Aktif(n=15)	Durmuş(n=14)	Rest.alt.(n=7)	Sınıflandırma dışı n=40
pH	4.9 ± 0.2	5.7± 0.5[c]	5.8 ± 0.7[c]	5.6 ± 0.4
Asit % oranları				
Laktat	88.2 ± 8.3	7.5 ± 6.5[b]	5.6 ± 8.3[c]	49.0 ± 22.6
Asetat	9.6 ± 5.9	64.0 ± 14.4[a]	54.0 ± 8.9[a]	36.3 ± 20.2
Propionat	1.2 ± 1.1	18.2 ± 9.2[a]	27.7 ± 10.6[c]	9.6 ± 5.9
İ_Bütirat	nd	0.5 ± 1.7	1.8 ± 3.5	0.3 ± 0.5
N_Bütirat	0.6 ± 0.9	4.9 ± 5.4[a]	6.3 ±4.9[b]	3.5 ± 4.7
İ_Valerat	0.1 ± 0.1	0.9 ± 1.4	0.9 ±1.0	0.7 ± 0.9
N_Valerat	0.1 ± 0.4	1.7 ± 2.7	1.8 ±3.0	0.9 ± 1.6
İ_Kaproat	nd	0.3 ± 0.7	0.6 ±1.6	0.7 ± 1.1
N_Kaproat	0.2 0.7	2.0 ±4.1	1.3 ±2.2	0.6 ±1.4

n=örnek sayısı %=mol % nd=tespit edilememiş

pH ve organik asit yüzdeleri; aktif lezyonlarla, restorasyon altı ve ilerlemesi durmuş olan çürüklerle kıyaslanmıştır.([a] p<0.05; [b] p<0.01; [c] p<0.001)

Dental plaktaki oral laktik asit bakterilerinin (Laktobasiller, Streptokoklar ve Leukonostok) asit üretimleri sonucu pH kritik eşik olan 5.5 değerinin aşağısına indiği zaman mine yüzeyinde demineralizasyon başlar. Plak asiditesi sadece dişler üzerinde zarar oluşturmaz, aynı zamanda biyofilm mikroorganizmaları için de bir stres kaynağı oluşturur. Ve belli değerlerin altında (pH 3.0-2.5) karyojenik bakteriler için ölümcül olabilmektedir.

Bakterilerin metabolizmalarını ve canlılıklarını olumsuz yönde etkileyen faktörlerin başında; açlık (starvation) stresi, oksidatif stres, yüksek sıcaklık stresi ve asidik stres gelmektedir. Mikroorganizmalar bu tür etkilere karşı koyup canlılıklarını devam ettirebilmek için zaten yapılarında var olan ya da sonradan edindikleri savunma mekanizmalarına sahiptirler. Özellikle asidik strese karşı MS (Mutans Streptekok) türleri sahip oldukları ATR (asit tolerans cevabı) sayesinde biyofilm kompleksi içerisinde yüksek asidik ortamda bir adım öne çıkarak (asidojenik-asidürik) karyojeniteden birincil planda sorumlu olarak görülmüşlerdir.[15]

İşte bu ölümcül asit değerlerinde dental plak bakterilerinin nasıl hayatta kaldığı ve yeni asit sentezine nasıl katkıda bulunduğunu anlatan özellikleri genel olarak asidürik özellikleri olarak karşımıza çıkar.

Bu özellik mikroorganizmaların kendi ürettikleri asit ortamda yaşayabilme ve çoğalabilme kabiliyetleridir. Bazı bakteriler diğerlerine nazaran aside karşı daha dayanıklıdırlar. Laktobasiller ve S. mutans bu grubun önde gelen isimleridir ve pH stratejistleri olarak anılırlar. Hem oluşturdukları asit ortamda yaşarken hem de yeni asit sentezine katkıda bulunurlar. Böylece pH daha da düşer. Düşük pH ise mine demineralizasyonu için spesifik şarttır. Mikrofloranın bu asit toleransı minenin demineralizasyonunu dolayısıyla çürük gelişimini indükler. (Tablo 2)

Tablo 2. Asit toleransa sahip oral bakteriler

ASİT TOLERANS PH= 4	ASİT TOLERANS PH= 5
Streptokokus Mutans	*Streptokokus Sangius*
Streptokokus Sabrinus	*Streptokokus Oralis*
Laktobasillus spp.	*Streptokokus Gordonii*
Aktinomiçes Odontolikus	*Streptokokus Anjinosus*
Enterokokus Faekalis	*Streptokokus Konstellatus*
	Streptokokus İntermedius
	Streptokokus Mitis
	Streptokokus Salvaryus
	Streptokokus Vestibularis
	Aktinomiçes Visközüs

Dental plakta bulunan büyümesi ve çoğalması durmuş (non growing) bakterilerin pH 5.0 ve altında asit üretebilme kapasiteleri (asidojenik-asidürik) diş çürüğü ile doğrudan ilişkilidir. Diş yüzeyindeki minerallerin çözünmesi plak pH'sından dolayıdır. Bu çözülmeye neden olan düşük pH ise oral streptokokların minumum büyüyebilme pH'sından daha düşük seviyededir. Yani bu pH seviyesinde oral streptekoklar büyüme ve çoğalma fonksiyonlarını sağlayamazlar ancak asit tolerans yetenekleri sayesinde canlılıklarını koruyup asit üretimini de devam ettirebildiklerinden dolayı diş çürüğünün birincil nedenidirler. Bu asit stresi altında bakteriler glikolitik yolla elde ettikleri ATP'yi büyüme ve çoğalma için değil yaşamsal metabolizmalarını devam ettirebilmek için kullanırlar.

İşte bu ATP büyümeden ziyade, F-ATPase yoluyla hücre membranı boyunca asit-baz dengesini korumak amacıyla kullanılır. Yani protonları (H^+) F-ATPase ile membran dışına çıkarıp hücre içi alkalizasyonu sağlar. Hücre membranındaki pH değişim farkı asidik ortamda glikolizin devam edebilmesine olanak tanır. Ancak pH 3.0 ve altındaki oranlar karyojenik streptokoklar için dahi ölümcül olabilir. Asit öldürücülüğünün oranı üretilen asit miktarı ve derecesi ile ilişkili olduğu kadar bakterilerin doğal ve adaptif (edinsel) olan asit tolerans yetenekleri ile de ilişkilidir.[16]

Fermente olabilen karbonhidratların metabolizması esnasında asidojenik bakteriler, oluşan asitler sayesinde plak pH'sını 4 ve daha aşağı seviyelere dakikalar içerisinde düşürürler. Ve bu pH'sı plak biyofilminin yaşına bağlı olarak 1 saate kadar aynı şekilde koruyabilirler. İşte bu pH dalgalanmalarına karşı koyabilmek için çeşitli bakteriler, asit tolerans cevap yeteneğine sahiptirler. Bu bakteriler içerisinde S.mutans asit tolerans cevap yeteneği bakımından bir adım daha öne çıkmıştır.[17]

Çoğunlukla bu yanıt, plak ve çevresindeki pH düşüşlerinde mikroorganizmaların yaşamlarını sürdürebilmelerine, karbonhidratları fermente edip asit üretimlerine devam ettirebilmelerine olanak tanır.[18]

Neilands ve *ark.*[19] S. mutans'ın ATR sistemine "chitosan"ın etkisini incelemişlerdir. Çalışmalarının ilk aşamasında pH'sı 7.5 olan bakteri biyofilmini HCL kullanılarak asit şoka uğratıp lethal doz olan pH 3.5'e indirmişlerdir ve sadece çok az oranda bir hücrenin canlı kaldığını tespit etmişlerdir. İkinci aşamasında ise pH'sı 5.5 olan bakteri biyofilmini 2 saatlik inkübasyon periyodunun ardından yine lethal doz olan pH 3.5'de 30 dadika süre ile bekletmişler ve büyük oranda bakterinin canlılığını koruduğunu tespit etmişlerdir. Üçüncü aşamasında ise pH 7.5 olan bakteri biyofilmi, 15 dakika süre ile chitosan nanopartiküllerine maruz bırakılmış ve ardından yine 2 saat süre ile pH 5.5 de adaptasyon amacıyla inkübe edilmiştir. Ardından lethal doz olan pH 3.5'da 30 dakika bekletilmiş ve çoğu bakterinin canlılığını kaybettiği tespit edilmiştir. Bu çalışmadan çıkan sonuçlara göre araştırıcılar, normal şartlarda adaptasyon periyodu sonucunda ATR geliştirebilen mikroorganizmaların chitosan varlığında bu yeteneklerini ortaya koyamadıklarını iddia etmektedirler.

Welin ve *ark.*[20] yaptıkları çalışmada biyofilm oluşturmuş ve planktonik haldeki S.mutans hücrelerinin farklı türlerinin gerçekleştirdikleri asit toleranslarını incelemişlerdir. Buna göre araştırıcılar sert yüzeye tutunup biyofilm oluşturan bakteri popülasyonunun planktonik haldeki mutanslara kıyasla asit stresine karşı altı kat daha dirençli olduğunu belirtmektedirler. Böyle olmakla beraber 3 günlük matür biyofilmin asidik stres karşısında sağ kalım oranı %41.5 iken 3 saatlik immatür biyofilmin sağ kalım oranı %5.1 olduğunu bildirmişlerdir. Ayrıca biyofilm içerisindeki çeşitli mutans türlerinin asit tolerans cevaplarının genetik yapıları nedeniyle istatistiksel olarak anlamlı olmasa da farklılık gösterdiğini bildirmişlerdir. Bu çalışmanın sonucunda karyojenitenin biyofilm oluşumu ve bunun olgunlaşmasından doğrudan etkilendiği ortaya çıkmaktadır.

ATR asidik koşullar altında devreye giren bir mekanizmadır ve pH 5-5.5 ATR indüksiyonu için optimal seviyedir. ATR'de altmıştan fazla protein gen rol oynar. Bunların büyük çoğunluğu da asidik şokun ilk 30 dakikasında aktif haldedir. Tümünün aktif hale gelmesi ise 90 ile 120 dakika arasında gerçekleşmektedir.[6,7,21]

Biyofilm mikroorganizmalarının asit stresine karşı verdiği cevap mekanizmaları;

-Genel stress proteinlerinin indüklenmesi,

-Membran proton geçirgenliğinin azalması,

-Proton ekstrüzyonu (F_1-F_0-ATPase)

-Artmış glikolitik aktivite

-DNA ve makroproteinlerin tamiri,

-Anabolik reaksiyonlar baskılanması (daha yavaş büyüme ve daha az metabolik ürün)

-Sitoplazma alkalizasyonu:

- Membran F-ATPases
- Arjinin deaminaz sistem (ADS)
- Sitoplazmik Ureaz sistemi (st.salivarius)
- Agmatin deaminaz sistem (AgDS)

-Malolaktik fermentasyon (MLF), gibi birden çok reaksiyonla meydana gelebilmektedir.[17,22]

Genel stress proteinlerinin indüklenmesi

Bakterilerin açlık, oksidatif, yüksek sıcaklık ve asidik streslere maruz kaldıkları zaman koruyucu mekanizmalarını başlatabilmeleri ve devam ettirebilmeleri için gerekli olan genetik proteinlerin uyarımı, sitoplazmaya salınımı ve fonksiyonlarını gerçekleştirme aşamasıdır. Len ve *ark.*[21] yaptıkları çalışmalarında nötral şartlarda (pH:7) gelişen hücre kültürü ile asidik şartlarda (pH:5) gelişen hücre kültürleri arasında 30 farklı gen proteinini tespit ettiklerini bildirmektedirler. Bu genetik proteinlerin stres tolerans mekanizması kapsamındaki yollarda görev aldıklarını belirtmişlerdir.

Membran proton geçirgenliğinin azalması

Hücre membran bütünlüğünün ve kompozisyonunun önemi birçok araştırmacı tarafından bildirilmiştir. Şöyle ki hücre membranı bünyesinde bulunan doymuş yağ asidi sentezinin artması ve yoğunlaşması, azalmış proton (H^+) geçirgenliğiyle sonuçlanmaktadır. Bu yağ asitlerinin biyosentezi de stres uyaranlarına karşı aktifleşen genler vasıtasıyla gerçekleşmektedir. Ayrıca hücre membran yüzeyi ile ilişkili genetik proteinlerin de hücre duvarı biyosentezi, biyofilm sentezi ve stres tolerans mekanizmalarında çok önemli görevler aldıkları belirtilmektedir. Wen ve *ark.*[22] yaptıkları çalışmalarında membran yüzeyi ile ilişkili BrpA geni mutasyonlu hücrelerin yukarıda bahsedilen mekanizmalarının kontrol hücreleri ile kıyaslandığında belirgin biçimde olumsuz yönde etkilendiğini bildirmektedir.[23]

Proton ekstrüzyonu (F_1-F_0-ATPase)

S.mutans'ın sitoplazma ve ekstraselüler çevre arasındaki pH homeostazını sağlamak için primer mekanizması proton ekstrüzyonu yapan membran yapısı içerisindeki F_1-F_0-ATPase sistemidir. Mikroorganizmalar stres altında hücre içi pH'larını dengeleyebilmeleri ve yaşamlarını devam ettirebilmeleri için hücre içerisindeki protonları sitoplazma dışına çıkarmaları gerekmektedir. Özellikle proton taşıyan ATPase sistemleri membran boyunca protonları dışarı çıkararak aside karşı hassas olan glikolitik enzimleri korurlar ve hücrenin canlı kalıp asidik ortamda ve diğer stresler altında yaşayabilmelerini sağlarlar.[15,24,25]

Artmış Glikolitik aktivite

Çevresel stresler özellikle asidik strese maruz kalan bakteri hücrelerinin hayatlarını devam ettirebilmeleri için gerçekleştirdikleri bir diğer savunma mekanizması ise artmış glikolitik aktivitedir. Ph 5.0'da hücrelerin H^+ iyonlarını hücre dışına çıkarabilmeleri ve sitoplazmanın alkalizasyonunu sağlayabilmesi için ATP üretimine ihtiyacı vardır. İşte bu ATP ihtiyacını asidürik özelliğe sahip hücreler glikolitik aktivitelerini artırarak giderirler. Len ve *ark.*[21] yaptıkları çalışmada S.mutans'ın nötral pH'da(7.0) glikoz transferine kullandıkları EII^{man} ve EII^{glc} enzim kompleks sistemlerini, asidik pH'da(5.0) kullanmaktan kaçındıkları ve bu iki glikoz transfer sistemi haricinde sitoplazmanın daha fazla alkali olmasını sağlayan yeni bir non-PTS glikoz permeaz enzim komleksini tercih ettiklerini bildirmişlerdir. Iwami ve *ark.* [26] ise pH:5.5'de S.mutans hücrelerinin glikolizin pirüvata indirgendiği tepkimelerdeki kullanılan enzimlerden 3-fosfogliserat ve fosfoenolpürivat'ın seviyelerinin azaldığı, 2-

fosfogliserat enzim seviyesinin aynı kaldığı ve pirüvat miktarının arttığını bildirmişlerdir. Ayrıca yaptıkları diğer bir çalışmada ise hücre içi pH düştüğü zaman pirüvat/fosfoenolpürivat oranının belirgin bir şekilde arttığını göstermişlerdir ki bu da pirüvatın ortamda artması yani daha fazla enerji elde edilmesi anlamına gelmektedir.[27]

DNA ve makroproteinlerin tamiri

Oral biyofilm hücrelerinin maruz kaldıkları stresler (açlık, asidik, oksidatif, termal, uv radyasyon)[28] sonucu gerçekleşebilen zararlardan bir tanesi de DNA harabiyetidir. DNA sarmalında glikozil bağlayan deoksiribonükleotitler düşük pH'da kararsız ve değişken bir durumdadır. Asit atağa maruz kalmış hücrede glikosil bağlarının kopması sonucunda pürin ve primidinler sarmal yapıdan ayrılarak DNA'nın bozulmasına neden olabilirler. Tamir edilmemiş DNA hasarları hücre için ölümcül olabilir.[20] Hanna ve *ark.*[29] yaptıkları çalışmada düşük pH larda S. mutans'da beliren bir genin B.subtillis'in UV tamir genine benzer yapıda olduğunu ve bu gen mutant olan S.mutans suşlarının standart suşlarla kıyaslandığında asit ve UV ataklarına karşı çok duyarlı hale geldiklerini bildirmişlerdir.

Anabolik reaksiyonların baskılanması

pH seviyesinin 5.0 ve altına düşmüş olduğu asidik durumlarda, strese cevap olarak dental plak bakterileri elde ettikleri enerjiyi, büyüme ve çoğalma gibi anabolik reaksiyonlardan ziyade çevresel streslerle mücadele için kullanmaya başlar.[17]

Sitoplazma alkalizasyonu;

Membran F-ATPase

Asit toleransa sahip bakterilerin sitoplazma pH'sını alkali tutabilmelerinin en önemli yollarından bir tanesi de membran F-ATPase sistemidir. Bu enzim sistemi hücre içi alkalizasyonu sağlamanın yanında, protonları hücre dışına çıkararak hücrelerin yaşamını devam ettirebilmeleri için gerekli olan ATP üretimine de katkıda bulunmaktadır.[15,24,30,31]

Arjinin deaminaz sistem(ADS)

Asit zararına karşı ağız içi bakterilerin kendilerini korumaları için kullandıkları diğer bir yol da alkali üretimidir. Arjininin yıkıma uğrayıp amonyağın açığa çıkması hücresel ve çevresel pH'yı artırır ve asit stresine karşı bir rahatlama olur.[32,33]

Üre

Diğer bir major alkali üretim kaynağıdır. Streptococcus salivarius ya da Actinomyces naeslundii tükürüğün içerisinde bulunan ürenin hidrolizini katalize ederler ve CO_2 ve NH_3 oluşur. Üreaz üretimi asidifikasyondan ziyade azot açlığına karşı cevapları düzenleyen bir sistem olarak görülür. Asit zararlarına karşı görevi enzimin ikinci bir fonksiyonu gibidir. Tükürük ve dolayısıyla ağız ortamındaki üre ve arjinin konsantrasyonunun artmasının karyojenik mikro-organizmaları negatif etkilediği yapılan çalışmalarla belirtilmiştir. Araştırıcılar üreaz ve arjinin deaminaz sistemi sayesinde üretilen amonyağın, çürük gelişimi ve asidojenik mikrobiyom için endojen kaynaklı bir inhibitör olabileceğini savunmaktadırlar.[34]

Agmatin deaminaz sistem (AgDS)

S. mutans'ın yüksek seviyede asit toleransı olmasına karşın ADS (arjinin deaminaz sistem) ve üreaz negatiftir. Buna karşın düşük seviyelerde de olsa agmatin deaminaz sistemi vardır. Sistem düşük pH'larda aktiftir böylece asit toleransa da katkıda bulunur. Yüksek asit tolerans yeteneğine sahip olmasına rağmen S.mutans alkali üretme eğiliminde olan bir mikroorganizma değildir. Zaten agmatin deaminaz sistemi de baz üretmek için değil daha çok agmatinin detoksifikasyonunu sağlamak için yapılmaktadır.[35]

Sheng ve *ark.*[36] 2007 yılında S.mutans'ın da aralarında bulunduğu oral laktik asit bakterilerinin değişik bir alkali üretim

sistemlerinin olduğunu tanımlamışlardır. Bu sistem amonyak üretmez onun yerine L-malik asidin dekarboksilasyonunu katalize ederek alkalizasyonu sağlar ve bakterilerin asidik streslerin öldürücü etkilerine karşı korunmalarına yardımcı olur. Sheng ve Marquizin bulduğu bu sistem MLF (Malolaktik Fermantasyon)'dir.

MALOLAKTİK FERMANTASYON

Plak pH'sının düşmesi ile birlikte asit stresine maruz kalan mikroorganizmaların glikolitik aktivitelerinin azalmasıyla birlikte glikoliz ürünleri ve ATP üretimi de azalır. Mikroorganizmaların bu asidik ortamda canlı kalabilmeleri için gerekli enerjiyi sağlayabilmesi için glikoliz haricinde başka yollara ihtiyacı vardır. MLF karyojenik S.mutans'ın da aralarında bulunduğu bazı oral laktik asit bakterilerinin (Lactobacillus, Leuconostoc ve Streptococcus) asidik ortamda enerji gereksinimleri için ATP elde ettikleri çok önemli bir sistemdir. MLF aynı zamanda daha asidik olan malik asidi, laktik asit ve CO_2'e dönüştürerek ortamın alkalileşmesini sağlayan ve bakterilerin yaşamlarını devam ettirebilmelerine ciddi biçimde destek olan major sistemlerdendir. Yani MLF, hücreleri sadece asit zararına karşı korumakla kalmaz starvation (açlık) zararlarına karşı da korur. Bu reaksiyon malolaktik enzim (MLE) katalizörlüğünde gerçekleşen bir dekarboksilasyondur. S.mutans'ların düşük asidik şartlarda MLF gerçekleştirebilme yeteneği diğer streptokok türlerine kıyasla daha başarılıdır, bu nedenle MLF, S.mutans'ın çok özellikli dental plak kompleksi içerisinde baskın rol oynamasını sağlamaktadır. S.mutans'ın düşük pH'larda (Ph=4 veya Ph=5) malik asitten laktik asit üretim kapasitesi, glikoliz ile laktik asit üretim kapasitesinden daha yüksektir. S.mutans'ın bu yeteneği yüksek karyojenik özellik göstermesinde çok etkilidir.[36,37]

S.mutans'ın asit tolerans cevabı düşük pH seviyelerinde ortamda malat bulunmadığında da devreye girer. S.mutans'ın bu davranışı adaptif olabilir, çünkü bakteriyel biofilmde meydana gelen pH değişimleri ve malik asit mevcudiyeti biofilmin kendi metabolik faaliyetleri haricinde, asidik yapıya sahip meyve-sebze alımından da etkilenir. Sheng ve *ark.*[37] MLF'nin, elma ve diğer bazı yiyecekler içinde major bir asit olarak bulunan L-Malat tarafından uyarılabilen bir sistem olduğunu bildirmişlerdir.

S.mutans suşlarında MLF için optimal pH 4.0'tür. Ancak pH 2.5 – 3.0 seviyelerinde de MLF reaksiyonu gerçekleşmektedir. Optimum pH 4.0'e ulaştıktan sonra malik asit dekarboksilasyonu giderek azalır ve pH 7.0 seviyesinde reaksiyon nerdeyse tamamen durur. Dolayısıyla pH, MLF için adeta bir açma kapama düğmesi olarak görev yapar.[37]

Sheng ve *ark.*[37] yaptıkları çalışmada S.mutans, S.sabrinus, S.salvaryus, S.sangius ve L.kasei türlerinin gerçekleştirdikleri maksimal MLF aktiviteleri ve optimal pH değerlerini tespit etmişlerdir. Çalışmaya göre pH optimal değeri sırasıyla 4.0; 4.5; 4.5; 5.0; 3.0 dır. MLF aktiviteleri ise yine sırasıyla 9.91±2.37; 23.52±4.10; 14.19±0.95; 8.58±0.95; 46.34±4.71 olarak bildirmişlerdir. S.mutans' ın diğer streptekoklara göre daha düşük pH seviyelerinde maksimal aktivitesini sergilemesi, karyojeniteden birincil olarak sorumlu tutulmalarını destekler bir çalışma olmuştur.

S.mutans genomunda (Oralgen database; http://www.oralgen.lanl.gov) MLF ile ilişkili genler tespit edilmiştir. Buna göre mleR'nin (SMu0121) MLF'nin regülasyonundan, mleS'nin (SMu0123) malolaktik enzim aktivasyonundan (L-malatın, L-laktik asit ve CO_2'e dekarboksile olmasını katalize eder) ve mleP'nin (SMu0124) malat permeaz regülasyonundan (L-malatın hücre membranı boyunca transportunu katalize eder) sorumlu genler olduğu bildirilmiştir.[17,38]

İnhibitörler

Sheng ve *ark.*[36,37] yaptıkları çalışmada, DCCD (N'Ndicyclohexylcarbodiimid'in) 1.0 mM konsantrasyonda etkili bir şekilde MLF ile ilişkili ATP sentezini bloke ettiğini ve bunun da F(H)-ATPase'ın blokajından kaynaklandığını bildirmişlerdir. Yine aynı çalışmada yaygın bir şekilde ağız bakım ürünlerinde kullanılan florun, HF formunda, protonların transmembran kondüktörü olduğunu ve pH 4 de S.mutans UA159 hücrelerinin malattan ATP üretimini artan bir şekilde inhibe ettiğini ayrıca glikolitik enzim olan enolazı inhibe ederek ATP üretiminin azalmasına neden olduğunu belirtmişlerdir.[39] Ağız bakım ürünlerinde yaygın bir şekilde kullanılan triclosan da pH 4 ve 0.1 mM ID 50 de S.mutans UA159'un MLF'nunu etkili bir şekilde inhibe eder. Bu inhibisyonlar sonucu S.mutans hücreleri MLF gerçekleştiremez ve asit ataklara karşı savunmasız kalır.

Fozo ve *ark.*[24] yağ asidi biyosentezi inhibitörü olan cerulenin ile işlem görmüş hücrelerin yoğun asit ortamlarında yaşamlarını sürdüremediklerini bildirmişlerdir.

Bender ve *ark.*[15] ise gramicidin gibi antibiyotiklerin de hücre membranının protonlara karşı permeabilitesini artırdığını ve membran boyunca pH dengesinin bozulmasına yol açtığını dolayısıyla aside karşı hassas türlerin oluştuğunu belirtmişlerdir.

Matsui ve *ark.*[40] asit toleransdan sorumlu genlerden olan ComCDE, hk11/rr11 ve CiaH/K genlerinin baskılanmasının asite karşı hassas fenotiplerin oluşmasına yol açabileceğini bu da bakterilerin karyojenik potansiyellerini azaltabileceğini bildirmişlerdir.

Hasona ve *ark.*[41,42] sinyal tanımlama sistemi (SRP: signal recognition pathway) ile ilişkili genlerin mutant olmalarının da biyofilm oluşumunun azalmasına neden olduğu, stres tolerans ve biyofilm oluşumunun birbiri ile çakışan birçok yönü olduğunu bildirilmiştir.

Yine doymuş yağ asitlerinin biyosentezinden sorumlu olan fabM geninin inaktivasyonu da düşük pH ortamlarına hassas bakteri türlerin oluşmasına ve hücrelerin delta pH'ı sürdürememelerine neden olduğu bildirilmiştir.

Protein bağlanması, renatürasyon ve parçalanma gibi çeşitli hücresel süreçlerde rol alan GroEL ve DnaK gibi hassas genlerin inhibisyonu da yüksek sıcaklığa hassas türlerin ortaya çıkmasına neden olabilir. Yine trigger faktörolan RopA (ribozome-associated peptidly-prolyl isomerase) geninin adezyon ve biyofilm oluşumunda ve asit toleransda önemli görev aldığını bildirmişlerdir.[22]

Fe ve Mn gibi metalik iyonlar da S. mutans'ın virülans özelliklerinin düzenlenmesinde rol oynadıkları belirtilmiştir. Metalloregülatör geni olan SloR geni ise S.mutans'ın biyofilm formasyonu ve oksidatif streslere karşı hücrelerin mücadelesinde önemli rol oynar. Bu genin baskılanması ise yine düşük pH seviyelerine karşı hassas türlerin ortaya çıkmasına neden olur.[43,44]

Yine agmatinin aktivasyonundan sorumlu olan LuxR gen ailesinden olan AguR geninin inaktivasyonu da AgD metabolizmasını azaltır ve hücre içi alkalizasyonunun sağlanmasını zorlaştırır. Hücre içi alkali ortamı sağlayamayan bakteriler asit ataklarında savunmasız kalırlar.[37]

UvrA geninin inhibisyonu da yine UV zararlarına karşı DNA'nın kendini tamir edebilme yeteneğini engeller ve streslere karşı hücreler korumasız kalırlar.[31]

Çitosan nanopartiküllerinin de S.mutans'ın asit tolerans sistemi üzerinde inhibe edici etkisi bulunmaktadır. Çitosan bu etkisini hücre membranıyla etkileşerek ve membran geçirgenliğini değiştirerek gerçekleştirir. Ayrıca chitosan mRNA ve bazı proteinlerin sentezini inhibe edebilir. Yani çitosan varlığında mikroorganizmalar asit adaptasyonlarını sağlayamaz ve ölürler. Bu da dental plağın

kompleks yapısında bozulmalara neden olur.[19] Nguyen ve *ark.*[45] yaptıkları çalışmalarında α-mangostin bitkisinin, S.mutans'ın MLF'sini ve F-ATPase'ı da içeren membran enzimlerini etkili bir şekilde inhibe ettiğini bildirmişlerdir. Bu özelliklerinden dolayı α-mangostin'nin antikaryojen ajanlarda kullanışlı olabileceğini belirtmişlerdir.

Duarte ve *ark.*[46] ise polifenol içeriği bakımından zengin olan kızılcık meyvasının da S.mutans'ın glikoziltransferaz ve F-ATPase enzimlerinin inhibisyonuna neden olduğunu böylece s.mutans'ın asidojenititesi ve biyofilm oluşturmasını olumsuz etkilediklerini bildirmişlerdir.

Yine pürivat dehidrogenaz enzim kopleksi nden sorumlu gen olan pdhA geninin baskılanması asit toleransı azaltmak ve S.mutans'ın karyojenitesini düşürmek için geliştirilebilecek yeni bir strateji olabilir.

Sonuç olarak S.mutans'ın gerçekleştirdiği bütün asit tolerans özelliklerinin inhibisyonu, çürük önleme stratejilerinin geliştirilmesinde yeni bir hedef olarak değerlendirilebilir.

SONUÇ

Dental çürük, dünya genelinde yaygın olarak görülen ve asidojenik & asidürik bakterilerin diş yüzeyindeki kolonizasyonları sonucu oluşan multifaktöriyel bir enfeksiyöz hastalıktır.[1-3] Karyojen mikroorganizmalar içerisinde ATR yeteneği bakımından en etkili olanlar S.mutans ve Laktobasil türleridir.[36, 37] Bu yetenekleri sayesinde sözü geçen bakteriler çok düşük asidik ortamlarda dahi canlılıklarını devam ettirebildiklerinden dolayı yüksek karyojen özellik gösterirler.[18,47] Başlıca ATR mekanizmalarının biri olan MLF S.mutans'da major alkali üretim kaynağıdır.[36] ATR ve MLF inhibitörü moleküllere örnek olarak DCCD, flor, triklosan, serulenin, çitosan, mangostan, kızılcık ve gramisidin verilebilir. Bu moleküllerin antikaryojenik özelliklerini gösteren çalışmalar mevcuttur.[20, 26, 37] Ayrıca ATR'den sorumlu olduğu düşünülen genlerle ilgili yapılan çalışmalarda bu genlerin baskılanması sonucunda antikaryojenik sonuçlar elde edildiği bildirilmiştir.[38-48] Yapılan bu çalışma sonuçlarına göre araştırıcılar MLF'nin de içerisinde bulunduğu ATR sisteminin çürük oluşumunda ne denli önemli bir yerinin bulunduğu ve çürük önleme stratejileri geliştirilmesinde birincil hedeflerden olması gerektiğini belirtmektedirler.

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