

Peri-İmplanter Sağlığın Peri-İmplant Oluk Sıvısında Matriks Metalloproteinaz-8 Düzeyi ile Belirlenmesi

Assessment of Peri-Implant Health by Detecting Matrix Metalloproteinase-8 Level in Peri-Implant Crevicular Fluid

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Abstract

The aim of this study was to determine whether a correlation existed between matrix-metalloproteinase (MMP-8) level in peri-implant crevicular fluid (PICF) and clinical parameters and also to assess whether the detection of MMP-8 in PICF might be useful in diagnosing early tissue breakdown. 20 healthy implants and 20 implants with peri-implant mucositis consisted the study groups. Plaque and gingival indices and probing depths were recorded. PICF was collected onto filter strips. MMP-8 level in PICF was evaluated using enzyme immunoassay method. Mann Whitney U test and Pearson Correlation Analysis were used for statistical analysis. All clinical and biochemical parameters were found to be significantly higher in the test group ($P<0.001$). MMP-8 demonstrated positive correlations with gingival index and probing depth ($P<0.001$). It may be concluded that the detection of MMP-8 in PICF serves as a useful biochemical method for monitoring the course of peri-implant disease.

Key Words

Matrix metalloproteinase-8, dental implants, peri-mucositis

Özet

Bu çalışmanın amacı, peri-implant oluk sıvısı'ndaki (PİOS) matriks metalloproteinaz-8 (MMP-8) düzeyinin klinik parametrelerle ilişkisinin ve PİOS' ndaki MMP-8 düzeyinin belirlenmesinin erken doku yıkımını tespit etmede kullanılabilir bir yöntem olup olmadığının değerlendirilmesidir. 20 sağlıklı ve 20 peri-implant mukositis'e sahip implant çalışma kapsamına alınmıştır. Plak ve gingival indeksler ve sondalama derinlikleri kaydedilmiştir. PİOS filtre kağıtları ile toplanmıştır. PİOS' ndaki MMP-8 düzeyi enzim immünoassay yöntemiyle değerlendirilmiştir. İstatistiksel analizde Mann Whitney U testi ve Pearson Korelasyon Analizi kullanılmıştır. Tüm klinik ve biyokimyasal parametrelerden grubunda anlamlı olarak daha yüksek bulunmuştur ($P<0.001$). MMP-8 gingival indeks ve sondalama derinliğiyle pozitif korelasyon göstermiştir ($P<0.001$). Çalışmanın bulgularına göre, PİOS' nda MMP-8 düzeyinin belirlenmesi peri-implant iltihabın gidişatını değerlendirmede kullanılabilir bir yöntem olarak kabul edilebilir.

Key Words

Matrix metalloproteinaz-8, dental implant, peri-implant mukositis

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Introduction

The predictability of osseointegrated dental implants for the replacement of missing teeth has resulted in their widespread use (1, 2). However, complications involving pathologic changes in the peri-implant tissues may take place following osseointegration and loading in unfortunate cases. Presence of microbial dental plaque and bacterial infection, biomechanical factors associated with occlusal overloading, micro-gap, biologic width, traumatic implant surgery and smoking can lead to inflammatory peri-implant processes (3-6). Peri-mucositis is defined as the reversible mucosal inflammatory condition where as peri-implantitis is the advanced stage of the disease characterized by destruction of bone and soft tissue around the implant (7). The diagnosis of these conditions is usually established by gingival index, probing pocket depth and radiographic bone resorption examinations (8). When the subjectivity of these signs is taken into consideration, a need for different diagnostic methods that supplement clinical signs with objective tests arises (9). Analyses of the constituents of peri-implant crevicular fluid (PICF) may provide crucial information about the inflammatory processes around dental implants. Although extensive research has been done regarding inflammatory mediators in gingival crevicular fluid (GCF) which are responsible for periodontal breakdown, few studies have focused on the presence and the role of these mediators in PICF of patients with peri-mucositis or peri-implantitis. Previous articles report that, aspartate aminotransferase and proteolytic enzymes including matrix metalloproteinases (MMP), some inflammatory cytokines and mediators such as interleukin-1 β and prostaglandin E₂ (PGE₂), had been studied for the detection of the inflammation around dental implants (10-14). MMPs are structurally related but genetically distinct proteolytic enzymes that can collectively degrade almost all extracellular matrix and basement membrane molecules during both tissue repair and destruction (19, 20). MMP-8 (neutrophil-type collagenase) is implicated as the major tissue destructive MMP in the crevicular fluid of individuals with chronic periodontitis and elevated levels of MMP-8 have been demonstrated also in peri-implantitis (21-23). Recently, Kivelä-Rajamäki et al.

have reported elevated levels of MMP-8 in diseased PICF and the existence of a strong correlation between MMP-8 levels and gingival index scores in diseased peri-implantitis sites (24, 25). However, Aboyoussef et al. had not found differences in MMP levels in early peri-implantitis and in healthy implants (13).

In the light of the established knowledge, the present study was planned to further determine whether a correlation existed between MMP-8 level in PICF and clinical parameters in peri-mucositis and also to assess whether the detection of this mediator in PICF might be useful in diagnosing early tissue breakdown.

Materials and Methods

Study Population

The study group consisted of 40 osseointegrated dental implants placed in 19 patients who had attended the Oral Implantology Department in the Faculty of Dentistry, University of Istanbul (Table 1). Each patient had been treated with one or more dental implants (Brånemark, Nobel Biocare, Westmont, IL) and all the implants had approximate function periods of 13 months. The patients were under maintenance therapy.

The subjects enrolled in the study were systemically healthy, had not taken antibiotics, known drugs with matrix metalloproteinase inhibitors or anti-inflammatory drugs and had not received any dental prophylaxis within the previous 6 months.

All patients signed an informed consent prior to participation.

Each implant was assigned to a diagnostic category based on the following criteria similar to previously described (13):

- 1. Healthy:** No gingival inflammation as evidenced by the low scores of gingival index and no pockets greater than 3 mm.
- 2. Peri-implant mucositis:** Presence of gingival inflammation as evidenced by an increase in gingival index scores and no pockets greater than 5 mm.

Table 1. The demographic data of the study population.

	Test	Control
Mean Age	51,25	46,15
Number of Subjects	10	9
	7 Females, 3 Males	5 Females, 4 Males
Number of Implants	20	20

SD: Standard deviations

Table 2. The comparison of clinical parameters in the test and control groups.

Parameter	Group	n	Median	(Min Max)	Z	P
PI	Test	20	1,00	(0-1)	-2,842	,004*
	Control	20	0,00	(0-1)		
GI	Test	20	1,00	(0-2)	-3,351	,001*
	Control	20	0,00	(0-1)		
PPD	Test	20	4,00	(2-5)	-3,504	,000*
	Control	20	3,00	(2-5)		

* Statistical significance at P<0.01.

Table 3. The comparison of PICF MMP-8 level in the test and control groups.

	Group	n	Median	Min-Max	Z	P
MMP-8	Test	20	3,57	(1,92-4,62)	-4,383	,000*
	Control	20	2,00	(1,15-3,50)		

* Statistical significance at P<0.01.

Table 4. Pearson correlations of PICF MMP-8 total amounts and clinical parameters in the study groups.

r	PI	GI	PPD
MMP-8	0,237	0,406*	0,407*

* Statistical significance at P<0.01.

According to these criteria, 30 dental implants with peri-implant mucositis, which were placed in 16 systemically healthy subjects comprised the test group. Another 30 dental implants with probing depths ≤ 3 mm that were placed in 13 healthy patients served as the control group.

Clinical Measurements

The clinical measurements and peri-implant crevicular fluid were obtained by the same examiner. Plaque index (PI) ⁽²⁶⁾ and gingival index (GI) ⁽²⁷⁾ scores and probing pocket depths (PPD) were recorded at 6 sites (mesio-facial, disto-facial, mid-facial, mesio-lingual, disto-lingual, mid-lingual) per implant.

Peri-implant Crevicular Fluid Sample Collection

Sites selected for PICF sampling were isolated using cotton rolls. A gentle stream of air was directed parallel to the root surface for 5 to 10 seconds to dry the area. PICF samples were collected using standardised paper strips (Periopaper, Proflow, Amityville, NY) as described by Ingman et al. ⁽²⁸⁾. The strips were placed into 1 mm depth in peri-implant crevice for 1 min. Samples containing blood were discarded. Then, the wet part of each paper strip was cut off with scissors and placed individually into uniquely labelled 0.5-ml microcentrifuge sterile tubes and stored under -30°C until the assay procedures.

Laboratory Procedures

Human MMP-8 enzyme immunoassay kit (Quantikine, R&D Systems, Minneapolis, USA) was used for detecting MMP-8 in PICF samples. Immediately prior to the assay, the paper strips were removed from storage and a 50 µL of corresponding assay buffer (tris buffered saline), which was provided within each kit, was pipetted onto each tube. The tubes were left at room temperature for 30 minutes and vortexed every 5 minutes. The aliquots of the extracted samples were used in the assay procedures, as described within the kit instructions. Finally, enhancement solution was added to stop the reaction and the optical density of the color produced was immediately measured using a 450 nm microplate reader. The level of MMP-8 in PICF samples were calculated from standard curves using standard proteins supplied by the kit and expressed as ng/ml.

Data Analysis

The differences between two groups regarding plaque

index, gingival index, probing pocket depths, PICF MMP-8 level was evaluated using Mann Whitney U test. The relationship between clinical parameters and MMP-8 level in PICF was statistically evaluated using Pearson Correlation Analysis. Statistical analysis of the study was carried out using a software program (SPSS for Windows, Release 10.0, 1999, SPSS Inc., USA) and statistical significance was established at $P < 0.05$.

Results

The demographic data of the study population and the number of dental implants placed are presented in **Table 1**.

Clinical data including the means and standard deviations and mean differences of clinical parameters and MMP-8 level in the test and control groups are summarized in **Table 2 and Table 3**. All clinical and biochemical parameters were found to be statistically significantly higher in the test group ($P < 0.001$). MMP-8 exhibited a positive correlation with GI and PPD at the level of $P < 0.001$ (**Table 4**).

Discussion

Osseointegrated dental implants are widely used to successfully restore dentition in either partially or totally edentulous individuals (1, 2). One of the difficulties experienced in implant dentistry is the failure of some initially healthy and functional dental implants. Untreated bacterial infection around implants leads to inflammatory peri-implant disease processes which in return may cause the implants to lose osseous support, become mobile and eventually require removal (7). The criteria for detecting disease activity around dental implants are based on subjective clinical and radiographic findings (9). For this reason, early detection of the inflammation with more objective methods such as the use of PICF constituents provides minimum tissue damage and increased therapeutic success. Some inflammatory mediators and proteolytic enzymes had been studied for the detection of inflammation around dental implants (10-14). Among these MMP-8 has been shown to exhibit higher levels in peri-implant inflammatory processes comparing with

healthy implants (21-23). Incompatibly, Aboyoussef et al. had not observed differences between the health state and early peri-implantitis regarding MMP levels. However the investigators claimed that a more critical analysis of MMP levels should be carried out using a more specific assay than zymography (13). Recently, Kivelä-Rajamäki et al. have reported elevated levels of MMP-8 in diseased PICF and the existence of a strong correlation between MMP-8 levels and gingival index scores in diseased peri-implantitis sites (24, 25). Consistent with these recent reports, the findings of the present study revealed higher MMP-8 levels in diseased sites being significantly related to PI, GI and PPD.

As in periodontal disease, presence of microbial dental plaque and bacterial infection can lead to peri-implant disease process which is clinically presented by bleeding on probing and/or pocket formation (3-6). For this reason it is not surprising that, the clinical aspect of this inflammation is accompanied with an elevation in inflammatory mediators such as MMP-8 in PICF as it is in GCF of individuals affected by periodontal disease (16, 29).

In conclusion, the findings of the present investigation further support the opinion that MMP-8 in PICF serve as a useful biochemical marker for monitoring the course of peri-implant disease. However, since this study is cross-sectional, the subjects should be followed up longitudinally. In the future, more cases with deeper peri-implant probing depths must be evaluated in order to confirm the diagnostic benefit of MMP-8 in peri-implant disease.

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