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Effects of potassium aluminum sulfate on TNF-α, MMP-1 and MMP-8 levels at gingival crevicular fluid in periodontally healthy subjects: a pilot study

[Periodontal sağlıklı bireylerde potasyum alüminyum sülfatın dişeti oluğu sıvısı TNF-α, MMP -1 ve MMP-8 düzeylerine etkisi: pilot çalışma]

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ABSTRACT

Objectives: The aim of this study was to investigate the possible effects of fixed dental prosthesis procedures with and without gingival retraction containing potassium aluminum sulfate on TNF-α, MMP -1 and MMP-8 levels at gingival crevicular fluid in periodontally healthy subjects and to demonstrate that these markers can be used for the assessment of disease activity.

Methods: Eight healthy subjects, each with at least two abutment teeth, were included in our research. Gingival crevicular fluid (GCF) samples were obtained before initiation of fixed dental prosthesis treatment and repeated on days 2, 3, 7 and first month after cementation. GCF concentrations of MMP-1, MMP-8 and TNF-α were determined by ELISA. ANOVA was used for statistical analysis.

Results: The GCF MMP-8 concentrations revealed significant differences among study groups ($p<0.05$), but not among the sampling days ($p>0.05$). The MMP-8 concentrations of control group was significantly decreased in the first month ($p<0.05$). The TNF-α concentrations showed significant differences in all study groups ($p<0.05$). In contrast to ret(-) and control groups, TNF-α concentrations of ret(+) group was significantly different among sampling days ($p<0.05$). The TNF-α concentration peaked in the first month in ret(+) group.

Conclusion: Within the scope of this study, it may be suggested that well-fitted fixed dental prosthesis and its procedures performed to periodontally healthy subjects do not affect the periodontal tissue health negatively.

Key Words: Cytokine, matrix metalloproteinases (MMPs), fixed dental prosthesis, gingival retraction, potassium aluminum sulfate.

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

ÖZET

Amaç: Periodontal olarak sağlıklı bireylerde, potasyum alüminyum sülfat içeren gingival retraksiyonlu ve retraksiyonsuz sabit dental protez işlemlerinin dişeti oluğu sıvısı TNF-α, MMP-1 ve MMP-8 seviyeleri üzerine etkisini araştırmak ve bu belirteçlerin hastalık aktivitesini değerlendirmede kullanılabileceklerinin gösterilmesi amaçlanmıştır.

Yöntem: Ağızlarında tedavi edilecek en az iki dayanak dişi bulunan toplam 8 sağlıklı birey çalışmaya dahil edildi. Dişeti oluğu sıvı örnekleri sabit dental protez işlemlerinin başlangıcından önce elde edildi ve 2., 3., 7. günler ve simantasyonun ardından 1. ayda tekrarlandı. TNF-α, MMP-1 ve MMP-8 konsantrasyonları ELISA ile değerlendirildi. İstatistiksel değerlendirme için varyans analizi kullanıldı.

Bulgular: Dişeti oluğu sıvısı MMP-8 konsantrasyonları çalışma gruplarında anlamlı farklılıklar gösterirken ($p<0.05$), örnekleme günlerinde anlamlı farklılık göstermemiştir ($p>0.05$). Kontrol grubunda MMP-8 konsantrasyonları 1. ayda anlamlı azalma göstermiştir ($p<0.05$). Tüm çalışma gruplarında TNF-α konsantrasyonları anlamlı farklılıklar göstermiştir ($p<0.05$). Ret(-) ve kontrol grubunun aksine, ret(+) grubunda TNF-α konsantrasyonları örnekleme günleri arasında anlamlı farklılıklar göstermiştir ($p<0.05$). Ret(+) grubunda 1. ayda TNF-α konsantrasyonu pik yapmaktadır.

Sonuç: Çalışmanın sınırları içerisinde, periodontal sağlıklı bireylere uygulanan iyi uyumlu sabit dental protez işlemleri ve protez aşamaları periodontal doku sağlığını olumsuz etkilememektedir.

Anahtar Kelimeler: Sitokin, matriks metalloproteinaz (MMP), sabit dental protez, gingival retraksiyon, potasyum alüminyum sülfat

Çıkar Çatışması: Yazarların makale konusu ile ilgili çıkar çatışmaları bulunmamaktadır.

Introduction

Despite the enhanced technology in dentistry, tooth loss may occur as a result of dental caries, trauma and periodontal disease. Prosthetic treatment is frequently being used as a solution to loss of teeth, partial destruction of tooth tissues and aesthetic problems. Success of prosthetic treatment mostly depends on maximum outcome with minimal invasion of tooth and adjacent tissues. However, some stages of prosthetic treatment procedure, especially in fixed dental prosthesis (FDP) treatment, may cause undesirable injury on adjacent tissues, especially on periodontium and gingiva [1]. Gingival retraction cords are the most commonly used materials for the retraction procedure. While the physical properties of retraction cords make gingival tissues retract laterally and vertically, chemical ingredients such as aluminum-potassium sulfate, epinephrine, and etc. may also provide chemical retraction via vasoconstriction and homeostasis [2, 3].

Gingival crevicular fluid (GCF) contains a large repertoire of serum proteins, inflammatory mediators, host cell degradation products and microbial metabolites and can be collected at the orifice or from within the gingival crevice. The potential diagnostic importance of gingival fluid was recognized more than 60 years ago. The mechanism of gingival crevicular fluid (GCF) production, especially in the presence of very early inflammatory changes, has been investigated. The GCF composition permanently altered by sampling and while some temporary disruption of the subgingival plaque may occur and a transient microscopic alteration in the gingival vasculature may be observed in the diseased gingiva. Enzymes, especially proteinases play a central role in the control of periodontal tissue turnover in health and in the tissue destruction that characterizes diseases of the periodontium [4].

Inflammatory mediators, such as TNF-α, IL-1β and PGE-2 play a critical role in the pathogenesis of periodontal disease and are used as markers in diagnosis and assessment of the level of disease activity, as well as in the efficacy of therapy [5-8]. TNF-α, a proinflammatory cytokine produced primarily by monocytes/macrophages, has been identified as a lethal mediator of acute and chronic infection [9, 10]. Several investigators have detected significant elevated TNF-α level in GCF in patients with gingivitis and periodontitis [8, 9, 11]. Matrix metalloproteinases (MMPs) are the major group of enzymes responsible for degradation of extracellular matrix (ECM), which leads to periodontal tissue destruction. MMPs represent a structurally related but genetically distinct superfamily of proteases acting not only in physiological development and tissue remodelling but also in pathological tissue destruction. MMPs can be divided into 5 major groups: collagenases (MMP-1, -8, -13), gelatinases (MMP-2, -9), stromelysins (MMP-3, -10, -11), membrane-type MMPs (MMP-14, -15, -16, -17) and others. MMPs can collectively degrade almost all components of extracellular matrix and basement membrane and their excess activity lead to periodontal tissue destruction [12].

MMP-1 (interstitial collagenase, collagenase-1), synthesized and secreted ubiquitously by connective tissue cells (fibroblast) and macrophages, is more often associated with normal tissue remodelling and hydrolyses mainly type III collagen [13], where MMP-8 is the most effective in hydrolyzing type I collagen [14]. MMP-1 and MMP-8 share the unique ability to cleave types I, II, and III collagen and probably serve as initiators of the majority of extracellular matrix destruction in periodontal disease. Thus, synthesis and activation of MMP-1 and MMP-8 are important steps in the pathological extracellular matrix destruction associated with inflammatory periodontal disease [15].

The effects of operative procedures including tooth preparation and gingival retraction, interactions between metallic crowns and the periodontium may cause significant increases in cytokine levels that may be further influenced by fixed prosthodontic operative procedures [16]. There were conflicting findings those stated a relationships between periodontal indices and intracrevicular cytokine levels. Much further studies were required to analyze the relationship between GCF cytokine levels and prosthodontic procedures. Additionally, the effect of prosthodontic procedures, such as gingival retraction which is used for clear denture margins and their effects to the ECM of periodontium are not clearly identified yet. Therefore, the aim of this study was to investigate the possible effects of fixed partial denture procedures with and without gingival retraction to the TNF-α, MMP-1, and MMP-8 levels in the gingival crevicular fluid of periodontally healthy subjects.

Material and Methods

A total number of 8 periodontally healthy subjects (5 female and 3 male subjects, aging between 28-72 years) who needed fixed dentures and had at least 2 abutment teeth to prepare were included in the study. All consecutive subjects were recruited from the Ege University School of Dentistry Department of Prosthodontics. The study protocol was approved by the ethics committee of the Ege University School of Medicine (08-7/18, Protocol code: 2008-DIS-018). Prior to participation, the purpose and procedures were fully explained to all patients and all participants gave written informed consent in accordance with Helsinki Declaration. The study was designed, conducted, analyzed and reported according to guidelines for Good Clinical Practice.

All subjects included to the study were periodontally healthy and were diagnosed in accordance with the clinical criteria stated in the consensus report of the World Workshop in Periodontics [17]. Subjects had ≥ 90% of the

measured sited exhibited PD < 3 mm and CAL ≤ 1 mm and having bleeding on probing (BOP) less than in the 10% of the probing sites, no radiographic sign of alveolar bone loss (i.e., a distance of < 3 mm between the CEJ and bone crest at > 95% of the proximal tooth sites). Medical and dental histories were taken and patients received clinical and radiographic evaluation at pre-screening visit. Subjects who had never smoked or who smoked < 10 cigarettes per day for < 5 years were included in the present study. Exclusion criteria were as follows: oral diseases other than caries and periodontal disease, ongoing orthodontic therapy, a history of systemic or local disease with influence on the immune system (cancer, cardiovascular and respiratory diseases), diabetes mellitus, hepatitis or HIV infection, immunosuppressive chemotherapy or current pregnancy or lactation.

Study protocol

Prosthetic treatment days and procedure;

Preparations for total number of 16 abutment teeth (4 maxillary canines, 9 maxillary premolars, 2 maxillary molars, and 1 mandibular premolar) were performed by two prosthodontists with at least 10 years of clinical experience. Tooth preparations were made with flame-shaped diamond burs (CN: 251, Acurata-Dental AG, Germany) under local anesthesia (Ultracain D-S Forte, Sanofi-Aventis Inc., Istanbul, Turkey) and preparation margins were set subgingivally. Two treated teeth per each patient were evaluated in this study. Gingival retraction procedure was performed to one of the abutment teeth per each patient with potassium-aluminum-sulfate braided retraction cord (Ultrax medium-braided, Sultan Healthcare Inc., NJ, USA) according to the manufacturer's recommendations (t=12 minutes). The teeth which the gingival retraction was applied to were recorded as ret(+), while the other prepared teeth as ret(-). Impressions were made with a conventional c-silicone impression material (Speedex, Colthéne-Whaledent AG, Germany) in two stages (wash technique). For each patient one tooth was recorded as negative control. Time table for FDP treatment stages were shown in Figure 1.

Gingival crevicular fluid (GCF) sampling days;

Subjects were examined in baseline (day 0) before preparation of their teeth. GCF samples were collected from buccal aspects of the teeth which needed denture and a negative control tooth. The following clinical parameters were recorded at six sites of each tooth: Plaque index (PI) [18], dichotomous measurement of presence of bleeding on probing (BOP) and probing pocket depth (PPD). All measurements were performed by a single calibrated and blind examiner without knowing whether the retraction is positive or negative. The patients were examined in the day 2, 3, 7 and one month after finishing denture and GCF samples were taken repeatedly. Time table of GCF sampling was shown in Figure 1.

Gingival crevicular fluid sampling

GCF samples were collected with filter paper strips (Periopaper ProFlow, Inc., Amityville, NY, USA) (each from retraction negative, retraction positive and control sites) in periodontally healthy subjects who needed fixed denture. Prior to GCF sampling, the supragingival plaque was removed from the interproximal surfaces with a sterile curette; these surfaces were dried gently by an air syringe and were isolated by cotton rolls. Paper strips were carefully inserted approximately 1 mm into the crevice and left there for 30 seconds [19]. Care was taken to avoid mechanical injury. Strips contaminated with blood were discarded [20]. The absorbed GCF volume of each strip was determined by Periotron 8000 (IDE Interstate,

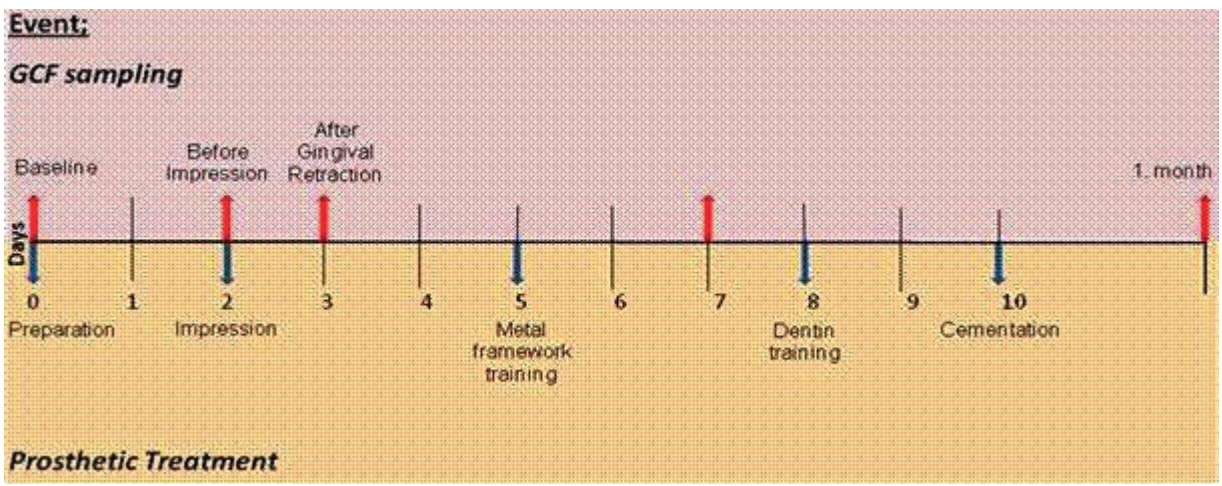

Figure 1. Time schedule for fixed dental prosthesis (FDP) treatment stages
Red arrow represents: Gingival crevicular fluid (GCF) sampling days 'Baseline, before impression, after gingival retraction, after metal framework training, 1 month after cementation'
Blue arrow represents: Prosthetic treatment phases 'Preparation, impression, metal framework training, dentin training, cementation'

Amityville, NY) and placed into a sterile polypropylene tube and kept at -80°C until being analyzed. The readings from the Periotron 8000 were converted to an actual volume (µl) by reference to the standard curve.

Analysis of MMP-1, MMP-8 and TNF-α

GCF samples were eluted from the strips by placing them in 250 µl of phosphate buffered saline. GCF samples were analyzed for MMP-1, MMP-8 and TNF-α levels by enzyme-linked immunosorbent assay (ELISA) with commercially available MMP-1, MMP-8 and TNF-α ELISA kits (Ray Biotech Inc., Norcross, GA, USA; Ray Biotech Inc., Norcross, GA, USA; Bender Medsystem, Burlibame, CA, USA; respectively). Procedures were performed according to the manufacturer's instructions. The minimum detectable limits of MMP-1, MMP-8 and TNF-α were 8 pg/ml, 6 pg/ml and 2.3 pg/ml, respectively, according to the manufacturer. The amounts of MMP-1, MMP-8 and TNF-α in each sample were calculated based on the dilutions according to the minimum and maximum detection limits of standard curve. The results were expressed as total cytokines in the 30 second GCF sample. Calculation of the concentration data for each mediator was performed by dividing the amount of each mediator by the GCF volume.

Statistical Analysis

The standard deviations of GCF concentrations were found to be too high after Kolmogorov-Smirnov test was performed for normality of distributions of study groups. Logarithmic transformation was therefore performed to GCF concentrations of the assayed proteins and then the concentrations were analyzed by analysis of variance (ANOVA). *P* values <0.05 were considered as statistically significant.

Results

The MMP-1, MMP-8 and TNF-α concentrations of GCF samples were easily quantified by the ELISA according to calculated LODs and LOQs. The LOD of MMP-1, MMP-8 and TNF-α in GCF samples were determined as 7.7 pg/ml, 6.3 pg/ml and 1.9 pg/ml, respectively. The LOQ of MMP-1, MMP-8 and TNF-α in GCF samples were determined as 20.3 pg/ml, 17.1 pg/ml and 2.6 pg/ml, respectively.

The GCF MMP-1 concentrations showed slight differences in control, ret(-) and ret(+) groups, when same sampling days were compared, these differences were not found to be significant (p>0.05) (Table 1a).

The GCF MMP-8 concentrations showed significant differences among study groups (p<0.05) (Table 1b), the differences between GCF MMP-8 concentrations of both ret(-) and ret(+) groups were not significant among the sampling days (p>0.05). The GCF MMP-8 concentrations of control group was significantly decreased at the first month (p<0.05) (Table 1b).

Table 1a. GCF Log MMP-1 (pg/ml) concentrations of study periods

Sampling Days	Control n=8	Ret (-) n=8	Ret (+) n=8
Day 0	2.55±0.67	1.98±0.41	1.97±0.34
Day 2	1.94±0.13	2.19±0.61	2.28±0.73
Day 3	2.08±0.69	2.12±0.63	1.95±0.53
Day 7	2.29±0.81	1.91±0.38	2.02±0.40
1st month	2.05±0.24	2.03±0.33	2.17±0.38

No significant differences between study groups and GCF sampling days (p>0.05)

Table 1b. GCF Log MMP-8 (pg/ml) concentrations of study periods

Sampling Days	Control n=8	Ret (-) n=8	Ret (+) n=8
Day 0	4.15±0.23	3.79±0.46	3.75±0.16
Day 2	3.83±0.36	3.76±0.20	3.84±0.23
Day 3	3.48±0.29	3.64±0.44	3.64±0.19
Day 7	4.03±0.28	3.49±0.47	3.58±0.44
1st month	3.91±0.29[a]	3.95±0.27	3.35±0.63

[a]; Significant differences at the 1st month compared to baseline (Day 0) (p<0.05)

The GCF TNF-α concentrations showed significant differences in between study groups (P<0.05) (Table 2). In contrast to ret(-) and control groups, the GCF TNF-α concentrations of ret(+) group was significantly different among sampling days (p<0.05) (Table 2). The GCF TNF-α concentrations reached a peak at the first month in ret(+) group.

The GCF flow rate in ret(+) group was significantly higher than those of ret(-) and control groups on day 3 (after gingival retraction) (p<0.05). The differences of GCF flow rates in all groups at all time intervals were found to be statistically significant (p<0.05) (Table 3).

Discussion

In this present study, we analyzed the GCF levels of TNF-α, MMP-1 and MMP-8 in the periodontally healthy individuals those needed fixed partial dentures in an attempt to at least partly explain possible effects of FDP procedures and gingival retraction. Operative procedures including tooth preparation and gingival retraction, interactions between metallic crowns and the periodontium are reported to exhibit an increased GCF cytokine levels. Accordingly, we hypothesized that the inflammatory response significantly increases during the FDP procedures and application of gingival

Table 2. GCF Log TNF-α (pg/ml) concentrations of study periods

Sampling Days	Control n=8	Ret (-) n=8	Ret (+) n=8
Day 0	1.66±0.62	1.07±0.32	1.00±0.25[a]
Day 2	1.05±0.26	1.08±0.49	0.98±0.19[b]
Day 3	1.13±0.66	0.92±0.39	0.81±0.30[c]
Day 7	1.39±0.70	0.86±0.15	0.96±0.21[d]
1st month	1.18±0.25	1.11±0.26	1.34±0.37[a,b,c,d]

[a,b,c,d]; Significant differences between GCF sampling days in the Ret (+) group ($p<0.05$)

Table 3. GCF flow rates (µl/sample)

Sampling Days	Control n=8	Ret (-) n=8	Ret (+) n=8
Day 0	1.20±0.66[a]	1.65±0.32[b,c]	1.75±0.22[a,b]
Day 2	1.74±0.24	1.82±0.32	1.78±0.21
Day 3	1.65±0.63[c]	1.92±0.31[d]	1.97±0.25[c,d]
Day 7	1.34±0.70	1.93±0.21	1.81±0.21
1st month	1.56±0.25	1.62±0.28	1.49±0.35

[a,b]; Ret(+) group was significantly higher than those of ret(-) and control groups on day 0 ($p<0.05$)
[c]; Ret(-) group was significantly higher than control groups on day 0 ($p<0.05$)
[c,d]; Ret(+) group was significantly higher than those of ret(-) and control groups on day 3 ($p<0.05$)

retraction. However, our findings did not reveal significant differences in GCF TNF-α, MMP-1, and MMP-8 levels between ret(+) and ret(-) groups during the FDP treatment sessions. This may be explained by the relatively small number of our study group.

This present study constitutes pioneering by investigating the effects of FDP treatment procedures on the periodontal parameters and TNF-α, MMP-1, and MMP-8 levels in the GCF in periodontally healthy individuals. In periodontitis, as an inflammatory disease, several proteases degrade collagen and extracellular matrix which leads to destruction of connective tissue attachment and alveolar bone. TNF-α, PGE2 and some other inflammatory mediators play a critical role in the pathogenesis of periodontal disease, and are used as markers in diagnosis and assessment of the level of disease activity, as well as in the efficacy of therapy [5, 8].

Conflicting results have been reported concerning the possibility of a circadian pattern in the flow of GCF. One group of investigators used the deep intracrevicular technique for collection of fluid in healthy subjects and found that the average flow was greater in the evening and minimal early in the morning [21]. Another group collected gingival fluid with orifice technique in healthy subjects and did not find any significant differences between the flow of fluid measured at 9 am and that of the fluid collected at 3 pm [22]. Therefore, in the present study GCF samples were collected with orifice technique and with filter paper strips. Paper strips were carefully inserted approximately 1 mm into the crevice and left there at standard times in each subject. In this way, the GCF volume and the fluid concentrations in each time were standardized. However, the small volumes of fluid collected from healthy gingiva had a protein concentration similar to interstitial fluid [23] with a different range of proteins [24]. This would be consistent with the hypothesis of Alfano [25] that the initial fluid accumulation represents a transudate of interstitial fluid produced by an osmotic gradient and that the later fluid represents a true exudate.

The effects of gingival retraction on periodontium was previously investigated by Feng *et al.* by evaluation of TNF- α levels in GCF collected at different days and five time intervals (days 1, 3, 7, 14, and 28) after the retraction procedure, and it has been observed that TNF-α levels reached a peak at day one after retraction, then gradually declined at days 3, 7, 14, and 28. This immediate increase at the first day demonstrated the acute effect of the retraction procedure whereas the decline of TNF-α levels at control intervals (days 3, 7, 14, and 28) stated that this stabilization were independent from the GCF sampling. But even at day 28, TNF-α did not recover to the baseline level [2]. The findings of Feng *et al.* suggested that the injury from cord placement may be more significant than previously considered. It is stated that no medicaments were infused in the cords. Besides, the investigators emphasized the fact that under clinical conditions, medicaments placed in the sulcus may result in a more severe gingival injury and under those circumstances it may be likely that the GCF cytokine levels may be higher than those observed in that experiment. Although the TNF-α levels seemed to be decreased throughout the stages of FDP treatment procedure, it reached high levels on the first month when compared to the baseline and negative control group in both ret(-) and ret(+) groups, but the levels recorded in the ret(+) group seemed to be greater than those of ret(-). These findings may be due to the mechanical trauma in cord placement, or the vasoconstrictor and homeostatic effects of the medicament infused, or the combination of both. Proinflammatory cytokines like TNF-α are signs of inflammation, in addition, they influence the tissue repair and healing function. On the other hand, TNF-α level on day 28 were the lowest during entire procedure and baseline [2], which also supports our results. But the TNF-α GCF levels of ret(+) group were not found to reach a peak 1 day after retraction compared to the baseline measurements. The homeostatic effect of aluminum-potassium-sulfate may be responsible for this outcome via inhibition of blood flow in periodontal tissues [3].

The immune mediators originating from a site of infection or from a site of severe trauma may activate hepatocytes in the liver to produce large quantities of acute-phase proteins [26]. In the present study the proinflammatory cytokines such as TNF-α were investigated to determine the acute phase trauma that can be expected after phases of prosthetic procedures. However, this may be one of the limitations of this study, proinflammatory cytokines can stimulate prostaglandins and lytic enzymes and modulate the expressions of other mediators such as acute phase proteins [27]. These cytokines such as TNF- α were shown effectively to induce the release of proMMP-1 and MMP-3 from gingival fibroblasts [15]. In agreement with these results, Beklen *et al.* stated that IL-1β and TNF-α have a potent effect on the production of MMPs [28].

MMP-1 (interstitial collagenase, collagenase-1), synthesized and secreted ubiquitously by connective tissue cells (fibroblast) and macrophages, is more often associated with normal tissue remodelling and hydrolyses mainly type III collagen [16], where MMP-8 is the most effective in hydrolyzing type I collagen [17] and is the major interstitial collagenase in inflamed human gingiva [29, 30]. In contrast to healthy patient's gingiva, extracts of untreated gingival tissue and GCF from periodontitis patients contain pathologically elevated levels of collagenase-2 (MMP-8) in catalytically active form [31, 32].

MMP-1 and MMP-8 share the unique ability to cleave types I, II, and III collagen and probably serve as initiators of the majority of extracellular matrix destruction in periodontal disease. Thus, synthesis and activation of MMP-1 and MMP-8 are important steps in the pathological extracellular matrix destruction associated with inflammatory periodontal disease [15].

On the other hand, we haven't found any significant differences in GCF MMP-1 levels in both ret(-) and ret(+) groups, compared to baseline and negative control group, at all time intervals. Although there were no significant differences between the study groups, the GCF MMP-8 levels were significantly different between the study periods.

In conclusion, within the scope of this present study, it may be suggested that well-fitted fixed dental prosthesis and its procedures performed to periodontally healthy subjects do not affect the periodontal tissue health negatively. One possible limitation of this study is the rather low patient numbers. Therefore, it is hard to state that potassium-aluminum-sulfate braided retraction cord causes periodontal inflammation after the gingival retraction procedure. Larger scale and intervention studies are required to better address these issues.

Ethical Considerations

The study protocol was approved by the ethics committee of the Ege University School of Medicine (08-7/18, Protocol code: 2008-DIS-018). Prior to participation, the purpose and procedures were fully explained to all patients and all participants gave written informed consent in accordance with Helsinki Declaration. The study was designed, conducted, analyzed and reported according to guidelines for Good Clinical Practice.

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Author Contributions

All authors contributed extensively to the work presented in this paper. In particular, N. Bıçakcı and Ö. Özçaka were responsible for study conception and design; A. Akcalı and Ö. Özçaka were responsible for periodontal data collection and T. Bıçakcı and R. Tüzünsoy-Aktaş were responsible for prosthetic treatments; A. Nalbantsoy was responsible for laboratory analysis; T. Köse was responsible for statistical analysis; and all authors extensively contributed in manuscript preparation.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

[1] Sherif AH, el Mahassen Badawi BA, el-Sayed SM. Biological influence of some crown and bridge restorative materials finished and polished by different techniques. Egypt Dent J 1993; 39:559-68.

[2] Feng J, Aboyoussef H, Weiner S, Singh S, Jandiski J. The effect of gingival retraction procedures on periodontal indices and crevicular fluid cytokine levels: a pilot study. J Prosthodont 2006; 15:108-12.

[3] de Gennaro GG, Landesman HM, Calhoun JE, Martinoff JT. A comparison of gingival inflammation related to retraction cords. J Prosthet Dent 1982; 47:384-6.

[4] Uitto VJ, Overall CM, McCulloch C. Proteolytic host cell enzymes in gingival crevice fluid. Periodontol 2000 2003; 31:77-104

[5] Biesbrock A, Yeh CH. Relationship of surface epithelium concentrations of IL-1 alpha and IL-1 beta to clinical inflammation during experimental gingivitis. Monogr Oral Sci 2000; 17:20-31.

[6] Mogi M, Otogoto J, Ota N, Inagaki H, Minami M, *et al.* Interleukin 1 beta, interleukin 6, beta 2-microglobulin, and transforming growth factor-alpha in gingival crevicular fluid from human periodontal disease. Arch Oral Biol 1999; 44:535-9.

[7] Tzannetou S, Efstratiadis S, Nicolay O, Grbic J, Lamster I. Interleukin-1 beta and beta-glucuronidase in gingival crevicular fluid from molars during rapid palatal expansion. Am J Orthod Dentofacial Orthop 1999; 115:686-96.

[8] Salvi GE, Brown CE, Fujihashi K, Kiyono H, Smith FW, *et al.* Inflammatory mediators of the terminal dentition in adult and early onset periodontitis. J Periodontal Res 1998; 33:212-25.

[9] Stashenko P, Dewhirst FE, Peros WJ, Kent RL, Ago JM. Synergistic interactions between interleukin 1, tumor necrosis factor, and lymphotoxin in bone resorption. J Immunol 1987; 138:1464-8.

[10] Baqui AA, Meiller TF, Jabra-Rizk MA, Zhang M, Kelley JI, *et al.* Enhanced interleukin 1 beta, interleukin 6 and tumor necrosis factor alpha in gingival crevicular fluid from periodontal pockets of patients infected with human immunodeficiency virus 1. Oral Microbiol Immunol 2000; 15:67-73.

[11] Heasman PA, Collins JG, Offenbacher S. Changes in crevicular fluid levels of interleukin-1 beta, leukotriene B4, prostaglandin E2, thromboxane B2 and tumour necrosis factor alpha in experimental gingivitis in humans. J Periodontal Res 1993; 28:241-7.

[12] Sorsa T, Tjäderhane L, Salo T. Matrix metalloproteinases (MMPs) in oral diseases. Oral Dis 2004; 10:311-8.

[13] Welgus HG, Jeffrey JJ, Eisen AZ. Human skin fibroblast collagenase. Assessment of activation energy and deuterium isotope effect with collagenous substrates. J Biol Chem 1981; 256:9516-21.

[14] Hasty KA, Jeffrey JJ, Hibbs MS, Welgus HG. The collagen substrate specificity of human neutrophil collagenase. J Biol Chem 1987; 262:10048-52.

[15] Birkedal-Hansen H, Moore WG, Bodden MK, Windsor LJ, Birkedal-Hansen B, *et al.* Matrix metalloproteinases: a review. Crit Rev Oral Biol Med 1993; 4:197-250.

[16] Nelson SK, Wataha JC, Cibirka RM, Lockwood PE. In vitro TNF-alpha release from THP-1 monocytes in response to dental casting alloys exposed to lipopolysaccharide. J Prosthet Dent 2001; 85:466-71.

[17] Armitage GC. Development of a classification system for periodontal diseases and conditions. Ann Periodontol 1999; 4:1-6.

[18] Quigley GA, Hein JW. Comparative cleansing efficiency of manual and power brushing. J Am Dent Assoc 1962; 65:26–9.

[19] Lamster IB, Oshrain RL, Gordon JM. Enzyme activity in human gingival crevicular fluid: considerations in data reporting based on analysis of individual crevicular sites. J Clin Periodontol 1986; 13:799–804.

[20] Cimasoni G. Crevicular fluid updated. Monogr Oral Sci 1983;12:1-152.

[21] Bissada NF, Schaffer EM, Haus E. Circadian periodicity of human crevicular fluid flow. J Periodontol 1967; 38:36-40.

[22] Suppipat N, Johansen JR, Gjermo P. Influence of "time of day", pocket depth and scaling on gingival fluid flow. J Clin Periodontol 1977; 4:48-55.

[23] Curtis MA, Griffiths GS, Price SJ, Coulthurst SK, Johnson NW. The total protein concentration of gingival crevicular fluid. Variation with sampling time and gingival inflammation. J Clin Periodontol 1988; 15:628–32.

[24] Curtis MA, Sterne JA, Price SJ, Griffiths GS, Coulthurst SK, *et al.* The protein composition of gingival crevicular fluid sampled from male adolescents with no destructive periodontitis: baseline data of a longitudinal study. J Periodontal Res 1990; 25:6–16.

[25] Alfano MC. The origin of gingival fluid. J Theor Biol 1974; 47:127–36.

[26] Steel DM, Whitehead AS. The major acute phase reactants: C-reactive protein, serum amyloid P component and serum amyloid A protein. Immunol Today 1994; 15:81-8.

[27] Loos BG. Systemic markers of inflammation in periodontitis. J Periodontol 2005;76:2106-15.

[28] Beklen A, Ainola M, Hukkanen M, Gürgan C, Sorsa T, *et al.* MMPs, IL-1, and TNF are regulated by IL-17 in periodontitis. J Dent Res 2007; 86:347-51.

[29] Kiili M, Cox SW, Chen HY, Wahlgren J, Maisi P, *et al.* Collagenase-2 (MMP-8) and collagenase-3 (MMP-13) in adult periodontitis: molecular forms and levels in gingival crevicular fluid and immunolocalisation in gingival tissue. J Clin Periodontol 2002; 29:224-32.

[30] Ingman T, Apajalahti S, Mäntylä P, Savolainen P, Sorsa T. Matrix metalloproteinase-1 and -8 in gingival crevicular fluid during orthodontic tooth movement: a pilot study during 1 month of follow-up after fixed appliance activation. Eur J Orthod 2005; 27:202-7.

[31] Mäntylä P, Stenman M, Kinane DF, Tikanoja S, Luoto H, *et al.* Gingival crevicular fluid collagenase-2 (MMP-8) test stick for chair-side monitoring of periodontitis. J Periodontal Res 2003; 38:436-9.

[32] McCulloch CA. Host enzymes in gingival crevicular fluid as diagnostic indicators of periodontitis. J Clin Periodontol 1994; 21:497-506..