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ORIGINAL ARTICLE

IL-6 and IL-8 levels in GCF of the teeth supporting fixed partial denture

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OBJECTIVES: To evaluate the gingival crevicular fluid (GCF) contents of interleukin-6 (IL-6) and interleukin-8 (IL-8) and the clinical parameters of the teeth supporting fixed partial denture (FPD) and the contralateral teeth and to assess the effect of scaling and root planning (SRP) on clinical parameters and the GCF levels of cytokines.

MATERIALS AND METHODS: The study population included 23 patients. Probing depth (PD), clinical attachment level (CAL), plaque index (PI), and gingival index (GI) were recorded, and GCF samples were collected for analysis of cytokine levels from the teeth with FPD (Test Group), the contralateral teeth (Control Group) of each participant at baseline. After initial measurements, all participants received primary phase of non-surgical treatment including oral hygiene instruction and scaling and root planning (SRP). At the 1st month and the 3rd month after SRP, these procedures were repeated.

RESULTS: In both groups, all clinical parameters and the total amount of IL-8 showed decreases from initial to the 3rd month ($P < 0.05$), but from the 1st month to the 3rd month; PD, PI, and GI values significantly increased in the test group ($P < 0.05$).

CONCLUSION: The non-surgical periodontal treatment reduced the total amount of IL-8, not IL-6, and the clinical parameters of the teeth with FPD and contralateral teeth. But, there was a trend to the higher levels of PD, PI, and GI in the teeth with FPD. Therefore, a regular program for dental prophylaxis is also important for the maintenance of periodontal health in patients with FPD.
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Keywords: IL-6; IL-8; Gingival crevicular fluid; fixed partial denture; non-surgical therapy

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Introduction

Increased levels of cytokines including interleukin-1, -2, -4, -6, -8 (IL-1, IL-2, IL-4, IL-6, IL-8), and tumor necrosis factor (TNF) in the gingival crevicular fluid (GCF) of patients with periodontal diseases have been reported (Rossomando *et al*, 1990; Geivelis *et al*, 1993; Reinhardt *et al*, 1993, Lee *et al*, 1995; Tsai *et al*, 1995; Wilson *et al*, 1996; Hirose *et al*, 1997).

Interleukin-6 is a multifunctional cytokine, of which biological activities include B-lymphocyte differentiation, T-lymphocyte proliferation, and stimulation of immunoglobulin (Ig) secretion by B lymphocytes, stimulation of acute phase protein synthesis, and complement cascade activation (Hirano *et al*, 1990). Of particular significance is the ability of IL-6 to induce bone resorption, both by itself and in conjunction with other bone-resorbing agents (Ishimi *et al*, 1990). Both pro- and anti-inflammatory effects have been observed. These different effects develop especially in cases of bacterial infection and are thus also of crucial importance in inflammatory periodontal diseases (Mengel *et al*, 2002). IL-8 is a potent chemokine with a distinct target for recruitment and activation of human granulocytes and mediation of inflammatory processes (Baggiolini *et al*, 1989). It can be secreted from many different host cells, including monocytes/macrophages, lymphocytes, fibroblasts, endothelial cells, and epithelial cells (Baggiolini *et al*, 1989; Lawlor *et al*, 1992). IL-8 plays an important role in regulation of neutrophil function (Tonetti, 1997).

The information available to dentists who are evaluating the periodontal health of abutment teeth that support prosthodontic restorations is conflicting. Investigations of fixed partial denture (FPD)s indicate that the retainers accumulate more plaque, exhibit more severe inflammation, and demonstrate greater periodontal pocket depth when compared with unrestored, non-abundment teeth. However, it has also been demonstrated that with proper oral hygiene, minimal periodontal changes develop around abutment teeth

that support FPD restorations (Lindhe and Nyman, 1977; Nyman and Lindhe, 1979; Nyman and Ericcson, 1982; Guyer *et al*, 1985). The intracrevicular location of restoration can hinder mechanical removal by routine oral hygiene measures and potentially create an environment conducive to colonization by periodontal pathogens. The common concept is: the smaller marginal discrepancy, the better the periodontal response. However, correlation of the size of marginal discrepancy with the degree of inflammation in adjacent gingival tissues has not been demonstrated. It is still doubtful whether an intracrevicular margin is per se is an etiologic factor for periodontal disease. Certain authors have suggested that, for patients with acceptable oral hygiene, an intracrevicular margin is not a critical factor. If patients are also on a regular recall program for dental prophylaxis, the potential for inflammation adjacent to a crown margin below the free gingival tissue is reduced further (Mueller, 1986; Flores-de- Jacoby *et al*, 1989). An adequate understanding of the relationship between periodontal tissues and restorative dentistry is paramount to ensure adequate form, function, esthetics, and comfort of the dentition (Padbury *et al*, 2003).

Therefore, the objectives of this study were to measure the GCF contents of IL-6 and IL-8 and clinical parameters at the teeth supporting FPD and the contralateral teeth in subjects with chronic periodontitis at baseline and at the 1st month and the 3rd month after non-surgical periodontal treatment.

Materials & methods

Selection of patients

The study population included 23 patients, 13 men and 10 women, in the age range of 30–60 years (43.5 ± 7.22). The patients had moderate to severe periodontal disease as evidenced by multiple sites with a probing depth of 5.0 mm or more, clinical attachment loss 3.0 mm or more, and bone loss at least two teeth per quadrant by radiographs. The inclusion criteria were: the presence of teeth supporting FPD in one posterior side of maxilla and natural teeth on the other posterior side. Their general health was good and all participants had no periodontal therapy in the preceding 3 months including root surface debridement or the adjunctive use of local or systemic antimicrobials and were drawn from the waiting list of patients with untreated chronic periodontitis at the Department of Periodontology at Kirikkale University, Faculty of Dentistry.

The purpose and nature of the study, including the types of clinical measurements and sample collection, were explained to all potential participants. After reading and signing the consent form, the subjects were enrolled into the study. The study was approved by the Medical Ethical Committee of Kirikkale University Faculty of Dentistry.

Clinical recordings were recorded and GCF samples were collected from two teeth with metal ceramic FPD (porcelain fused to metal restoration) (Test Group), two contralateral teeth (Control Group) of each participant at baseline. After baseline measurements, all participants received a primary phase of non-surgical treatment including oral hygiene instruction, scaling and root plan-ing. Full-mouth supragingival professional tooth cleaning (scaling and polishing) was performed in a single session for 60 min and oral hygiene instructions were given at this time. One week later, the subsequent non-surgical treatment consisted of subgingival debridement using Gracey curettes (Hu-Friedy, Chicago, IL, USA) under local anesthesia in one session for 60 min. At the 1st and the 3rd month, clinical recordings and sampling procedures were repeated following the intervention.

Clinical recordings

Prior to crevicular fluid collection, supragingival plaque was scored using Plaque index (PI) (Silness and Löe, 1964). Gingival inflammation was scored following crevicular fluid collection using gingival index (GI) (Löe and Silness, 1963). Probing depth (PD) and clinical attachment level (CAL) measures were obtained from sample sites (mesial or distal midpoints) of teeth using a conventional periodontal probe (Hu-Friedy). The probe was directed parallel to the long axis of the tooth. CAL measurements were made from the cemento-enamel junction to the bottom of the sulcus. All clinical data were recorded by one examiner.

Crevicular fluid sampling

After supragingival plaque was removed from each tooth, the individual tooth site was gently air-dried and isolated with cotton rolls. A saliva ejector was used to avoid salivary contamination of the samples. Each GCF sample was collected with paper strips (Periopaper, Amityville, NY, USA) from the two sites of the teeth supporting FPD. This procedure was undertaken for two sites of contralateral teeth in each patient. The paper strips were consecutively inserted into the crevice at the mesial or distal midpoints until mild resistance was felt. The strips were left *in situ* 30 s and then transferred, for volume determination, to the chair-side located Periotron 8000 (Oraflow Inc., Plainview., NY, USA), which was calibrated using known volumes of phosphate-buffered saline (PBS). Two strips of each site of each patient were immediately placed in a labeled tube containing 500 μl PBS and transported to the laboratory. Following 10 s vortexing and 20 min shaking, the strips were removed and the eluates centrifuged for 5 min at 5800 g to remove plaque and cellular elements. The samples were stored at −80°C for subsequent assays.

Cytokine assay

Levels of IL-6 and IL-8 in samples were determined by using an appropriate commercial ELISA kit (Biosource, California, USA) according to manufacturer's instructions. The results were read using a microplate reader at 405 nm wavelength. Concentrations of the cytokines in each 400 μl sample were determined by generation of a standard curve for comparison. Concentrations of the cytokines were corrected for GCF volume and were

defined as pg μl^{-1}. Total amounts of cytokines were expressed as pg/2 sites.

Statistical analysis
Data were expressed as mean values and standard deviations. The statistical significance of differences in GCF cytokine levels and clinical parameters between groups at the 1st month and the 3rd month was analyzed by Mann–Whitney *U* Test, and intra-group measurements over time were analyzed by using Wilcoxon Signed Ranks Test. Simple pair wise correlation coefficients were calculated according to the product-moment correlation method of Pearson. The examiner was not calibrated and the authors did not perform power analysis to determine the sample size in the study groups. The null-hypothesis was rejected at $P < 0.05$.

Results

Clinical characteristics
The clinical characteristics of this study at baseline, the 1st month and the 3rd month after non-surgical periodontal treatment, are shown in Table 1. When the clinical parameters were compared between groups, PD and PI values were significantly higher in the test group compared with control group at the 3rd month ($P < 0.05$).

Once comparisons of clinical parameters were evaluated in each group, in test group and the control group, all clinical parameters including PD, CAL, PI, and GI showed decreases from initial to the 1st month and the 3rd month ($P < 0.05$), but from the 1st month to the 3rd month, PD, PI, and GI values significantly increased in the test group ($P < 0.05$).

GCF sample levels of IL-6 and IL-8
The concentration and total amount measurements of GCF levels of IL-6 and IL-8 and intra-group comparisons of these cytokines are shown in Table 2.

In both groups, the volume of GCF decreased from initial to the 1st month and the 3rd month ($P < 0.05$). The total amount of IL-6 was lower at baseline in the control group than the test group. The concentration of GCF levels of IL-8 decreased from initial and the 1st month to the 3rd month in the test group, and it decreased from initial to the 3rd month in the control group ($P < 0.05$). In the test group, the total amount of IL-8 in GCF decreased from initial and the 1st month to the 3rd month, and in the control group it decreased from initial to the 3rd month ($P < 0.05$).

Correlations
Correlations between the mean total amount of IL-6, IL-8 levels and the volume of GCF and clinical parameters are shown in Table 3.

Table 1 The mean values of clinical parameters at initial, the 1st month, and the 3rd month in sampling sites (mean ± s.d.)

	Initial values		*1st month values*		*3rd month values*	
Parameters	*Test Group (n = 23)*	*Control Group (n = 23)*	*Test Group (n = 23)*	*Control Group (n = 23)*	*Test Group (n = 23)*	*Control Group (n = 23)*
PD	4.48 ± 0.43	4.31 ± 0.42	3.69 ± 0.30[a]	3.61 ± 0.25[a]	3.81 ± 0.29*[,a,b]	3.65 ± 0.24[a]
CAL	3.80 ± 0.76	3.69 ± 0.62	3.27 ± 0.43[a]	3.17 ± 0.43 [a]	3.34 ± 0.49[a]	3.22 ± 0.45[a]
PI	1.92 ± 0.34	1.92 ± 0.33	1.51 ± 0.27[a]	1.42 ± 0.29[a]	1.62 ± 0.25*[,a,b]	1.45 ± 0.26[a]
GI	1.91 ± 0.34	1.92 ± 0.31	1.60 ± 0.28[a]	1.58 ± 0.31[a]	1.70 ± 0.26[a,b]	1.56 ± 0.27[a]

PD, probing depth; CAL, clinical attachment level; PI, plaque index; GI, gingival index.
*$P < 0.05$ according to groups.
[a]$P < 0.05$ according to initial values.
[b]$P < 0.05$ according to the 1st month values.

Table 2 The mean values of the volume of GCF and the concentration and total amount of IL-6 and IL-8 in GCF at initial, the 1st month, and the 3rd month in sampling sites (mean ± s.d.)

		Initial values		*1st month values*		*3rd month values*	
	Parameters	*Test Group (n = 23)*	*Control Group (n = 23)*	*Test Group (n = 23)*	*Control Group (n = 23)*	*Test Group (n = 23)*	*Control Group (n = 23)*
	GCF	0.33 ± 0.13	0.29 ± 0.10	0.26 ± 0.09[a]	0.23 ± 0.09[a]	0.27 ± 0.10[a]	0.22 ± 0.11[a]
Concentration (pg μl^{-1})	IL-6	0.86 ± 1.21	0.33 ± 0.19	0.99 ± 1.30	0.37 ± 0.20	0.57 ± 0.68	0.53 ± 0.42
	IL-8	0.93 ± 0.81	1.13 ± 0.76	0.91 ± 0.65	1.19 ± 1.03	0.50 ± 0.34[a,b]	0.78 ± 0.82[a]
Total amount (pg/2 sites)	IL-6	0.15 ± 0.24*	0.04 ± 0.01	0.12 ± 0.16	0.04 ± 0.01	0.08 ± 0.11	0.05 ± 0.04
	IL-8	0.16 ± 0.18	0.18 ± 0.15	0.10 ± 0.08	0.14 ± 0.13	0.06 ± 0.03[a,b]	0.07 ± 0.05[a]

*$P < 0.05$ according to groups
[a]$P < 0.05$ according to initial value
[b]$P < 0.05$ according to 1st month value

Table 3 Correlations between total amount (pg/2sites) of IL-6, IL-8 and the volume of GCF and clinical parameters in the test group and the control group

		PD		*CAL*		*PI*		*GI*	
	Month	*Test Group (n = 23)*	*Control Group (n = 23)*	*Test Group (n = 23)*	*Control Group (n = 23)*	*Test Group (n = 23)*	*Control Group (n = 23)*	*Test Group (n = 23)*	*Control Group (n = 23)*
GCF	0	0.686**	0.358	0.797**	0.511*	0.538**	0.424*	0.518*	0.303
	1	0.455*	0.042	0.344	0.239	0.183	−0.064	0.445*	0.233
	3	0.211	0.012	0.242	0.301	0.311	0.115	0.457*	0.145
IL-6	0	0.254	0.090	0.307	0.171	0.240	0.108	0.141	−0.084
	1	0.293	−0.056	0.336	−0.179	0.166	−0.356	0.511*	−0.088
	3	0.101	−0.098	0.254	0.324	0.388	0.497	0.277	0.089
IL-8	0	0.416*	0.402	0.538**	0.647**	0.087	0.232	0.245	0.264
	1	0.357	0.327	0.012	0.443*	−0.031	−0.057	−0.102	0.508*
	3	−0.170	0.147	−0.457*	−0.058	−0.408	0.039	−0.330	−0.131

Pearson correlation coefficients.
PD, probing depth; CAL, clinical attachment level; PI, plaque index; GI, gingival index; GCF, gingival crevicular fluid.
*The correlation at the 0.05 level.
**The correlation at the 0.01 level.

In the test group, the volume of GCF showed a positive correlation with initial PD, CAL, PI ($P < 0.01$), and GI values ($P < 0.05$), with the 1st month PD and GI values and with the 3rd month GI values ($P < 0.05$). The mean total amount of IL-6 showed a positive correlation only with the 1st month GI values ($P < 0.05$). The mean total amount of IL-8 showed positive correlations with the1st month PD ($P < 0.05$) and CAL values ($P < 0.01$) and negative correlation with the 3rd month CAL values ($P < 0.05$).

In the control group, positive correlations were found between the volume of GCF and the initial CAL and PI values ($P < 0.05$). There were positive correlations between the mean total amount of IL-8 and initial CAL values ($P < 0.01$) and the 1st month CAL and GI values ($P < 0.05$).

Discussion

It has been strongly suggested that the detection of specific subgingival bacteria together with the identification of specific substances in GCF as a measure of host response may develop useful co-biomarkers for identification of disease progression and monitoring of treatment responses (Lamster *et al*, 1994; Wolff *et al*, 1994).

This study examined the GCF contents of IL-6 and IL-8 and clinical parameters at the teeth supporting FPD and contralateral teeth in subjects with chronic periodontitis and further evaluated the effect of scaling and root planning on IL-6 and IL-8 levels in GCF and treatment response among a group of chronic periodontitis patients, who had not received any prior periodontal treatment, at the 1st month and the 3rd month.

Although some of the studies have evaluated the effects of FPD on gingival health (Knoernschild and Campbell, 2000; Kancyper and Koka, 2001; Reitemeier *et al*, 2002), they did not document the periodontal disease history of the patient. But the classification of periodontal diseases is important because of the different clinical and host responses in different periodontal diseases. Therefore, in our study, all of the patients had chronic periodontitis.

The results of this study suggested that all clinical parameters were similar in the teeth with FPD and the contralateral teeth, but the teeth with FPD showed an increase in all clinical parameters at the 3rd month. Scaling and root planning generally reduced the levels of all clinical parameters from baseline to the 3rd month, but from the 1st month to the 3rd month; PD, PI, and GI values significantly increased in the teeth with FPD. Although the clinical baseline measurements were similar between these groups, the teeth with FPD responded less favorably at this evaluation period. This result is consistent with the studies that artificial crowns and FPDs increase the incidence of advanced gingival inflammation, probing depth and plaque index (Knoernschild and Campbell, 2000; Reitemeier *et al*, 2002). Evidence suggests that restoration factors such as poor margin adaptation, intracrevicular margin placement, rough surfaces, and overcontouring could contribute to localized gingival inflammation, increased probing depths, and bone resorption (Knoernschild and Campbell, 2000). In our study, all FPDs had intracrevicular margins. Patient factors may also be effective on the clinical parameters of FPDs. Thus, FPDs may not be cleaned easily such as natural teeth.

Gingival crevicular fluid volume is influenced by many factors such as flow rate (Challacombe, 1980), gingival trauma (Curtis *et al*, 1988), and repeat sampling (Lamster *et al*, 1989). It is logical to expect that with some cytokines, total amounts in gingival fluid will correlate better with disease than cytokine concentrations. Saliva contamination is a potential problem when sampling GCF with filter paper strips and this could clearly influence results expressed as cytokine concentration. The total amounts of cytokines are more associated with disease activity than concentration (Nakashima *et al*, 1994). Therefore, the comparisons of cytokines between groups, the changes of cytokines intra-groups, and the correlations between cytokines

and clinical parameters were evaluated and discussed according to the total amount in our study. There are many ways of collecting GCF and some of them are paper strips, capillary tubes, paper cones, and gingival washing. We preferred to use paper strips as used earlier by some of the authors (Rossomando *et al*, 1990; Geivelis *et al*, 1993; Reinhardt *et al*, 1993; Lee *et al*, 1995; Atilla and Kütükçüler, 1998). There is no commonly accepted method for depth of insertion, time of sampling or the most appropriate expression and computation of cytokine levels (i.e., total amount *vs* concentration). Recently, GCF collection is usually made by paper strips, and 30 s time of sampling is generally preferred (Rossomando *et al*, 1990; Reinhardt *et al*, 1993; Atilla and Kütükçüler, 1998). In our study, the volume of GCF decreased from initial to the 1st and the 3rd month in both groups.

IL-6 is a multifunctional cytokine (Hirano *et al*, 1990), which has the ability to induce bone resorption, both by itself and in conjunction with other bone-resorbing agents (Ishimi *et al*, 1990). In this study, although there was not any significant difference in the mean levels of clinical measurements, as previous studies have shown that there was a positive correlation between inflammation degree and GCF cytokine levels (Masada *et al*, 1990; Geivelis *et al*, 1993), the total amount of IL-6 was lower in the contralateral teeth than the teeth with FPD at baseline. Scaling and root planning did not affect the levels of IL-6 in the teeth with FPD and the contralateral teeth. Our findings were consistent with the results of some studies that scaling and root planning did not affect the amount of IL-6 in GCF. (Erdemir *et al*, 2004; Talbert *et al*, 2006). The mean total amount of IL-6 of the teeth with FPD showed correlation only with baseline GI scores.

Interleukin-8 has been shown to be important for the initiation and development of inflammation and tissue destruction in periodontal diseases (Tsai *et al*, 1995; Chung *et al*, 1997; Gamonal *et al*, 2000). Different results in terms of the effect of periodontal treatment on IL-8 levels in GCF have been reported (Tsai *et al*, 1995; Chung *et al*, 1997; Gamonal *et al*, 2000). In this study, scaling and root planning generally reduced the levels of IL-8 in GCF from baseline to the 3rd month with corresponding reduction in probing depth and clinical attachment loss in the teeth with FPD and the contralateral teeth. The possible explanations for the difference among these studies could be attributed to several factors, e.g., patient and site selection, classification of the periodontal disease status, sampling method employed, timing of clinical re-assessment, and possible variations in periodontal treatment protocol and treatment outcomes achieved. Scaling and root planning also yielded different outcomes in the teeth with FPD relating to the total amount of IL-6 and IL-8 in GCF. This may indicate different roles of IL-6 and IL-8 in the pathogenesis of the disease. The mean total amount of IL-8 showed correlations with the1st month PD and CAL values and the 3rd month CAL values in the teeth with FPD. There were correlations between the mean total amount of IL-8 and initial CAL values and the 1st month CAL and GI values in the teeth with clinical attachment loss. We were unable to compare our data with other studies as we were unable to find published reports on the GCF cytokine levels in chronic periodontitis patients with FPD.

In conclusion, our results indicate that there was a difference at the beginning of the study in the total amount of IL-6 in GCF between the teeth with FPD and the contralateral teeth and there was no any difference in the total amount of IL-8 in GCF between these two groups. The non-surgical periodontal treatment reduced the total amount of IL-8, not IL-6, and the clinical parameters of the teeth with FPD and contralateral teeth. It can be stated that if patients have acceptable oral hygiene, tooth-supported FPDs with intracrevicular margins were not predisposed to unfavorable gingival responses. However, there was a trend to the higher levels of PD, PI, and GI in the teeth with FPD. Therefore, a regular program for dental prophylaxis is also important for the maintenance of periodontal health in patients with FPD and patients should be informed that the FPDs need care as their natural teeth. We believe that such studies will make great contribution to understand the effects of FPDs on the gingival health.

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