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Effect of scaling and root planing on interleukin-1β, interleukin-8 and MMP-8 levels in gingival crevicular fluid from chronic periodontitis patients

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Background and Objective: There are few data concerning the effect of scaling and root planing on the levels of immune and inflammatory mediators in gingival crevicular fluid from patients with chronic periodontitis. Therefore, in this study the influence of scaling and root planing was determined on amounts of interleukin (IL)-1β, IL-8 and MMP-8 in gingival crevicular fluid from patients with chronic periodontitis, in relation to clinical parameters.

Material and Methods: A total of 51 patients were enrolled in this study. The study population consisted of 30 patients with generalized advanced chronic periodontitis, while 21 periodontally healthy subjects were recruited for the control group. The clinical parameters included approximal plaque index, gingival index, pocket depth and clinical attachment loss. The amounts of IL-1β, IL-8 and MMP-8 in gingival crevicular fluid were measured by ELISA. Periodontal parameters as well as gingival crevicular fluid humoral factor amounts were evaluated in the control group and in chronic periodontitis patients at baseline and at 1 and 4 wk after scaling and root planing treatment.

Results: At baseline, there were significant differences between control subjects and chronic periodontitis patients in terms of clinical attachment loss, pocket depth, gingival index ($p < 0.001$) and approximal plaque index ($p < 0.01$). The amounts of IL-1β, MMP-8 ($p < 0.001$) and IL-8 ($p < 0.01$) in gingival crevicular fluid were significantly lower in healthy subjects than in chronic periodontitis patients. Scaling and root planing led to improvement in all examined clinical parameters, apart from clinical attachment loss. Periodontal treatment also resulted in a significant decrease in the amounts of IL-1β, IL-8 and MMP-8 in comparison to baseline, especially 4 wk after scaling and root planing ($p < 0.001$); however, the amounts of these humoral factors were still higher than those in control group.

Conclusion: Our observations indicated that short-term nonsurgical therapy resulted in a significant improvement in periodontal indices and in a marked decrease of IL-1β, IL-8 and MMP-8 gingival crevicular fluid levels. Nevertheless, no significant correlations were found between clinical parameters and amounts of humoral factors after therapy.

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Nowadays, it is well established that periodontal diseases, including chronic periodontitis, are inflammatory conditions of the supporting tissues of the teeth induced by micro-organisms that stimulate the host immune and inflammatory responses (1,2). The interplay between periodontal pathogens and the inflammatory–immune system leads to development of chronic inflammation, progressive destruction of connective tissues and resorption of alveolar bone (3,4). Generally, diagnosis of periodontal diseases is based almost solely on clinical parameters (5,6); however, increasing amounts of data indicate that the intensity of inflammation and destruction within periodontal tissues can be evaluated objectively by analysis of gingival crevicular fluid components, because several inflammatory and immune mediators implicated in periodontal destruction have been identified in gingival crevicular fluid (7,8).

Of the humoral factors influencing immuno-inflammatory reactions within periodontal tissues, a crucial role is played by interleukin (IL)-1β and IL-8 (CXCL8). Interleukin-1β promotes development of an inflammatory response, amplifies inflammation and modulates a lot of immunological processes. Some of its biological effects include stimulation of fibroblast proliferation, stimulation of prostaglandin E_2 production by monocytes and fibroblasts and activation of different cell populations to release MMPs that degrade extracellular matrix proteins. This cytokine also promotes osteoclast formation and is a potent inducer of bone demineralization. It affects neutrophil chemotaxis and activation. An increasing body of evidence indicates that all of these IL-1β-dependent mechanisms may contribute to the inflammation and destruction of bone and to attachment loss, which are characteristic features of periodontal disease (9–11). Interleukin-8 is a potent chemoattractant for neutrophils and exerts various effects on neutrophil activity, including stimulation of granule exocytosis and release of myeloperoxidase, elastase and β-glucuronidase. As neutrophils undeniably play a crucial role in the development of inflammatory injury, IL-8 is of considerable importance for neutrophil-induced tissue destruction. The role of IL-8 in the pathological processes within periodontal tissues has been investigated, and it has been stated that excessive IL-8-mediated processes within the periodontal tissues may contribute to local periodontal tissue destruction (12,13).

Type I collagen is the main component of extracellular matrix in the soft and hard periodontal tissues, and thus its degradation is regarded as an important process involved in the uncontrolled destructive lesions. It is well established that MMPs are key proteolytic enzymes for periodontal tissue destruction, and it seems that MMP-8 (collagenase-2) plays a central role in the turnover and degradation of periodontal tissues (14,15). However, it should be emphasized that MMP-8, especially at physiologically levels, can also exert anti-inflammatory effects by processing some anti-inflammatory cytokines and chemokines (16,17). Golub *et al.* (18) stated that MMP-8 accounts for 80% of the total collagenase protein found in gingival crevicular fluid of chronic periodontitis patients.

An increased level of IL-1β has been observed in gingival crevicular fluid of periodontitis patients, and the amount of this cytokine in the gingival crevicular fluid is closely associated with the severity of periodontal disease and periodontal tissue destruction (10,19–22). In addition, raised levels of MMP-8 have been found in gingival crevicular fluid of periodontitis patients, especially in gingival crevicular fluid of subjects with chronic periodontitis (23–25). Therefore, it is suggested that both IL-1β and MMP-8 can serve as markers of periodontal tissue destruction. Some data also suggest that the level of IL-8 in gingival crevicular fluid is correlated with the severity of periodontitis; however, observations are inconsistent (26–28).

Scaling and root planing is a nonsurgical initial therapy for periodontal disease and, in association with adequate supragingival biofilm control, has well-documented success, especially regarding clinical parameters (29). Our hypothesis was that the relationship between the clinical changes after nonsurgical periodontal therapy and IL-1β, IL-8 and MMP-8 levels in gingival crevicular fluid may be an effective tool in management of chronic periodontitis. Therefore, the aim of this study was first, to determine the short-term effect of scaling and root planing on IL-β, IL-8 and MMP-8 levels in gingival crevicular fluid from patients with chronic periodontitis and second, to establish the correlation between clinical parameters and IL-β, IL-8 and MMP-8 levels in gingival crevicular fluid prior to and after periodontal treatment.

Material and methods

Subject population

A total of 51 patients were enrolled in this study. Patients were chosen at random from those attending the Dental Institute at the Medical University of Łódź. Informed consent was obtained from all patients in accordance with the Declaration of Helsinki. The protocol was approved by the ethics committee of the Medical University of Łódź, Poland.

The study population consisted of 30 patients (16 women and 14 men; mean age 48.7 ± 9.1 years) with generalized, advanced chronic periodontitis based on the current classification of the American Academy of Periodontology (30). Twenty-one periodontal healthy subjects (13 women and 8 men; mean age 33.7 ± 8.2 years) were recruited for the control group. Patients were designated healthy if their mean whole-mouth attachment loss value was < 1 mm and there was no radiographic evidence of alveolar bone loss. Complete medical and dental histories were taken from all patients. Criteria for patient inclusion were as follows: (i) no treatment of periodontitis for the last 12 mo that could affect periodontal treatment outcomes; (ii) no use of antibiotics and anti-inflammatory drugs for 3 mo prior to treatment; and (iii) no systemic diseases (e.g. diabetes, osteoporosis and immunological disorders). Criteria for exclusion from the study

were as follows: (i) pregnancy or breastfeeding for women; (ii) cigarette smoking; (iii) < 20 teeth; and (iv) orthodontic therapy.

Clinical examination

The clinical periodontal parameters were recorded at baseline and at 1 and 4 wk after treatment for each test and control tooth. The clinical examination included approximal plaque index, gingival index, pocket depth and clinical attachment loss. All clinical parameter measurements were conducted using a manual periodontal probe. For evaluation of approximal plaque index, a periodontal probe was gently placed through approximal spaces of the first and third quadrants from the oral side and of the second and fourth quadrant from the buccal side. The plaque remnants were noted as '+'. A maximum of 28 points were measured. Gingival index measurements were assessed at four surfaces per tooth (mesial, distal, buccal, lingual and palatal surface), while pocket depth and clinical attachment loss measurements were taken at six surfaces per tooth (mesiobuccal, mid-buccal, distobuccal, mesio-oral, mid-oral and disto-oral). Approximal plaque index, gingival index, pocket depth and clinical attachment loss are given as average values. All clinical parameters were assessed by one examiner.

Gingival crevicular fluid sampling

Prior to gingival crevicular fluid collection, the supragingival plaque was carefully removed. At baseline and each follow-up time point, gingival crevicular fluid samples were collected from the mesiobuccal site. The sites to be sampled were isolated with cotton rolls and gently air dried. Gingival crevicular fluid samples were collected with sterile Periopaper strips (Oraflow Inc., Plainview, NY, USA) that were inserted into the gingival crevice until mild resistance was felt and left in place for 30 s. Mechanical irritation was avoided, and strips visually contaminated with blood were discarded. After gingival crevicular fluid collection, strips were placed in Eppendorf vials and immediately frozen at −80°C until use.

Treatment procedure

Following the collection of gingival crevicular fluid and clinical data prior to treatment, chronic periodontitis patients received full-mouth scaling and root planing with curettes and ultrasonic instruments under local anesthesia for not more than 2 h. Full-mouth scaling was done using piezoelectric ultrasonic scalers, and root planing was performed using specific Gracey curettes. No antibiotics were prescribed following this treatment. Oral hygiene instruction was provided.

Assay of IL-1β, IL-8 and MMP-8 in gingival crevicular fluid

Gingival crevicular fluid samples were analysed for IL-β, IL-8 and MMP-8 by an ELISA (Quantikine R&D Systems Inc., Minneapolis, MN, USA). For gingival crevicular fluid extraction, strips were placed in tubes containing 500 μL of phosphate-buffered saline (pH 7.2) and the tubes shaken gently for 1 h at room temperature. The strips were removed and the fluids assayed by ELISA for IL-1β, IL-8 and MMP-8. All ELISA procedures were carried out according to the manufacturer's instructions. The ELISA plates were then assessed spectrophotometrically at an optical density of 450 nm. Interleukin-1β, IL-8 and MMP-8 determinations were carried out in duplicate for each sample. The gingival crevicular fluid IL-1β, IL-8 and MMP-8 amounts were calculated from the standard curves. Interleukin-1β, IL-8 and MMP-8 were determined as the total amount per sample (in picograms for IL-1β and IL-8 and in nanograms for MMP-8). The sensitivities for IL-1β, IL-8 and MMP-8 ELISAs were 1 pg/mL, 3.5 pg/mL and 0.02 ng/mL, respectively. Sites with IL-1β, IL-8 or MMP-8 amounts below the limits of detectability were scored as 0.

Statistical analysis

Data are presented as means and SD. Differences in clinical parameters and in IL-1β, IL-8 and MMP-8 levels between the control group and chronic periodontitis patients were compared using the Mann–Whitney *U*-test. The statistical significance of the difference in clinical parameters and in IL-1β, IL-8 and MMP-8 levels between before and after treatment was analysed using the Wilcoxon test. The Pearson correlation test was used for normally distributed variables, whereas the Spearman correlation test was used for correlation when non-normally distributed data were analysed. A value of $p < 0.05$ was considered statistically significant.

Results

Effect of scaling and root planing on clinical parameters

The clinical findings are shown in Table 1. A total of 51 patients

Table 1. Clinical parameters in healthy control subjects and in patients with chronic periodontitis at baseline and 1 and 4 wk after scaling and root planing treatment

		Chronic periodontitis patients (n = 30)		
	Control group (n = 21)	Baseline	1 wk after scaling and root planing	4 wk after scaling and root planing
No. of teeth	25.4 ± 3.6	21.6 ± 1.3	21.6 ± 1.3	21.4 ± 1.2
Clinical attachement loss (mm)	< 1	5.4 ± 0.9	5.4 ± 0.9	5.3 ± 0.9
Pocket depth (mm)	1.5 ± 0.9	6.4 ± 0.6	5.8 ± 0.7	4.8 ± 0.7
Approximal plaque index (%)	45.8 ± 12.7	64.4 ± 18.8	47.8 ± 17.8	51.0 ± 20.5
Gingival index	0.7 ± 0.3	2.3 ± 0.8	1.6 ± 0.7	1.2 ± 0.7

Data are shown as means ± SD.

completed the baseline examination. Twenty-one control subjects received no treatment and were included in the baseline comparison only. At baseline, there were statistically significant differences between the control subjects and the chronic periodontitis patients in all evaluated clinical parameters. Differences between control subjects and chronic periodontitis patients in the clinical attachment loss, pocket depth and gingival index reached $p < 0.001$, and differences in approximal plaque index reached $p < 0.01$.

Periodontal treatment led to improvement in all examined clinical parameters, apart from clinical attachment loss. Scaling and root planing resulted in a statistically significant ($p < 0.001$) decrease in the mean pocket depth on days 7 and on 28 compared with day 0. A significant ($p < 0.001$) reduction in average approximal plaque index occurred at 1 and 4 wk after scaling and root planing treatment, compared with baseline. The mean gingival index was significantly lower 1 and 4 wk after scaling and root planing ($p < 0.001$). In contrast, no significant changes in the mean clinical attachment loss values were observed in chronic periodontitis patients during 4 wk after periodontal therapy. It should be emphasized that the post-treatment healing was uneventful in all cases and no complications, such as abscesses or infections, were observed throughout the study period.

Effect of scaling and root planing on amounts of IL-1β, IL-8 and MMP-8 in gingival crevicular fluid

At baseline, mean amount of IL-1β in gingival crevicular fluid of control subjects was statistically lower than in chronic periodontitis patients (15.5 ± 14.0 vs. 72.5 ± 37.0 pg/sample, respectively; $p < 0.001$). Scaling and root planing resulted in a significant decrease in the mean amount of IL-1β in gingival crevicular fluid, from 72.5 ± 37.0 pg/sample prior to treatment to 34.1 ± 13.9 pg/sample 4 wk after therapy ($p < 0.001$; Fig. 1 and Table 2). The amount of IL-8 in gingival crevicular fluid of control subjects varied from 0 to 54.2 pg/sample and in chronic periodontitis patients before scaling and root planing it varied from 7.4 to 96.1 pg/sample, which was significantly higher than in control group ($p < 0.01$). One week after scaling and root planing, the mean amount of IL-8 decreased significantly compared with baseline ($p < 0.01$). Four weeks after treatment, the mean amount of IL-8 in gingival crevicular fluid was 22.7 ± 20.3 pg/sample and was significantly lower than at baseline ($p < 0.001$) but not than 1 wk after scaling and root planing ($p > 0.05$; Fig. 2 and Table 2). The amount of MMP-8 in gingival crevicular fluid of the control group ranged from 0 to 10.1 ng/sample and was significantly lower ($p < 0.001$) than in gingival crevicular fluid of chronic periodontitis subjects (10.6–34.4 ng/sample). Scaling and root planing resulted in a significant decrease in the amount MMP-8 in the gingival crevicular fluid ($p < 0.001$). One week after treatment, the mean amount of MMP-8 was 11.0 ± 6.6 ng/sample, and 4 wk after scaling and root planing it was 7.3 ± 3.3 ng/sample (Fig. 3 and Table 2).

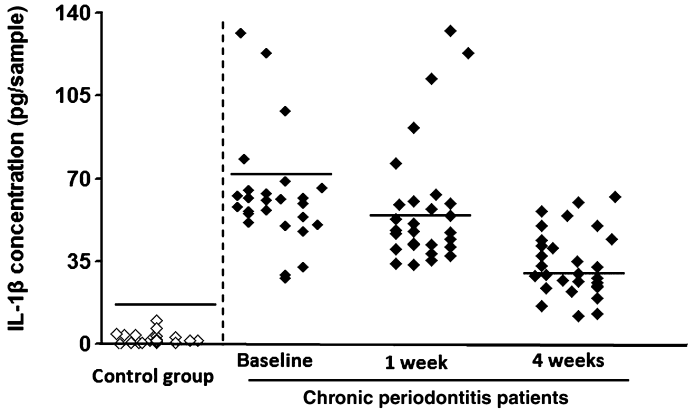

Fig. 1. Amount of interleukin (IL)-1β in gingival crevicular fluid from healthy subjects and from chronic periodontitis patients prior to and following scaling and root planing treatment.

Correlation analysis

Correlation data between the amounts of IL-1β, IL-8 and MMP-8 in gingival crevicular fluid and clinical parameters are shown in Table 3. In control subjects, correlations were found between the amount of IL-1β and approximal plaque index and gingival index ($p < 0.01$) and betweent the amount of MMP-8 and pocket depth ($p < 0.05$). In patients with chronic periodontitis at baseline, there were correlations between the amount of IL-1β and pocket depth and approximal plaque index, with a negative correlation between the amount of IL-8 and gingival index ($p < 0.05$). There were no significant correlations between clinical parameters and amounts of IL-1β, IL-8 or MMP-8 after scaling and root planing therapy. The correlation analysis also indicated that there were no relationships between amounts of MMM-8 and IL-1β, amounts of MMP-8 and IL-8 and amounts of IL-1β and IL-8 in gingival crevicular fluid at any time point studied.

Discussion

An increasing amount of data indicate that assessment of certain humoral factors, including IL-1β, IL-8 and MMP-8, in gingival crevicular fluid might provide a good diagnostic tool to monitor the course of periodontitis (20–27,31). It is also suggested that the measurement of these mediators in gingival crevicular fluid could be helpful to estimate the effect of periodontal treatment; however, limited information exists on the influence of periodontal therapy on gingival crevicular fluid levels of IL-1β, IL-8 and MMP-8.

We found that, compared with the healthy control subjects, patients with chronic periodontitis demonstrated

Table 2. Amounts of interleukin (IL)-1β, IL-8 and MMP-8 in gingival crevicular fluid from healthy control subjects and from patients with chronic periodontitis at baseline and 1 and 4 wk after scaling and root planing treatment

		Chronic periodontitis patients (n = 30)		
	Control group (n = 21)	Baseline	1 wk after scaling and root planing	4 wk after scaling and root planing
IL-1β (pg/sample)				
Range	2.4–56.1	27.8–168.9	33.3–132.6	11.8–62.2
Mean ± SD	15.5 ± 14.0	72.5 ± 37.0	57.2 ± 26.0	34.1 ± 13.9
IL-8 (pg/sample)				
Range	0–54.2	7.4–96.1	0–58.8	0–101.0
Mean ± SD	11.3 ± 14.7	26.3 ± 21.5	21.4 ± 16.1	22.7 ± 20.3
MMP-8 (ng/sample)				
Range	0–10.1	10.6–34.4	3.4–27.1	2.3–14.0
Mean ± SD	2.6 ± 2.6	18.6 ± 6.4	11.0 ± 6.6	7.3 ± 3.3

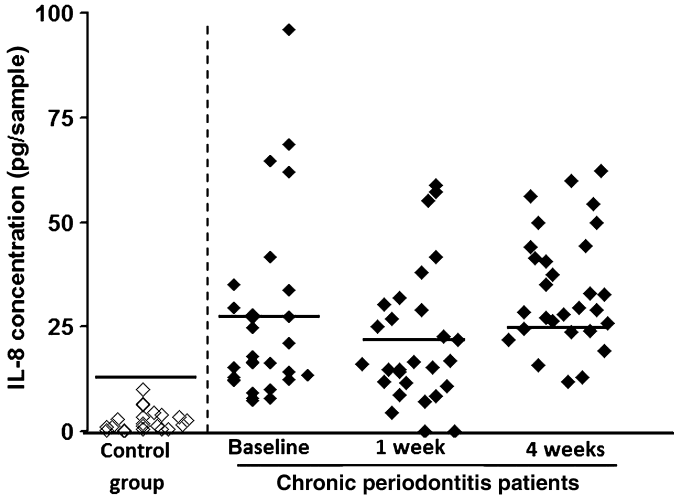

Fig. 2. Amount of IL-8 in gingival crevicular fluid from healthy subjects and from chronic periodontitis patients prior to and following scaling and root planing treatment.

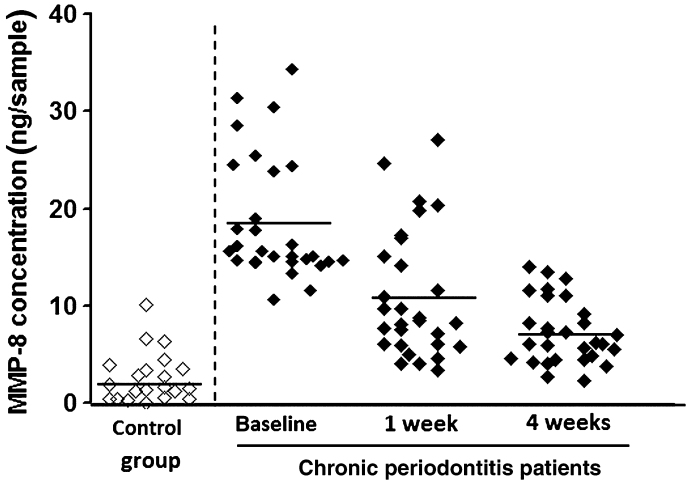

Fig. 3. Amount of MMP-8 in gingival crevicular fluid from healthy subjects and from chronic periodontitis patients prior to and following scaling and root planing treatment.

significantly higher levels of IL-1β, IL-8 and MMP-8 in gingival crevicular fluid. It should be pointed out, however, that in control subjects only correlations between IL-1β and approximal plaque index and gingival index and between MMP-8 and pocket depth were found. In chronic periodontitis patients, there were correlations between IL-1β and pocket depth and approximal plaque index, as well as a negative correlation between IL-8 and gingival index. Our observations are in line with previous studies. It has been found that patients with chronic periodontitis have higher IL-1β gingival crevicular fluid levels compared with control subjects (19,20,22,26,31–36), and the amounts of this cytokine do not correlate with clinical parameters (19,22,31,33). Only Goutoudi *et al.* (32) stated that the total amounts of IL-1β were positively correlated with gingival index, while Hou *et al.* (37) observed a positive correlation between the total amount of IL-1β, but not IL-1β concentration, and gingival index as well as pocket depth. It was also found that the level of IL-8 in gingival crevicular fluid correlated with the severity of periodontitis (13,19,26,27). The higher levels of MMP-8 in gingival crevicular fluid from periodontitis patients, in comparison to healthy subjects, were observed by others (23,25,38–44); however, the correlations between clinical parameters and amounts of MMP-8 were rarely found in subjects with chronic periodontitis (23,40, 41,45). It should be stressed, however, that assay techniques and, in particular, antibodies used for the detection of MMP-8 in oral fluids affect the measurement outcome (46–51). It this study, we have used a commercially available ELISA, with employment of monoclonal antibodies against MMP-8, designated to measure both active and latent forms of MMP-8 in body fluids. We did not test whether similar results would have been obtained using other MMP-8 antibodies.

In our study, we also found that scaling and root planing significantly reduced the amounts of IL-1β, IL-8 and MMP-8 gingival crevicular fluid. It should be stressed, however, that 4 wk

Table 3. Correlations between amounts of IL-1β, IL-8 and MMP-8 in gingival crevicular fluid and clinical parameters in healthy control subjects and in chronic periodontitis patients at baseline and 1 and 4 wk after scaling and root planing treatment

		Chronic periodontitis patients (n = 30)		
Evaluated parameter	Control group (n = 21)	Baseline	1 wk after scaling and root planing	4 wk after scaling and root planing
IL-1β				
Pocket depth	0.42*	0.44†	−0.11*	0.08*
Approximal plaque index	0.62‡	0.48†	0.25*	−0.14*
Gingival index	0.58‡	0.26*	0.13*	−0.13*
Clinical attachment loss	0.12*	0.07*	−0.06*	−0.06*
IL-8				
Pocket depth	0.12*	0.15*	0.30*	0.07*
Approximal plaque index	0.10*	−0.19*	0.01*	0.09*
Gingival index	0.16*	−0.36†	−0.20*	−0.10*
Clinical attachment loss	0.18*	0.21*	−0.02*	−0.03*
MMP-8				
Pocket depth	0.53†	0.29*	0.30*	0.20*
Approximal plaque index	0.28*	−0.03*	0.08*	−0.23*
Gingival index	0.22*	0.10*	−0.09*	−0.15*
Clinical attachment loss	0.23*	0.23*	0.13*	−0.15*

*$p > 0.05$; †$p < 0.05$; ‡$p < 0.01$.

after nonsurgical therapy the amounts of examined humoral factors were still significantly higher ($p < 0.001$) than in control groups. We also found that the levels of IL-1β, IL-8 and MMP-8 following scaling and root planing therapy did not correlate with clinical parameters. The effect of nonsurgical therapy on IL-1β levels has been studied previously, and it has been shown that scaling and root planing resulted in reduction of gingival crevicular fluid IL-1β (19,22, 26,33,34,52,53). Gamonal *et al*. (19) stated that there was a weak correlation between clinical parameters and the level of IL-1β 2 mo after scaling and root planing, and Toker *et al*. (33) did not detect any significant correlations between the level of this cytokine and clinical parameters 6 wk following therapy. Different results have been reported regarding the effect of periodontal treatment on the IL-8 level in gingival crevicular fluid. Gamonal *et al*. (19) noticed a decrease in the level of IL-8 in gingival crevicular fluid 2 mo after scaling and root planing; however, only a weak relationship between clinical parameters and the level of IL-8 was observed. Jin *et al*. (13) observed a reduction in IL-8 level 4 wk following therapy. On the contrary, Chung *et al*. (28) observed no changes in the level of IL-8 in gingival crevicular fluid 2 wk after therapy. Previously, a decrease in the amount of MMP-8 in gingival crevicular fluid was observed following periodontal therapy (23,25,38,41,45,54). Figueredo *et al*. (38) indicated that no correlation exists between the level of MMP-8 and clinical parameters.

Numerous studies have reported beneficial outcomes of scaling and root planing treatment in both clinical and microbial parameters (13,29,33,38,55). Our observations indicated that 4 wk following nonsurgical therapy there was significant improvement in clinical parameters. However, the gingival crevicular fluid levels of markers of inflammation, i.e. IL-1β, IL-8 and MMP-8, were still elevated. Thus, these findings might suggest that short-term nonsurgical therapy resulted in an improvement in clinical signs of inflammation but that the inflammatory and destruction processes within periodontal tissues are not entirely eliminated.

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