GCF Elastase and Interleukin-8, and the Treatment Outcome of Scaling and Root Planing

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This six-month longitudinal study quantified gingival crevice fluid elastase and interleukin-8 and analysed their relationship to clinical outcome of mechanical therapy. Eighteen patients with chronic periodontitis, presenting 209 sites, were included in the study (123 periodontitis, 45 gingivitis and 41 healthy sites). After oral hygiene instructions and supragingival scaling, periodontitis sites were root planed. Gingival crevice fluid was collected twice before treatment within 10 days, and two weeks, two and six months thereafter. Elastase was determined using a fluorogenic substrate, and interleukin-8 was quantified using enzyme-linked immunosorbent assay. Clinical parameters included pocket probing depths, bleeding on probing and plaque scores. Therapy resulted in highly significant reductions of elastase and interleukin-8 in all three categories of sites. Eleven periodontitis sites, distributed in six subjects, remained at six months with pockets greater than 5mm. At baseline and at two months these sites showed significantly higher levels of pocket probing depths and bleeding on probing ($p < 0.01$), whereas no difference was noted for elastase and interleukin-8. On the subject level, however, no difference was noted at baseline between these six patients and the other ones. Clinical improvement after periodontal therapy was associated with a highly significant reduction in elastase and interleukin-8 levels. Whereas probing depth and bleeding on probing at baseline reflected the clinical outcome, the biochemical parameters did not.

Key words: scaling and root planing, predictive value, elastase, interleukin-8, gingival crevice fluid

INTRODUCTION

Clinical periodontal diagnosis consists essentially in the assessment of periodontal tissue damage (loss of attachment and alveolar bone) occurred in the past. Ideally, diagnostic procedures should also yield information regarding the activity of the lesion, and the probable outcome of a standard therapy such as scaling and root planing. Information regarding the inflammatory state of periodontal tissues may have a prognostic value for the long term healing response. Although potential markers for disease activity have been sought by several researchers, mainly in the gingival crevice fluid (GCF) (Armitage, 2004), at present, the diagnostic usefulness of such markers remains controversial. Questions remain specifically with regards to the response patterns of individual sites in relation to patient specific findings. The expression of markers may show important variation from site to site, and may depend on the local clinical status of the sites sampled (Que et al, 2004).

Periodontitis is an inflammatory process leading to the destruction of the dento-alveolar ligament, connective tissue and alveolar bone. Neutrophilic granulocytes migrate in high numbers towards the inflamed periodontal tissues (Christersson et al, 1987). In their interaction with microorganisms, present in the periodontal pocket, these cells are known to release high levels of lysosomal enzymes (Cimasoni and Kowashi, 1980; Havemann and Gramse, 1984; Zafiropoulos et al, 1991), such as elastase (Cergneux et al, 1982; Giannopoulou et al, 1992; Benedek-Spat et al, 1991), from their cytoplasmic azurophilic granules. This is believed to contribute significantly to the destruction of the periodontal tissues. In terms of quantity, elastase from neutrophilic granulocytes (EC 3.4.21.37; 28 kD) is by far the most important of all proteases detected in GCF (Cox and Eley, 1989). GCF elastase levels are significantly elevated in sites demonstrating periodontal inflammation (Kowashi et al, 1979; Murray and Patters, 1980; Zafiropoulos et al, 1991; Gonzales et al, 2001; Jin et al, 2002), and are known to decrease after conventional periodontal therapy (scaling and root planing) (Figueredo et al, 2004). Elastase is a marker of intracrevicular granulocyte activity (Lamster 1997), an indicator of periodontal disease progression (Palcanis et al, 1992; Armitage et al, 1994; Meyer et al, 1997), and low levels of elastase seem to reflect clinical stability during maintenance (Jin et al, 1995). However, variation in elastase expression from site to site has not been investigated and thus its site-specific diagnostic potential to predict treatment outcomes is not known.

Cytokines play an important role as mediators in the inflammatory process (Genco, 1992; Birkedal-Hansen, 1993; Fujihashi et al, 1993). IL-1β and Interleukin-8 (IL-8) have been shown to function in concert with other members of the cytokine network in order to regulate the cellular inflammatory response in the periodontium. IL-8 is a chemotactic agent produced by many cells, such as lymphocytes, epithelial cells, fibroblasts, macrophages and neutrophils. Neutrophils have the ability to secrete IL-8 in response to bacterial lipopolysaccharide (LPS) (Yoshimura et al, 1997). Because of its ability to attract and activate neutrophils into sites of tissue damage (Baggiolini et al, 1989; Bickel, 1993), IL-8 is of interest for the understanding of mechanisms leading to neutrophil-related tissue-breakdown. In addition, IL-8 has been demonstrated to amplify the inflammatory response. When primed by IL-1β, IL-8 activates neutrophils for elastase release (Brandolini et al, 1996; Brandolini et al, 1997).

Conflicting results concerning the association of IL-8 in GCF and the severity of periodontitis have been reported. A possible inverse relationship between IL-8 activity and PMN recruitment was suggested in short-term studies (Chung et al, 1997; Jin et al, 2002). The total amount of GCF IL-8 is significantly higher in diseased sites of patients with adult periodontitis compared to healthy sites of the control group (Mathur et al, 1996). Moreover, GCF IL-8 levels decrease after periodontal therapy in adult periodontitis patients (Tsai et al, 1995).

The purpose of this study was to quantify simultaneously elastase and IL-8 in the GCF on a long-term basis to determine the relationship between total amounts of elastase and IL-8 before and after periodontal treatment, and to explore their predictive value for clinical success of scaling and root planing, on the level of the patient, as well as the site.

STUDY DESIGN AND RESULTS

Patient Selection

Eighteen patients (16 Caucasians and two Asians), aged 35 to 68 years, were recruited. All of them consulted or were referred to the Division of Periodontology and Oral Physiopathology at the School of Dental Medicine of the University of Geneva for treatment of moderate to advanced periodontitis. They had never been treated for periodontal disease previously. Inclusion criteria for participation were: 1) Probing Pocket Depths (PPD) ≥ 5 mm, 2) Clinical Attachment Loss (CAL) ≥ 3 mm, 3) radiographic evidence of general alveolar bone loss, and 4) presence of at least six to eight sites with periodontal lesions, two to three sites with gingivitis and two to three sites with clinically healthy gingiva.

Pregnant women and subjects having received antibiotics, immunosuppressive drugs or periodontal treatment within the past six months were not included. All patients were systemically healthy, and six of them were smokers. The ethical commission of the School of Dental Medicine, University of

Geneva, approved the study protocol. Written informed consent was obtained from the patients prior to the beginning of the study.

Site Selection

Ten to 14 non-adjacent sites were selected in each subject. These sites were divided into three categories according to the degree of gingival inflammation and clinical parameters:

- Periodontitis sites (P):
  PPD ≥ 5 mm, CAL ≥ 3 mm, and Bleeding On Probing (BOP = 1)
- Gingivitis sites (G):
  5 mm > PPD > 3 mm, CAL ≤ 2 mm and BOP = 1
- Healthy sites (H):
  PPD ≤ 3 mm, CAL ≤ 1 mm and BOP = 0

Clinical Parameters

Six sites were evaluated around each tooth (mesial-buccal, mid buccal, distal-buccal, distal-palatal/lingual, mid-palatal/lingual, mesial-palatal/lingual). Dental plaque scores (PI) were recorded dichotomously and expressed as a percentage of total surfaces (PI%). Probing Pocket Depth (PPD) was measured from the gingival margin to the bottom of the pocket, using a standard probe. Bleeding On Probing (BOP) was recorded dichotomously and expressed as a percentage of total probing points (BOP%). PI was recorded at PTE1 as well as at D14, M2 and M6; PPD, and BOP were recorded at PTE1 as well at M2 and M6 (Table 1). The same examiner (I.M.D) recorded all parameters throughout the study. The smoking status was recorded as smoker or non-smoker.

Clinical Procedures

The time sequence of the study is shown in Table 1. At the first baseline examination (PTE1), the PI was recorded. Next, supragingival plaque was carefully removed. Then GCF samples were collected, and finally the other clinical parameters were recorded. At a second visit, 10 days later (PTE2), GCF sampling was repeated. All patients were given a case presentation and detailed instructions for proper supragingival plaque control measures. An oral hygiene level with more than 80% surfaces plaque free was required to start subgingival instrumentation. All teeth were thoroughly treated by scaling, and in addition P and G teeth by root planing. The mechanical instrumentation was continued until the operator felt that the surface was hard and smooth. After scaling, pockets were rinsed with a 0.2% chlorhexidine solution. These treatments required two to four sessions within one month, the last session representing D0.

Table 1 Time sequence of the study

Time points	Procedures
PTE1	PI, GCF collection, PPD, BOP, SUP
PTE2	GCF collection
Treatment	Motivation for proper oral hygiene Oral hygiene instructions → PI % ≤ 20 Scaling Root Planing (SRP), polishing
Day 0	Completion of therapy (no sampling)
Day 14	PI, GCF collection
Month 2	PI, GCF collection, PPD, BOP, SUP
Month 6	PI, GCF collection, PPD, BOP, SUP

PTE: Pre-Treatment Examination
PI: Plaque score
GCF: Gengival Crevice Fluid
PPD : Pocket Probing Depth
BOP : Bleeding On Probing
SUP: Suppuration

Patients were recalled after 14 days (D14), as well as after two and six months (M2, M6). At all these appointments study parameters were recorded. In addition, oral hygiene levels were checked and hygiene practices reinforced. Supragingival calculus removal and polishing were provided when necessary. However, no instrumentation was performed 1mm below the gingival margin.

Determination of Treatment Success

On the level of the site, the clinical outcome of the treatment was rated from 1 to 3 as follows (Site Success Index, SSI):

1: sites with PPD < 5 mm and BOP = 0
2: sites with PPD < 5 mm and BOP = 1
3: sites with PPD ≥ 5 mm.

On the level of the patient, the clinical outcome was rated on a scale of 1 to 3 based of P sites evaluation six months after treatment (Patient Success Index, PSI)

1: PPD < 5 mm and BOP = 0
2: PPD < 5mm and BOP = 1
3: persistence of at least one periodontal pocket ≥ 5mm.

GCF Sampling and Analysis

After careful removal of supragingival plaque the study sites were isolated from saliva with cotton rolls and air-dried. After two minutes, the GCF was collected by means of Durapore filter membranes (0.22 µm pore size, Millipore, Bedford, MA, USA). The strips were placed at the entrance of the sulcus or pocket and left in place for 15 seconds. The strips were placed into a microcentrifuge tube and immediately frozen at – 20°C until analysed. In case of visible contamination with blood, the strips were discarded. The day of analysis, 100 µl of phosphate buffered saline (PBS, pH 7.2) was added to each sample. The tubes were gently shaken for one minute and centrifuged at 2000 g for five minutes, with the strips kept at the collar of the tube in order to completely elute GCF components. After strip removal, the supernatants were divided into aliquots for the measurement of each biochemical compound.

For the determination of elastase activity, the fluorogenic substrate Meo-Suc-Ala-Ala-Pro-Val/7-amino-4-methylcoumarin (MW 627.69) (Bachem, Bubendorf, Switzerland) was used. The enzyme assay was conducted at 25°C with 0.1 mmol/l substrate in 50 mmol/l phosphate buffer, pH 7.4, containing 0.05% Triton X-100 and 0.5mol/l NaCl. 100 µl of the substrate solution was pre-incubated for five minutes, and 20 µl of the GCF sample was added. After six hours incubation time, the reaction was stopped with 5 µl of a selective enzyme inhibitor, phenylmethylsulfonylfluoride (PMSF), at a final concentration of 1.9 mM (Sigma, St. Louis, MO9). Fluorescence levels of the 7-amino-4-methylcoumarin (H-AMC) were measured by a spectrofluorimeter. A standard curve was established using H-AMC (Bachem, Bubendorf, Switzerland) to obtain a linear curve in the concentration range of 0.35–140µM. GCF-elastase activity was expressed as nmol of H-AMC cleaved from the substrate after six hours incubation. The amount of the cytokine IL-8 in the GCF was determined by enzyme-linked immunosorbent assay (ELISA) (Ruwag Diagnostics, Zurich, Switzerland). A standard curve with a range of 1-240 pg/ml was used and the total amount of IL-8 per sample determined. The assays were carried out in accordance to the manufacturer's instructions.

Data Analysis

The quantities of elastase and IL-8 were expressed as total amounts per 15s-samples. The values obtained at time point PTE1 were compared to those obtained at PTE2 by calculating, for each site, the difference between the two measurements, and then the mean of the differences. Standard deviations of the single measurements (Si) and coefficients of variation (CV) were determined. For the analysis on the level of the patient, a mean value was calculated for each person of all P, G and H sites. The Mann-Whitney U and the Wilcoxon matched pairs signed rank tests were used to determine longitudinal changes. The Friedman test was used to establish differences between P, G and H sites. The level of statistical significance was set at $p = 0.02$.

RESULTS

Six to eight periodontitis sites, two to three gingivitis sites and two to three healthy sites were available for the study in each subject. In total 209 sites (123 P, 45 G, 41 H) were investigated in 18 subjects. Table 2 shows the mean values of total amount of elastase and IL-8 in samples of P, G and H sites taken at time points PTE1 and PTE2, the means of the differences (PTE1-PTE2), the coefficient of variation of the first measurement (CV PTE1) and its significance (p), and the standard deviation of a single measurement (Si). The statistical unit in this table is the site. A general trend for lower values at PTE2 was noted.

Effect of SRP

Table 3 shows the clinical parameters of P, G and H sites before and after SRP. SRP reduced PI%, PPD and BOP% for all three categories of sites as expected. There was a continuous reduction of plaque levels from M2 to M6 for P and G sites. In P sites, PPD decreased by 2.5 mm from PTE1 to M2 ($p < 0.001$), and by 3 mm from PTE1 to M6 ($p < 0.001$). A significant PPD reduction was also observed in the G group.

Table 2 Mean values of elastase (nmol H-AMC/sample) and IL-8 (pg/sample) at P, G and H sites, assessed twice before treatment (PTE1 and PTE2), means of the differences PTE1-PTE2 (Diff.), coefficients of variation of the first measurements (CV PTE1), significance (P), and standard deviation of a single measurement (Si). NS: Not significant

		PTE1	PTE2	Diff.	CV PTE1	P	S_i
Elastase	P	4.67	3.69	0.98	0.98	0.004	2.87
	G	3.26	1.84	1.42	1.44	0.02	3.23
	H	2.50	1.38	1.12	1.36	0.02	2.57
IL-8	P	47.87	40.20	7.67	0.95	0.001	23.50
	G	46.16	32.48	13.68	1.26	NS	33.74
	H	35.19	31.58	3.61	1.16	NS	20.34

Table 3 Mean values of clinical parameters assessed at time points PTE1, M2 and M6, means of the differences between PTE1 and M2 (Diff.1) and between PTE1 and M6 (Diff.2) and their respective significances (P1, P2).
NS: Not significant

		PTE1	M2	Diff. 1	P1	M6	Diff. 2	P2
PI%	P	44 (± 5)	31 (± 46)	13	NS	17 (± 38)	27	< 0.001
	G	36 (± 48)	22 (± 42)	14	NS	9 (± 29)	27	0.001
	H	21 (± 41)	10 (± 3)	11	NS	10 (± 3)	11	NS
PPD	P	5.9 (± 1.3)	3.4 (± 1.6)	2.5	< 0.001	2.9 (± 1.0)	3.0	< 0.001
	G	3.2 (± 1.0)	2.6 (± 0.9)	0.6	< 0.001	2.3 (± 0.5)	0.9	< 0.001
	H	2.6 (± 0.7)	2.4 (± 0.6)	0.2	NS	2.3 (± 0.5)	0.3	0.003
BOP%	P	57 (± 50)	17 (± 37)	40	< 0.0001	12 (± 33)	45	< 0.0001
	G	38 (± 5)	9 (± 29)	29	0.002	2 (± 15)	36	< 0.001
	H	0 (± 0)	0 (± 0)	0	NS	0 (± 0)	0	NS

Table 4 shows mean elastase and IL-8 levels at PTE1, M2 and M6, using the site as the statistical unit. Elastase decreased significantly in P and G sites from PTE1 to M2 and M6. IL-8 decreased significantly within the two first months in P sites and was significantly lower in all three categories of sites at M6.

Mean elastase and IL-8 values were generated per patient for all P, G and H sites. Table 5 shows the mean values of patient specific parameters. Significant differences were observed in elastase levels between P and H sites at PTE1 ($p<0.01$). At M6 this difference was leveled out.

Fig 1 shows the relationship between elastase and IL-8 in P sites at PTE1 (black dots) and M6 (red dots). As can be seen, IL-8 and elastase levels were both highly variable before therapy. A linear relationship nevertheless existed between the two ($p < 0.001$, $R^2 = 0.233$). At month 6, a majority of sites demonstrated markedly reduced levels of

Table 4 Mean values of elastase (nmol H-AMC/sample) and IL-8 (pg/sample) at time points PTE1, M2 and M6, means of the differences between PTE1 and M2 (Diff.1), PTE1 and M6 (Diff.2) and their respective significances (P1, P2). NS: Not significant

		PTE1	M2	Diff. 1	P1	M6	Diff. 2	P2
Elasta-se	P	4.67 (± 4.37)	2.39 (± 3.6)	2.28	< 0.001	1.13 (± 2.23)	3.54	< 0.001
	G	3.26 (± 4.8)	1.5 (± 2.9)	1.76	0.004	1.06 (± 1.75)	2.2	0.001
	H	2.50 (± 3.57)	1.04 (± 1.54)	1.46	NS	0.68 (± 0.97)	1.98	< 0.001
IL-8	P	47.87 (± 46.88)	35.44 (± 35.4)	12.43	<0.001	25.76 (± 31.81)	22.11	< 0.001
	G	46.16 (± 56.05)	29.07 (± 29.33)	17.09	NS	20.96 (± 27.95)	25.2	0.001
	H	35.19 (± 41.3)	25.61 (± 24.5)	9.58	NS	14.5 (± 19.63)	20.69	< 0.001

Table 5 Patient means of P, G and H sites at PTE1 and M6 for elastase (nmol H-AMC/sample) and IL-8 (pg/sample)

	PTE1			M6		
	P	G	H	P	G	H
Elastase	4.62 (± 3.56)[a]	3.14 (± 4.07)	2.34 (± 3.15)	1.12 (± 1.4)	1.01 (± 1.4)	0.64 (± 0.7)
IL-8	48.3 (± 40.6)	44.77 (± 51.3)	25.87 (± 38)	25.58 (± 23.6)a	21 (± 25)	15.3 (± 19)

[a] *Difference P/H: p≤ 0.01*

both parameters. A small number of sites continued to display high levels of IL-8 or elastase, with a poor correlation between the two parameters ($p < 0.05$, $R^2 = 0.049$). The clinical response to therapy was specifically analysed for these sites. Except one, these sites showed a favorable treatment response. Furthermore half of these sites had low levels of the respective elevated parameter at baseline. Hence, high levels of either IL-8 or elastase at M6 had no diagnostic value.

Treatment Success

We assessed the power of parameters assessed at baseline or at M2 to predict the clinical success of SRP at M6, both on the level of the site and the patient. Out of 123 P sites, in 18 subjects, a total of 11 sites, in six subjects, remained with a PPD ≥ 5 mm (SSI 3) at M6. The mean PPD of these sites amounted to 5.55 ± 0.9 mm, in comparison to 2.93 ± 0.5mm of the other sites. 36% of these SSI 3 sites were bleeding on probing, compared to 12% of the SSI 1+2 sites. Already at baseline, they showed a higher mean PPD (7.64 mm/ 5.84 mm) and a higher mean BOP% (64/56) ($p < 0.01$) value than sites with a SSI 1 + 2. However, for elastase and IL-8, no difference was noted at baseline between these two categories of sites.

The 11 sites represented 50% of all sites with a PPD ≥ 5 mm at M2. The outcome of success, determined on the site level by the SSI at M2 and M6 are shown in Table 6. As can be seen, the specificity of M2 to predict success at M6 was 1 (none of the sites rated successful at M2 turned out to be no success at M6), and the sensitivity was 0.902.

On the patient level, the six patients with a PSI 3 showed generally higher mean values for all parameters than the subjects with a PSI score 1 or 2. Two weeks after treatment, a significant difference

Table 6 Predictive value of 'success' at M2 to predict 'success' at M6 («No success»: SSI=3). Sensitivity of M2 to predict M6: 0.902, specificity of M2 to predict M6: 1

Month 2	Month 6			
	No success	Success	Total	%
No success	11	11	22	17.9
IL-8	0	101	101	82.1
Total	11	112	123	
%	8,9	91.1		100

was noted between PSI 3 and PSI 1+2 for PI% ($p < 0.05$). At M2, PPD was the only parameter with a significant difference between these groups ($p < 0.01$). Patients with sites with a persisting PPD ≥ 5 mm (PSI = 3) at M6, showed PPD ≥ 5 already at M2. Apart from that, no parameter could be identified that would discriminate successful from unsuccessful therapy.

DISCUSSION

The present longitudinal study quantified GCF elastase and IL-8 before and after scaling and root planing, and examined their potential predictive value for the outcome of standard therapy. The first issue treated was variability of repeated sampling with 10 days of interval before treatment (Table 2). A significant trend for lower values was noted for the second assessment. This phenomenon has been observed previously for repeatedly measured parameters. The fact being enrolled in a study results in the improvement of patient oral hygiene habits ('Hawthorne effect'), leading to a decrease in local inflammation (Addy and Moran, 1997; Wickstrom and Benedix, 2000). In the present study, an improvement in oral hygiene was indeed observed, however not quantitatively analysed.

As expected, SRP reduced all clinical parameters significantly. PPD decreased 2.5 mm in the first two months in P sites and an additional 0.5 mm between M2 and M6. This further improvement is in agreement with previously published data (Badersten et al, 1981; Claffey et al, 1988) and reinforces the notion that clinical results of SRP should not be evaluated too early to see the full effect of therapy. Average pocket depth of P sites was reduced from 5.9 ± 1.3 mm to 2.9 ± 1.0 mm within six months. Therefore supplementary surgical therapy was not considered to be necessary in most cases after the completion of this study.

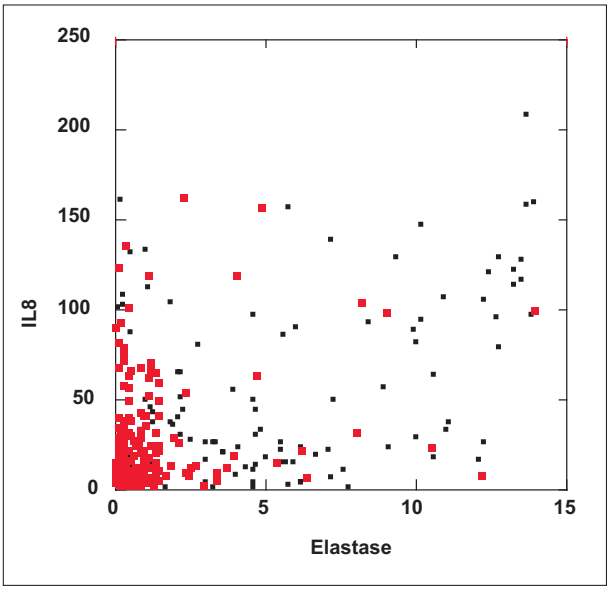

Fig 1 Scatter plot for elastase (nmol) and IL-8 (pg) levels of P sites at PTE1 (black dots) and M6 (red dots)

The levels of elastase and IL-8 were expressed as total amounts per 15s samples. As has been discussed in the literature, concentrations are more prone to error in samples of small volume whereas total amounts are more appropriate (Lamster, 1997). Correspondingly, highly significant reductions of both elastase and IL-8 levels in GCF were observed during the study (Tables 4, 5 and Figure 1). Similar results have been presented from a study extending over one month (Jin et al, 2002). Our results are also in agreement with a study monitoring IL-8 and β-glucuronidase following SRP, where a majority of subjects showed a reduction of both parameters (Chung et al, 1997). In this two-week study, a subgroup of 20% subjects

demonstrated an inverse relationship due to an increase, rather than a decrease, of IL-8 levels after treatment. In the present study, elastase and IL-8 levels of P sites decreased particularly within the first two months after treatment and showed a further decrease in the following four months. Despite of these highly significant reductions, both elastase and IL-8 did not reach the low levels observed in H sites at M6. They did, however, reach lower mean levels than the respective values of the H sites at baseline (Table 4).

Clinical improvement after periodontal therapy was associated with highly significant reduction in elastase and IL-8-levels; nevertheless, PPD and BOP were the only parameters at baseline reflecting the clinical outcome. Furthermore, based on the clinical parameters assessed at M2, it was possible to identify all 11 sites of the six subjects that would not be a success at M6. On the other hand, the mean values of all parameters further improved from M2 to M6, but half of the sites indicating no success at M2 still remained 'no success' at M6.

In conclusion, the present study could not substantiate a significant value of GCF elastase and IL-8 to predict clinical success of scaling and root planning; but an association could be observed between clinical improvement and biochemical levels reduction.

REFFERENCES

Addy M, Moran JM. Evaluation of oral hygiene products: science is true; don't be misled by the facts. Periodontol 2000 1997;15:40–51.

Armitage GC. Analysis of gingival crevice fluid and risk of progression of periodontitis. Periodontol 2000 2004; 34:109–119.

Armitage GC, Jeffcoat MK, Chadwick DE et al. Longitudinal evaluation of elastase as a marker for the progression of periodontitis. J Periodontol 1994; 65: 120–128.

Badersten A, Nilveus R, Egelberg J. Effect of nonsurgical periodontal therapy. I. Moderately advanced periodontitis. J Clin Periodontol 1981;8:57–72.

Baggiolini M, Walz A, Kunkel SL. Neutrophil-activating peptide-1/interleukin 8, a novel cytokine that activates neutrophils. J Clin Invest 1989;84:1045–1049.

Benedek-Spat E, Di Felice R, Andersen E, Cimasoni G. In vitro release of elastase from human blood and gingival crevicular neutrophils. Arch Oral Biol 1991;36:507–510.

Bickel M. The role of interleukin-8 in inflammation and mechanisms of regulation. J Periodontol 1993;64:456–460.

Birkedal-Hansen H. Role of cytokines and inflammatory mediators in tissue destruction. J Periodontol 1993;28:500–510.

Brandolini L, Bertini R, Bizzarri C et al. IL-1 beta primes IL-8-activated human neutrophils for elastase release, phospholipase D activity, and calcium flux. J Leukoc Biol 1996;59: 427–434.

Brandolini L, Sergi R, Caselli G et al. Interleukin-1 beta primes interleukin-8-stimulated chemotaxis and elastase release in human neutrophils via its type I receptor. Eur Cytokine Netw 1997;8:173–178.

Cergneux M, Andersen E, Cimasoni G. In vitro breakdown of gingival tissue by elastase from human polymorphonuclear leucocytes. An electron microscopic study. J Periodontal Res 1982;17:169–182.

Christersson LA, Albini B, Zambon JJ et al. Tissue localization of Actinobacillus actinomycetemcomitans in human periodontitis. I. Light, immunofluorescence and electron microscopic studies. J Periodontol 1987; 58: 529–539.

Chung RM, Grbic JT, Lamster IB. Interleukin-8 and beta-glucuronidase in gingival crevicular fluid. J Clin Periodontol 1997;24:146–152.

Cimasoni G, Kowashi Y. Proteinases of the gingival crevice and their inhibitors. In: Lehner T CG (ed). The Borderland between Caries and Periodontal Diseases. London: Academic Press 1980:31–49 vol II.

Claffey N, Loos B, Gantes B et al. The relative effects of therapy and periodontal disease on loss of probing attachment after root debridement. J Clin Periodontol 1988; 15: 163–169.

Cox SW, Eley BM: Detection of cathepsin B- and L-, elastase-, tryptase-, trypsin-, and dipeptidyl peptidase IV-like activities in crevicular fluid from gingivitis and periodontitis patients with peptidyl derivatives of 7-amino-4-trifluoromethyl coumarin. J Periodont Res 1989;24:353–361.

Figueredo CM, Areas A, Miranda LA et al. The short-term effectiveness of non-surgical treatment in reducing protease activity in gingival crevicular fluid from chronic periodontitis patients. J Clin Periodontol 2004;31:615–619.

Fujihashi K, Kono Y, Beagley KW et al. Cytokines and periodontal disease: immunopathological role of interleukins for B cell responses in chronic inflamed gingival tissues. J Periodontol 1993;64:400–406.

Genco RJ. Host responses in periodontal diseases: current concepts. J Periodontol 1992;63:338–355.

Giannopoulou C, Andersen E, Demeurisse C, Cimasoni G. Neutrophil elastase and its inhibitors in human gingival crevicular fluid during experimental gingivitis. J Dent Res 1992;71:359–363.

Gonzales JR, Herrmann JM, Boedeker RH et al. Concentration of interleukin-1beta and neutrophil elastase activity in gingival crevicular fluid during experimental gingivitis. J Clin Periodontol 2001;28:544–549.

Havemann K, Gramse M. Physiology and pathophysiology of neutral proteinases of human granulocytes. Adv Exp Med Biol 1984;167:1–20.

Jin LJ, Leung WK, Corbet EF, Soder B. Relationship of changes in interleukin-8 levels and granulocyte elastase activity in gingival crevicular fluid to subgingival periodontopathogens following non-surgical periodontal therapy in subjects with chronic periodontitis. J Clin Periodontol 2002;29:604–614.

Jin LJ, Soder PO, Asman B, Soder B, Puriene A, Bergstrom K. Variations in crevicular fluid elastase levels in periodontitis patients on long-term maintenance. Eur J Oral Science 1995;103:84–89.

Kowashi Y, Jaccard F, Cimasoni G. Increase of free collagenase and neutral protease activities in the gingival crevice during experimental gingivitis in man. Arch Oral Biol 1979;24:645–650.

Lamster IB. Evaluation of components of gingival crevicular fluid as diagnostic tests. Ann Periodontol 1997;2: 123–137.

Mathur A, Michalowicz B, Castillo M, Aeppli D. Interleukin-1 alpha, interleukin-8 and interferon-alpha levels in gingival crevicular fluid. J Periodont Res 1996;31:489–495.

Meyer J, Guessous F, Huynh C et al. Active and alpha-1 proteinase inhibitor complexed leukocyte elastase levels in crevicular fluid from patients with periodontal diseases. J Periodontol 1997;68:256–261.

Murray PA, Patters MR. Gingival crevice neutrophil function in periodontal lesions. J Periodont Res 1980;15:463–469.

Palcanis KG, Larjava IK, Wells BR et al. Elastase as an indicator of periodontal disease progression. J Periodontol 1992;63:237–242.

Que ML, Andersen E, Mombelli A. Myeloid-related protein (MRP)8/14 (calprotectin) and its subunits MRP8 and MRP14 in plaque-induced early gingival inflammation. J Clin Periodontol 2004;31:978–984.

Tsai CC, Ho YP, Chen CC. Levels of interleukin-1 beta and interleukin-8 in gingival crevicular fluids in adult periodontitis. J Periodontol 1995;66:852–859.

Wickstrom G, Bendix T: The 'Hawthorne effect' - what did the original Hawthorne studies actually show? Scand J Work Environ Health 2000; 26: 363–367.

Yoshimura A, Hara Y, Kaneko T, Kato I. Secretion of IL-1 beta, TNF-alpha, IL-8 and IL-1ra by human polymorphonuclear leukocytes in response to lipopolysaccharides from periodontopathic bacteria. J Periodont Res 1997;32: 279–286.

Zafiropoulos GG, Flores-de-Jacoby L, Todt G et al. Gingival crevicular fluid elastase-inhibitor complex: correlation with clinical indices and subgingival flora. J Periodont Res 1991;26:24–32.

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