

Neutrophil elastase activity, levels of prostaglandin E₂, and matrix metalloproteinase-8 in refractory periodontitis sites in smokers and non-smokers

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The study was aimed to determine elastase activity, levels of prostaglandin E₂ (PGE₂), and matrix metalloproteinase-8 (MMP-8) in gingival crevicular fluid (GCF) in 20 smokers and 20 non-smokers, mean age 47.4 (±2.9 SD) years with refractory periodontal diseases. GCF was collected with intracrevicular washing from four sites in each subject. Clinical assessments, included gingival index, probing depth, clinical attachment level, bleeding on probing, bone height, and plaque accumulation. Smokers had a significantly higher percentage of the gingival margin covered by plaque (P%Im), higher number of sites with probing pocket depth ≥5 mm, higher mean values of probing pocket depth and probing attachment level ($P < 0.01$). Smokers had significantly higher mean levels of neutrophil elastase activity ($P < 0.01$) in the supernatants than non-smokers did. In sites with matching pocket depths, neutrophil elastase activity was significantly higher in smokers ($P < 0.001$) than in non-smokers. In sites with high levels of MMP-8 the PGE₂ levels were significantly ($P < 0.001$) higher compared to sites with low levels in smokers as well as in non-smokers. A significant correlation was found between probing pocket depth and levels of MMP-8 ($P < 0.001$) and in non-smokers between probing pocket depth and levels of PGE₂ ($P < 0.05$). □ *Gingival crevicular fluid; non-surgical treatment*

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Several factors have been described which increase the risk of periodontitis, smoking probably being one of the major risk factors (1–3). Smoking diminishes cell-mediated and humoral immune responses. MacFarlane et al. (4) reported that 90% of refractory periodontitis patients were smokers with defects in peripheral polymorphonuclear leukocyte function.

A recent study by Zambon et al. (5) indicates that smokers are more likely to be infected with periodontal pathogens than non-smokers. Bacterial plaque on the tooth surfaces stimulates an inflammatory response in the gingival tissues, which are infiltrated by neutrophil granulocytes during gingivitis and periodontitis. Among the products of neutrophil granulocytes, elastase (EA) is of particular significance in these conditions. EA (EC 3.4.21.37) is a neutral protease stored in the azurophil granule of the granulocytes. It can degrade collagen, laminin, fibronectin, proteoglycans, and elastin and it plays a significant role in connective tissue destruction associated with inflammatory processes. Studies by Armitage et al. (6) and Jin et al. (7, 8) indicate that sites with high levels of EA are at significantly greater risk of progressive bone loss compared to sites with low levels.

Prostaglandin E₂ (PGE₂), one of the most potent biochemical mediators of inflammation, plays an important role in the pathogenesis of periodontal diseases (9).

This particular molecule indicates innate biological activity, which can reflect cellular and biological events occurring during the inflammatory and tissue-destructive responses as a direct result of the host-bacterial interaction (9, 10).

Matrix metalloproteinases (MMPs) and matrix-degrading serin proteinases are proteinases related to degradation of connective tissue (11–13). However, MMPs also participate in tissue remodeling in diseases of the skin, periodontium, as well as rheumatoid arthritis, and cancer (14). At least four MMPs, MMP-1, MMP-8, MMP-9, and MMP-13 have been found in gingival crevicular fluid (GCF) (15–17). MMP-8 along with MMP-9 may play a key role in the destruction of periodontal tissues during periodontal disease (15, 16, 18).

In a longitudinal study of a randomly selected sample, Jin et al. (8) found that subjects with severe periodontitis who did not respond to periodontal treatment after 5 years of maintenance therapy showed significantly higher mean EA activity than healthy controls. In that study it was suggested that increased neutrophil EA levels in GCF might serve as a diagnostic marker in refractory periodontitis patients.

In view of these previous findings, the aims of the present study were to compare smokers with non-smokers as regards the relation between EA activity, MMP-8, and the levels of PGE₂ and disease severity.

Material and methods

Subjects

The participants in this study were 40 subjects of mean age 47.4 (± 2.9 SD) years. Of these, 20 were smokers (8 males, 12 females, mean age 47.7 (± 3.0 SD) years) and 20 were non-smokers (11 males, 9 females, mean age 47.2 (± 2.9 SD) years).

The material originates from a randomly selected sample of subjects with severe periodontal diseases (1, 19). The subjects in the present study were consecutively selected from the group of patients with refractory periodontal diseases. They had received intensive initial therapy, including scaling and root planing, with regular maintenance treatment at 6-month intervals over a 7-year period. The smokers in the present study had smoked a mean of 17 (± 9.0 SD) cigarettes/day for at least 20 years. All subjects fulfilled the following criteria: not suffering from any systemic disease, had not received systemic or local antibiotic therapy during the 6 months prior to the start of the study, and had not undergone non-surgical or surgical treatment during the last 3 months. Refractory periodontitis was defined as presence of 3 teeth with probing depths ≥ 5 mm and radiographic evidence of alveolar bone loss.

Periodontal examination—selection of sites and sampling

The supragingival plaque area was measured by P%I (20). The percentage of tooth surfaces in contact with the gingival margin covered by plaque (P%Im) was determined. Determination of gingival inflammation was carried out using a non-invasive modification of the gingival index (GI) (21). Probing pocket depth and loss of attachment were measured at mesio-buccal, mid-buccal, mesio-lingual, disto-buccal, disto-lingual, and mid-lingual sites. Bleeding on probing was registered and was expressed as percentage of bleeding sites per patient. The deepest site in each quadrant was selected for GCF sampling and examined for EA activity, levels of PGE₂, and levels of MMP-8.

The sites to be sampled were isolated with cotton rolls, gently air-dried and supragingival plaque carefully removed at the place for insertion of the needle into the pocket. GCF was collected using the intracrevicular washing technique (7). The ejection needle of the instrument was carefully inserted into the crevice to a level of approximately 1 mm below the gingival margin. The sulcus or pocket was then flushed by the constant delivery system (10 μ l per flushing) with an aliquot of 15 μ l of phosphate-buffered saline (PBS, pH 7.4) and simultaneously drained through the collection needle into Eppendorf tubes by constant suction (flow rate 25 ml/h). The gingival washings in the Eppendorf tubes were diluted to a final volume of 500 μ l by washing the drain tubes. Thus, each 500- μ l sample represented the GCF of the individual site. The samples were immediately centrifuged

(8000 \times g) for 15 min at 4°C and the supernatants and pellets frozen to -70°C before the analyses.

Radiographs were taken and, using a computerized measuring system, bone height was determined as a percentage of the root length (BH%) from radiographs magnified $\times 5$ (22, 23).

Assay of neutrophil EA activity

Neutrophil EA activity was measured with a low molecular weight chromogenic substrate specific for neutrophil EA, L-pyroglutamyl-L-prolyl-L-valine-*p*-nitroanilide (S-2484, AB Kabi Diagnostica, Stockholm, Sweden) (24) according to the method of Jin et al. (8). EA was assessed in both the supernatants (EA-S) and the pellets, which were suspended in 250 μ l of PBS (pH 7.4) containing 0.1% Tween[®] for 10 min. The neutrophil EA in the pellets (EA-P) was then measured. The substrate was dissolved in dimethylsulfoxide to 8 mM and the working solution was made up to 2 mM by dilution with PBS (pH 9.2). To 50 μ l of sample, 34 μ l of the working solution was added. The absorbency at 405 nm was measured immediately in an Emax[™] Precision Microplate Reader (Molecular Devices Corporation, Menlo Park, CA, USA) controlled by a personal computer. The microplate was sealed and incubated at 37°C. The mAbs recorded represent the hydrolysis for 2 h and are presented as arbitrary units per site (mAbs/site). The ratio of EA-S to EA-P (S/P ratio) was used as a relative measure of the EA released by the neutrophils present.

Assay of PGE₂

GCF supernatants (30 μ l) were assayed for PGE₂ levels by radioimmunoassay (¹²⁵I RIA Kit, E. I. Du Pont de Nemours & Co., Inc., NEN[®] Research Products, Boston, MA, USA) according to the manufacturer's instruction manual. The levels of PGE₂ were determined as total amount per site (pg/site).

Assay of matrix MMP-8

GCF supernatants (20 μ l) were assayed for MMP-8 (both free and complexed) with the ELISA kit (Amersham Life Science Ltd, Little Chalfont, Buckinghamshire, UK). The MMP-8 assay was based on a two-sided ELISA "sandwich" format, which enabled measurements of active MMP-8 and MMP-8 complexed bound to tissue inhibitors of metalloproteinases (TIMPs), TIMP-1 and TIMP-2 (11), as well as the amount of proMMP-8. According to the manufacturer, it does not cross-react with MMP-1, -2, -3, -7, -9, or -13. Standards and GCF supernatants were incubated in microtiter wells precoated with anti-MMP-8 antibody. The MMP-8 was detected by the peroxidase-labeled Fab' to MMP-8. The amount of MMP-8 was measured at 450 nm in an Emax[™] Precision Microplate Reader (Molecular Devices Corporation, Menlo Park, CA,

Table 1. Clinical findings in smokers and non-smokers showing percentage of the gingival margin covered by plaque (P%Im), percentage of sites with gingival index (GI) scores 0, 1, 2, and 3 (% GI 0–3) (mean ± SE)

Parameter	Smokers (n* = 20)	Non-smokers (n = 20)	P-value
P%I m	79.8 ± 4.1	65.2 ± 2.6	<0.01
% GI = 0	3.6 ± 1.8	14.3 ± 2.5	<0.001
% GI = 1	16.3 ± 4.1	25.2 ± 3.5	<0.05
% GI = 2	43.9 ± 3.3	46.0 ± 3.5	NS†
% GI = 3	36.8 ± 5.4	14.5 ± 3.1	<0.01

* No. of subjects, † not significant.

Table 2. Clinical findings in smokers and non-smokers showing pocket depth (PD mm), loss of attachment (AL mm), and number of sites ≥5 mm (no sites ≥5 mm) (mean ± SE)

Parameter	Smokers (n* = 20)	Non-smokers (n = 20)	P-value
PD mm	3.5 ± 0.2	2.8 ± 0.1	<0.001
AL mm	4.9 ± 0.2	4.1 ± 0.1	<0.01
No. sites ≥5 mm	23.8 ± 4.4	9.5 ± 1.4	<0.01

No. of subjects.

USA) controlled by a personal computer and determined as total amount per site (ng/site).

Ethical approval

Approval was given by the Ethics Committee at Huddinge Hospital, Huddinge Sweden. The subjects gave their informed consent to participation in the study.

Statistical analysis

Clinical data were calculated as means of the values from the four test sites in each patient and the total number of sites in each patient (excluding the 3rd molars). The samples from individual sites and the patients were used as units of observations for the statistical analyses. Comparisons between smokers and non-smokers were made using the Mann-Whitney test. The Pearson correlation test was used to determine the correlation between pocket depth and PGE₂ levels, pocket depth and EA activity as well as between PGE₂ and neutrophil EA activity.

Results

No significant difference was found between males and females regarding the clinical parameters measured. The mean values for the clinical parameters in smokers and non-smokers are given in Tables 1 and 2. Smokers had significantly higher percentages of the gingival margin covered by plaque ($P < 0.01$) compared to non-smokers. In

Table 3. Number of neutrophils (PMNs), neutrophil elastase in supernatants (EA-S), pellets (EA-P), and S/P-ratio as well as prostaglandin E₂ (pGE₂) and MMP-8 in sites from smokers and non-smokers (mean ± SE)

Parameter	Smokers (n* = 20)	Non-smokers (n = 20)	P-value
No. of PMNs	28.9 ± 7.13	31.4 ± 10.44	NS
EA-S(mAbs/site)	0.278 ± 0.062	0.050 ± 0.016	<0.01
EA-P(mAbs/site)	1.360 ± 0.170	1.452 ± 0.149	NS†
S/P-ratio	0.324 ± 0.102	0.043 ± 0.019	<0.001
PGE ₂ (pg/site)	10.8 ± 2.3	14.4 ± 3.3	NS
MMP-8 (ng/site)	45.9 ± 15.1	41.3 ± 9.2	NS

* No. of subjects, † not significant.

Table 4. Clinical findings in the 80 sites from smokers and 79 sites from non-smokers, showing plaque index (P%I), gingival index (GI), pocket depth (PD mm), loss of attachment (AL mm), and percentage of remaining bone (BH%) (mean ± SE)

Parameter	Smokers (n* = 20)	Non-smokers (n = 20)	P-value
P%I	27.8 ± 2.1	20.89 ± 1.7	<0.01
GI	2.1 ± 0.1	1.9 ± 0.1	NS†
PD mm	5.1 ± 0.2	4.1 ± 0.2	<0.05
AL mm	6 ± 0.2	5.3 ± 0.2	<0.01
BH %	77.6 ± 1.0	82.5 ± 1.0	<0.01

* No. of subjects, † not significant.

non-smokers, the percentage of sites with GI 0 was significantly higher than in smokers ($P < 0.001$), while in smokers the percentage of sites with GI 3 was significantly higher than in non-smokers ($P < 0.01$) (Table 1). Smokers had significantly deeper probing pocket depth ($P < 0.001$), more loss of attachment ($P < 0.01$), and more sites with probing depth ≥5 mm ($P < 0.01$) compared to non-smokers (Table 2).

The neutrophil EA activity in the supernatants and pellets and the S/P ratio as well as the PGE₂ and MMP-8 in smokers and non-smokers are given in Table 3. Smokers had significantly higher EA activity in the supernatants than non-smokers ($P < 0.01$). The S/P ratio was significantly higher in smokers compared to non-smokers ($P < 0.001$).

Table 4 gives the clinical findings in the test sites. Significant differences were found between smokers and non-smokers for P%I ($P < 0.01$), pocket depth ($P < 0.05$), attachment loss ($P < 0.01$), and bone height ($P < 0.01$).

The release of neutrophil EA in the supernatant, pellet, S/P ratio, levels of PGE₂ and MMP-8 in GCF from sites with matching pocket depths are given in Table 5. Neutrophil EA activity in the supernatant and the S/P ratio were significantly higher in smokers than in non-smokers ($P < 0.001$).

The levels of MMP-8, similar in smokers and non-smokers, were divided into two subgroups: high and low levels of MMP-8, respectively. For smokers with low levels of MMP-8, the mean was 2.1 (±0.2 SE); in the high levels

Table 5. Pocket depth (PD mm), loss of attachment (AL mm), neutrophil EA in supernatants (EA-S), pellets (EA-P), S/P-ratio, matrix metalloproteinase-8 (MMP-8) and prostaglandin E₂ (PGE₂) (mean ± SE) in GCF from 49 sites with matching pocket depths from smokers and non-smokers

Parameter	Smokers (n* = 20)	Non-smokers (n = 20)	P-value
Pocket depth mm (PD)	4.8 ± 0.2	4.8 ± 0.2	NS†
Loss of attachment mm (AL)	5.8 ± 0.3	5.7 ± 0.2	NS
EA-S (mAbs/site)	0.30 ± 0.08	0.06 ± 0.03	<0.001
EA-P (mAbs/site)	1.31 ± 0.13	1.50 ± 0.19	NS
S/P-ratio	0.39 ± 0.16	0.06 ± 0.03	<0.001
MMP-8 (ng/site)	36.50 ± 9.90	49.40 ± 11.40	NS
PGE ₂ (pg/site)	9.94 ± 2.10	12.52 ± 2.52	NS

* No. of subjects, † not significant.

Table 6. Pocket depths (PD), neutrophil elastase in supernatants (EA-S), pellets (EA-P), S/P-ratio and prostaglandin E₂ (PGE₂) (mean ± SE) in GCF from smokers with sites containing low levels of MMP-8, mean 2.1 (±0.2 SE) ng/site and high levels of MMP-8, mean 102.7 (±20.1 SE) ng/site

Parameter	Low MMP-8 (n* = 10)	High MMP-8 (n = 10)	P-value
PD mm	4.6 ± 0.4	5.70 ± 0.3	<0.05
EA-S (mAbs/site)	0.31 ± 0.11	0.26 ± 0.10	NS†
EA-P (mAbs/site)	1.06 ± 0.12	2.03 ± 0.49	NS
S/P-ratio	0.30 ± 0.11	0.60 ± 0.39	NS
PGE ₂ (pg/site)	5.58 ± 1.42	23.54 ± 5.34	<0.001

* No. of subjects, † not significant.

group the mean was 102.7 (±20.1 SE). In non-smokers with low levels of MMP-8, the mean was 2.1 (±0.1 SE); in the high levels group the mean was 88.3 (±14.0 SE). In smokers, pockets with high values of MMP-8 were significantly deeper ($P < 0.05$) than sites with low values. Significantly higher PGE₂ levels were found in sites with high values of MMP-8 ($P < 0.001$) than in those with low values (Table 6). In non-smokers, the levels of PGE₂ were significantly higher ($P < 0.001$) in sites with high values of MMP-8 compared to sites with low values (Table 7).

A significant correlation was found between probing pocket depth and levels of PGE₂ in non-smokers ($P < 0.05$). There was a positive correlation between the levels of MMP-8 (ng/site) and probing pocket depths ($r = 0.43$; $P < 0.001$) for smokers ($r = 0.43$; $P < 0.01$) and for non-smokers ($r = 0.44$; $P < 0.01$). There was also a positive correlation between the levels of MMP-8 (ng/site) and attachment loss ($r = 0.46$; $P < 0.001$) for smokers ($r = 0.40$; $P < 0.01$) and for non-smokers ($r = 0.53$; $P < 0.001$).

Discussion

The data from this study clearly show the negative effect of smoking tobacco on periodontal health. The subjects in

Table 7. Pocket depths (PD), neutrophil elastase in supernatants (EA-S), pellets (EA-P), S/P-ratio and prostaglandin E₂ (PGE₂) (mean ± SE) in GCF from non-smokers with sites containing low levels of MMP-8, mean 2.1 (±0.1 SE) ng/site and high levels of MMP-8, mean 88.3 (±14.0 SE) ng/site

Parameter	Low MMP-8 (n* = 6)	High MMP-8 (n = 7)	P-value
PD mm	3.9 ± 0.4	4.4 ± 0.5	NS†
EA-S (mAbs/site)	0.05 ± 0.03	0.03 ± 0.01	NS
EA-P (mAbs/site)	0.94 ± 0.14	1.70 ± 0.36	NS
S/P-ratio	0.10 ± 0.07	0.03 ± 0.01	NS
PGE ₂ (pg/site)	5.98 ± 2.03	28.20 ± 6.49	<0.001

* No. of subjects, † not significant.

this randomly selected group of smokers and non-smokers were of similar age and suffering from refractory periodontal diseases. Factors such as poorer oral hygiene and less frequent professional dental care in smokers than in non-smokers were eliminated in the present study, because both groups had had regular maintenance treatment for scaling and root planing every 6 months during the 7 years. This may explain why no significant difference in the mean P%I was found between smokers and non-smokers. This is in agreement with the results of studies by Macgregor et al. (25) and Linden & Mullally (26). However, smokers had a significantly higher percentage of the gingival margin covered by plaque (Table 1). Some investigators have reported less gingival inflammation or no difference between smokers and non-smokers (27), while another study has shown greater gingival inflammation in smokers (5, 28). Smokers in this study had more sites with gingival inflammation, expressed as a mean of the percentage of GI score 3, and significantly fewer sites with no inflammation (GI 0) than non-smokers (Table 2). The significant difference in the severity of gingival inflammation indicates that smokers in the present study had an infection with bacterial plaque, probably depending on the finding that smokers had more plaque at the gingival margin, even if they harbored equal amounts of plaque on the tooth surfaces.

The data from this study show that in the two groups with refractory periodontitis, those which have been treated for their periodontal diseases for 7 years, smokers had higher neutrophil EA activity in the GCF supernatants after centrifugation and more severe periodontal diseases with deeper pockets than non-smokers did (Tables 2 and 3). Alavi et al. (29) reported that smokers had lower concentrations of EA in GCF. The findings differ probably because the subjects in their study and those reported in this paper were in a different stage of the severity of the periodontal diseases. Alavi et al. (29) investigated subjects with untreated moderate-to-advanced chronic inflammatory periodontal disease. In the present study the subjects were treated for refractory periodontitis for 7 years.

In smokers, the neutrophil EA activity was significantly higher than in non-smokers in sites with matched pocket

depths (Table 5). This may be a sign of fragility of the neutrophils in smokers which permitted leakage of EA to the surrounding tissues and could be a risk factor in progression of the disease even in shallow pockets. The neutrophil functions in refractory periodontitis and healthy controls were investigated by MacFarlane et al. (4), who found that in the refractory periodontitis group neutrophil phagocytic capacity was significantly impaired compared to the controls. It was also suggested that, in smokers, neutrophil dysfunction may be considered a co-factor in the increased susceptibility to periodontal diseases.

Neutrophil EA seemed to be released, activated, or inhibited in a different way from MMP-8 in smokers, as the level of MMP-8 did not simultaneously increase with increasing EA activity (Table 5). High levels of MMP-8 in smokers seem to reflect the possible degradation of collagen fibers and degree of inflammation in these sites (Table 6). In this study, sites in smokers had significantly higher neutrophil EA activity than in non-smokers. Sites in smokers with high levels of MMP-8 also had significantly higher levels of PGE₂ and deeper pockets. These sites may be at risk for progression of the periodontal diseases.

The release and activation of human neutrophil metallo- and serin proteases, such as MMP-8 and EA, are believed to be responsible for tissue breakdown in periodontal disease. However, MMP-8 mRNA has also been detected in fibroblasts and human endothelial cells (30, 31), and MMP-8 from these cells probably also participates in the process. The positive correlation between levels of MMP-8 and pocket depths, as well as the loss of attachment in this study, seems to confirm this statement. It also indicates that most of the activity of MMP-8 was presumably in free form, active or complexed bound to TIMP-1 and TIMP-2 in a 1:1 molar ratio (13). This balance between MMP-8 and TIMP-1 and TIMP-2, which is thought to be important in several diseases (14), needs to be further investigated in periodontal diseases.

The interactions between bacteria and the host are essential for development and progression of periodontal diseases. Taichman et al. (32) and Baehni et al. (33) showed that polymorphonuclear leukocytes (PMNs) in vitro released lysosomal enzymes when they were exposed to supra- and subgingival plaque (32, 33). Ding et al. (34, 35) reported that certain periodontopathogens in vitro can cause the release of MMP-8 and EA from PMNs. Bacterial proteinases can participate in the activation of proMMPs (36). The activation of MMPs, accompanied by inactivation of alpha-1-protease inhibitor, will enhance proteolytic damage to the matrix tissues of the infected sites by MMPs and EA (37). The results of the present study show that smokers who have high levels of MMP-8 as well as a higher amount of EA in GCF than non-smokers may be at greater risk for progression of periodontal disease. From the results in Tables 6 and 7, it could be speculated whether sites with high levels of MMP-8, large amounts of EA, and high levels of PGE₂ in GCF are at high risk for progression of periodontal breakdown, regardless of pocket depth.

The present study suggests that neutrophil EA, PGE₂, and MMP-8 may be used as markers for identifying subjects or sites at enhanced risk for periodontal diseases. This would make it possible to monitor the response to non-surgical treatment and to predict the progression of periodontal diseases.

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References

1. Söder P-Ö, Jin IJ, Söder B, Wikner S. Periodontal status in an urban adult population in Sweden. *Commun Dent Oral Epidemiol* 1994;22:106–111.
2. Grossi SG, Genco RJ, Machtei EE, et al. Assessment of risk for periodontal disease. II. Risk indicators for alveolar bone loss. *J Periodontol* 1995;66:23–9.
3. Genco RJ. Current view of risk factors for periodontal diseases. *J Periodontol* 1996;67:1041–9.
4. MacFarlane GD, Herzberg MC, Wolff LF, Hardie NA. Refractory periodontitis associated with abnormal polymorphonuclear leukocyte function. *J Periodontol* 1992;64:908–13.
5. Zambon JJ, Grossi SG, Machtei EE, Ho AW, Dunford RRJG. Cigarette smoking increases the risk for subgingival infection with periodontal pathogens. *J Periodontol* 1996;67:1050–4.
6. Armitage GC, Jeffcoat MK, Chadwick DE, et al. Longitudinal evaluation of elastase as a marker for the progression of periodontitis. *J Periodontol* 1994;65:120–8.
7. Jin IJ, Söder P-Ö, Åsman B, Söder B, Bergström K. Granulocyte elastase in gingival crevicular fluid: improved monitoring of the site-specific response to treatment in patients with destructive periodontitis. *J Clin Periodontol* 1995;22:240–6.
8. Jin IJ, Söder P-Ö, Åsman B, Söder B, Puriene A, Bergström K. Variations in crevicular fluid elastase levels in periodontitis patients on long-term maintenance. *Eur J Oral Sci* 1995;103:84–9.
9. Offenbacher SS, Odle BM, Van Dyke TE. The use of crevicular fluid prostaglandin E₂ levels as a predictor of periodontal attachment loss. *J Periodont Res* 1986;21:101–12.
10. Offenbacher S, Heasman PA, Collins JG. Modulation of host PGE₂ secretion as a determinant of periodontal disease expression. *J Periodontol* 1993;64:432–44.
11. Cawston TE, Galloway A, Mercer E, Murphy G, Reynolds JJ. Purification of rabbit bone inhibitor of collagenase. *Biochem J* 1981;195:159–65.
12. Fosang AJ, Last K, Knäuper V, et al. Fibroblast and neutrophil collagenases cleave at two sites in the cartilage aggrecan interglobular domain. *Biochem J* 1993;295:273–6.
13. Knäuper V, Osthuus A, DeClerck YA, Langley KE, Bläser J, Tschesche H. Fragmentation of polymorphonuclear leukocyte collagenase. *Biochem J* 1993;291:847–54.
14. Birkedal-Hansen H. Role of matrix metalloproteinases in human periodontal diseases. *J Periodontol* 1993;64:474–84.
15. Sorsa T, Uitto VJ, Suomalainen K, Vauhkonen M, Lindy S. Comparison of interstitial collagenases from human gingiva, sulcular fluid and polymorphonuclear leukocytes. *J Periodont Res* 1988;23:386–93.
16. Ingman T, Tervahartiala T, Ding Y, et al. Matrix metalloproteinases and their inhibitors in gingival crevicular fluid and saliva of periodontitis patients. *J Clin Periodontol* 1996;23:1127–32.
17. Golub LM, Lee HM, Greenwald RA, et al. A matrix metalloproteinase inhibitor reduces bone-type collagen degradation fragments and specific collagenases in gingival crevicular fluid during adult periodontitis. *Inflamm Res* 1997;46:310–9.
18. Westerlund U, Ingman T, Lukinmaa PL, et al. Human

- neutrophil gelatinase and associated lipocalin in adult and localized juvenile periodontitis. *J Dent Res* 1996;75:1553–63.
19. Söder B, Jin LJ, Söder P-Ö, Wikner S. Clinical characteristics of destructive periodontitis in a risk group of Swedish urban adults. *Swed Dent J* 1995;19:9–15.
 20. Söder P-Ö, Jin LJ, Söder B. Computerized planimetric method for clinical plaque measurement. *Scand J Dent Res* 1993;101:21–5.
 21. Löe H, Silness J. Periodontal disease in pregnancy (I). Prevalence and severity. *Acta Odontol Scand* 1963;21:533–51.
 22. Wouters FR, Lavstedt S, Frithiof L, Söder P-Ö, Helldén LB, Salonen LWE. A computerized system to measure interproximal alveolar bone levels in epidemiologic radiographic investigations (I). Methodologic study. *Acta Odont Scand* 1988;46:25–31.
 23. Wouters FR, Lavstedt S, Frithiof L, Söder P-Ö, Helldén LB, Salonen LWE. A computerized system to measure interproximal alveolar bone levels in epidemiologic radiographic investigations (II). Intra- and inter-examiner variation study. *Acta Odont Scand* 1988;46:33–9.
 24. Kramps JA, Twisk CH, Van der Linden AC. L-Pyroglyutamyl-L-prolyl-L-valine-p-nitroanilide, a highly specific substrate for granulocyte elastase. *Scand J Clin Lab Invest* 1983;43:427–32.
 25. Macgregor IDM, Edgar WM, Greenwood AR. Effects of cigarette smoking on the rate of plaque formation. *J Clin Periodontol* 1985;12:35–41.
 26. Linden GJ, Mullally BH. Cigarette smoking and periodontal destruction in young adults. *J Periodontol* 1994;65:718–23.
 27. Bergström J, Floderus-Myrhed B. Co-twin control study of the relationship between smoking and some periodontal disease factors. *Commun Dent Oral Epidemiol* 1983;11:113–6.
 28. Preber H, Kant T, Bergström J. Cigarette smoking, oral hygiene and periodontal health in Swedish army conscripts. *J Clin Periodontol* 1980;7:106–13.
 29. Alavi AL, Palmer RM, Odell EW, Coward PY, Wilson RF. Elastase in gingival crevicular fluid from smokers and non-smokers with chronic inflammatory periodontal disease. *Oral Dis* 1995;1:110–4.
 30. Sorsa T, Lukinmaa P-L, Westerlund U, et al. The expression, activation and chemotherapeutic inhibition of matrix metalloproteinase-8 (neutrophil collagenase/collagenase-2) in inflammation. In: Davidovitch Z, Norton LA, Davidovitch Z, Norton LA, editors. *Biological mechanisms of tooth movement and craniofacial adaptation*. Boston: Harvard Society for the Advancement of Orthodontics; 1996. p. 317–23.
 31. Hanemaaijer R, Sorsa T, Kontinen YT, et al. Matrix metalloproteinase-8 is expressed in rheumatoid synovial fibroblasts and endothelial cells. Regulation by tumor necrosis factor-alpha and doxycycline. *J Biol Chem* 1997;272:31504–9.
 32. Taichman NS, Tsai CC, Baehni PC, Stoller N, McArthur WP. Interaction of inflammatory cells and oral microorganisms. IV. In vitro release of lysosomal constituents from polymorphonuclear leukocytes exposed to supragingival and subgingival bacterial plaque. *Infect Immun* 1977;16:1013–23.
 33. Baehni P, Tsai CC, Taichman NS, McArthur W. Interaction of inflammatory cells and oral microorganisms. V. Electron microscopic and biochemical study on the mechanisms of release of lysosomal constituents from human polymorphonuclear leukocytes exposed to dental plaque. *J Periodontol Res* 1978;13:333–48.
 34. Ding Y, Uitto VJ, Haapasalo M, et al. Membrane components of *Treponema denticola* trigger proteinase release from human polymorphonuclear leukocytes. *J Dent Res* 1996;75:1986–93.
 35. Ding Y, Haapasalo M, Kerosuo E, Lounatmaa K, Kotiranta A, Sorsa T. Release and activation of human neutrophil matrix metallo- and serine proteinases during phagocytosis of *Fusobacterium nucleatum*, *Porphyromonas gingivalis* and *Treponema denticola*. *J Clin Periodontol* 1997;24:237–48.
 36. Okamoto T, Akaike T, Suga M, et al. Activation of human matrix metalloproteinases by various bacterial proteinases. *J Biol Chem* 1997;272:6059–66.
 37. Maeda H, Okamoto T, Akaike T. Human matrix metalloprotease activation by insults of bacterial infection involving proteases and free radicals. *Biol Chem* 1998;379:193–200.

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