J Periodont Res 2006; 41; 491–497

JOURNAL OF PERIODONTAL RESEARCH
doi:10.1111/j.1600-0765.2006.00887.x

The associations between gingival crevice fluid matrix metalloproteinase-9, tissue inhibitor of metalloproteinase-1 and periodontitis in human immunodeficiency virus-positive patients

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Alpagot T, Suzara V, Bhattacharyya M. The associations between gingival crevice fluid matrix metalloproteinase-9, tissue inhibitor of metalloproteinase-1 and periodontitis in human immunodeficiency virus-positive patients. J Periodont Res 2006; 41: 491–497. © Blackwell Munksgaard 2006.

Background and Objective: The study aimed to determine whether matrix metalloproteinase-9 (MMP-9) and tissue inhibitor of metalloproteinase-1 (TIMP-1) in gingival crevice fluid could serve as prognostic factors for the progression of periodontitis in human immunodeficiency virus (HIV) -positive patients. Activated inflammatory cells produce inflammatory mediators, which stimulate the production of MMPs and their inhibitors. It is likely that the compromised immune system contributes to the pathogenesis of periodontitis in HIV-positive patients.

Methods: Clinical measurements including gingival index, plaque index, bleeding index, probing depth, attachment loss, and gingival crevice fluid samples were taken from two healthy sites (including sites with gingival recession, gingival index = 0; probing depth $\leq$ 3 mm; attachment loss $\leq$ 2 mm), three gingivitis sites (gingival index $>$ 0; probing depth $\leq$ 3 mm; attachment loss = 0) and three periodontitis sites (gingival index $>$ 0; probing depth $\geq$ 5 mm; attachment loss $\geq$ 3 mm) of each of the 35 patients at baseline visits and 6-month visits by means of paper strips. Gingival crevice fluid levels of MMP-9 and TIMP-1 were determined by sandwich enzyme-linked immunosorbent assays.

Results: The mean amounts of MMP-9 and TIMP-1 in the gingivitis and periodontitis sites sites were significantly higher than in the healthy sites ($P < 0.0001$). The progressing site was defined as a site that had 2 mm or more attachment loss during the 6-month study period. Gingival crevice fluid levels of MMP-9 were significantly correlated with probing depth, attachment loss, TIMP-1, age, smoking pack years, and viral load values at baseline and 6-month visits ($0.0001 < P < 0.001$). TIMP-1 levels were only correlated with CD4, viral load,

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Key words: matrix metalloproteinase-9; tissue inhibitor of metalloproteinases-1; gingival crevice fluid; human immunodeficiency virus; periodontitis; smoking; age; viral load; CD4

Accepted for publication December 23, 2005

attachment loss, and MMP-9 ($0.001 < P < 0.01$). Repeated measures analysis of 11 active sites vs. 269 inactive sites indicated that MMP-9 and TIMP-1 levels were significantly higher in active sites than in inactive sites ($P < 0.0001$). These data indicate that sites with high ginigval crevice fluid levels of MMP-9 and TIMP-1 in HIV-positive patients are at significantly greater risk for progression of periodontitis.

Periodontal diseases are common in human immunodeficiency virus (HIV) -infected individuals. It is well recognized that the development of periodontal disease depends on the interaction between the resident oral microbiota found in the dentogingival plaque and the host response. The bacteria colonize and invade the periodontal tissue while the host uses a variety of defense mechanisms to maintain a dynamic equilibrium with the resident oral microbial flora. As a result of these interactions between the bacteria and the host, a sequence of host immune mechanisms may be activated even at the expense of damaging the periodontal tissues. Most of the tissue damage is caused by the host response to infection. The etiology of periodontal disease in HIV-positive patients remains unclear. It is likely that the compromised immune system contributes to the pathogenesis of the lesions.

Components of microbial plaque have the capacity to induce the initial infiltrate of inflammatory cells including lymphocytes, macrophages, and polymorphonuclear leukocytes. These activated inflammatory cells produce inflammatory mediators, which stimulate the production of matrix metalloproteinases (MMPs) from mast cells, fibroblasts, polymorphonuclear leukocytes, and epithelial cells. MMPs are secreted in latent, inactive pro-enzyme forms (1). Myint *et al.* (2) reported high numbers of mast cells in gingival tissues taken from patients with chronic periodontitis. The number of mast cells was found to be even higher in HIV-positive patients with chronic periodontitis compared to HIV-negative counterparts (2). The increase in the number of mast cells was 10-fold even in the early stages of HIV infection, suggesting that this increase could be an HIV-induced effect. Chymase is a serine proteinase of mast cells and may degrade basal membranes and neuropeptides, activate latent interleukin-1β or MMP-9 (3). It has been reported that HIV infection can increase MMP expression in polymorphonuclear leukocytes (4,5). Mellanen *et al.* (6) reported an increase in MMP-2, MMP-9, tissue inhibitor of metalloproteinse-1 (TIMP-1), and TIMP-2 levels in the saliva of HIV-positive patients. MMPs play important roles in cell migration, wound healing, and tissue remodeling and have pathogenic roles in periodontitis (1). They can cause rapid extracellular matrix degradation and periodontal tissue destruction. The presence of MMP-1, MMP-2, MMP-3, MMP-8, and MMP-9 has been identified in gingival tissues and gingival crevice fluid samples from non-HIV subjects with periodontitis (7–10). MMP activity is further modulated by TIMP-1, -2, and -3. Fibroblasts can also produce TIMPs (11).

To our knowledge, there are no longitudinal data available with respect to the role of MMP-9 and TIMP-1 in the progression of untreated periodontitis sites in HIV-positive patients.

Materials and methods

Thirty-five HIV-infected patients were randomly selected from the database of an ongoing epidemiological project. The demographic, clinical, and immunological data related to these 35 HIV-positive patients were used from the same database. The HIV-positive patients were recruited from the CARE clinic at the UOP School of Dentistry. The inclusion criteria included the following: study subjects were older than 18 years of age, had two healthy, three gingivitis, and three periodontitis sites, did not require premedication with antibiotics for a periodontal examination, and had not gone through periodontal therapy within the last 6 months. The protocol for all procedures was approved by the Institutional Review Board of the California Pacific Medical Center. All study participants signed the committee-approved consent.

Medical and demographic variables, including medical history, age, race, cigarette smoking, alcohol use, oral hygiene practices, dental care utilization, level of education, and income, were obtained using a structured interview with the subject. Current CD4 cell count and viral load values (within 2 months of the initial study visit) were recorded from the chart review.

Clinical measurements including gingival index, plaque index, bleeding on probing depth, attachment loss and gingival crevice fluid samples were taken from two healthy sites (including sites with gingival recession, gingival index = 0; probing depth ≤ 3 mm; attachment loss ≤ 2 mm), three gingivitis sites (gingival index > 0; probing depth ≤ 3 mm; attachment loss = 0) and three periodontitis sites (gingival index > 0; probing depth ≥ 5 mm; attachment loss ≥ 3 mm) of each patient at baseline and 6-month visits by means of paper strips.

Levels of MMP-9 and TIMP-1 in the gingival crevice fluid were determined by sandwich enzyme-linked immunosorbent assays The plaque index (12), gingival index (13), probing depth, attachment level, and bleeding on probing were recorded for each experimental site by a calibrated examiner. Gingival crevice fluid sampling was carried out using sterile paper strips. A sterile periopaper (IDE Interstate, Amityville, NY, USA) was gently inserted 1–2 mm into the orifice of the gingival crevice and left in place for 30 s. Sample volume was

measured with a calibrated Periotron 6000 before transfer of the strip to a microfuge tube containing 300 μl of elution medium (physiological saline–0.1% Tween-20). The gingival crevice fluid samples were stored at −80°C.

The same procedures were repeated at the 6-month visit. All study subjects received scaling-polishing and oral hygiene instructions immediately after the completion of their baseline visit. No additional periodontal treatment was performed during the course of the 6-month study period.

Sample sites were classified as:

- Healthy sites (including sites with gingival recession): gingival index = 0, probing depth ≤ 3 mm and attachment loss ≤ 2 mm.
- Gingivitis sites: gingival index > 0, probing depth ≤ 3 mm, attachment loss = 0 mm.
- Periodontitis sites: gingival index > 0, probing depth ≥5 mm, attachment loss ≥3 mm.

A progressing site was defined as a site with ≥2 mm new attachment loss during the 6-month study period.

MMP-9 and TIMP-1 determinations

Gingival crevice fluid MMP-9 was quantified using sandwich enzyme-linked immunosorbent assay (R & D Systems, Minneapolis, MN, USA). The standards and samples were incubated in the 96-well polystyrene microplate precoated with anti-MMP-9 antibody. Any MMP-9 present was bound to the wells, other components of the sample being removed by washing and aspiration. The MMP-9 was detected by a peroxidase-labeled Fab antibody to MMP-9. The amount of peroxidase bound to each well was determined by the addition of tetramethylbenzidine substrate. The reaction was stopped by adding 1 M sulfuric acid. The plate was read at 450 nm.

The concentration of MMP-9 in the gingival crevice fluid samples was determined by interpolation from a standard curve. The sensitivity, defined as two standard deviations above the mean optical density of 10 zero standard replicates, was determined as 0.45 ng/ml.

TIMP-1 in the gingival crevice fluid was also quantified using sandwich enzyme-linked immunosorbent assays (R & D Systems, Minneapolis, MN, USA). The standards and samples were incubated in the 96-well polystyrene microplate precoated with anti-TIMP-1 antibody. Any TIMP-1 present was bound to the wells, other components of the sample being removed by washing and aspiration. A peroxidase-labeled antibody to TIMP-1 was used to detect TIMP-1. The amount of bound peroxidase was determined by the addition of tetramethylbenzidine substrate. The reaction was stopped by the addition of 1 M sulfuric acid. The plate was read at 450 nm.

The concentration of TIMP-1 in the gingival crevice fluid sample was determined by interpolation from a standard curve. The sensitivity, defined as two standard deviations above the mean optical density of 10 zero standard replicates was determined as 0.25 ng/ml.

Statistical analysis

Levels of MMP-9 and TIMP-1 in the gingival crevice fluid were subjected to square root transformation to render variances more homogeneous and to reduce skewness. The SAS statistical package was used to analyze the data. Power analysis was performed to determine the sample size of the study population. The associations between the demographic variables, clinical measurements, MMP-9 and TIMP-1-values were determined by Pearson's correlation coefficients. Analysis of variance for repeated measures and paired *t*-test were used to perform within-patient comparisons of healthy, gingivitis and periodontitis sites.

Gingival crevice fluid MMP-9 and TIMP-1-values of active and inactive sites were compared using *t*-tests.

A Bonferroni correction was applied. A P-value < 0.05 was considered indicative of a true difference. No adjustment was made for multiple testing. History of smoking (including current smokers) was reported as pack-years (number of packs of cigarettes smoked per day multiplied by number of years smoked). Smoking pack-years, CD4 cell counts, and viral load values were analyzed as continuous variables.

Results

The descriptive statistics of the study population are shown in Table 1. A total of 35 HIV-positive male subjects were enrolled in this 6-month longitudinal study. At the time of baseline examination, 25 (71.4%) subjects were on an antiretroviral therapy. The mean clinical measurements, MMP-9, TIMP-1 and gingival crevice fluid values of healthy, gingivitis, and periodontitis sites are given in Table 2. For the periodontitis sites, mean ± SD probing depths and attachment loss measurements at baseline were 5.73 ± 0.68 mm and 4.11 ± 1.06 mm, respectively. For the healthy sites, mean ± SD probing depths and attachment loss measurements at baseline were 1.85 ± 0.63 mm and 0.50 ± 0.50 mm, respectively. At baseline, 52 of 105 periodontitis sites (49%) showed bleeding upon probing. The baseline clinical measurements

Table 1. Descriptive statistics at baseline and 6-month visits

Variables	Baseline	6-month
Age	39.3 ± 10	39.8 ± 10
Pack years	4.4 ± 3.3	4.7 ± 3.4
CD4 (per μl)	344 ± 107	337 ± 147
Viral load (per ml)	7371 ± 9387	8863 ± 10746
Probing depth (mm)	3.50 ± 0.32	3.78 ± 0.35
Attachment loss (mm)	1.99 ± 0.40	2.13 ± 0.38
MMP-9 (ng/site)	0.45 ± 0.16	0.51 ± 0.18
TIMP-1 (ng/site)	0.32 ± 0.06	0.36 ± 0.08

Mean and SD of the entire subject group $N = 35$; MMP-9, matrix metalloproteinase-9; TIMP-1, tissue inhibitor of metalloproteinase-1.

Table 2. Mean and SD of clinical measurements, matrix metalloproteinase (MMP-9) and tissue inhibitor of metalloproteinase-1 (TIMP-1) in healthy, gingivitis and periodontitis sites (N = 35 subjects) at baseline and 6-month visits

	Healthy sites (n = 70)		Gingivitis sites (n = 105)		Periodontitis sites (n = 105)	
Variables	Baseline	6-month	Baseline	6-month	Baseline	6-month
Plaque index	0.36 ± 0.35	0.70 ± 0.30	1.08 ± 0.14	1.20 ± 0.26	1.78 ± 0.42	2.07 ± 0.37
Gingival index	0	0	1.05 ± 0.12	1.09 ± 0.18	1.44 ± 0.40	1.52 ± 0.34
Bleeding index	0	0	0.23 ± 0.31	0.38 ± 0.23	0.49 ± 0.12	0.59 ± 0.16
Probing depth (mm)	1.85 ± 0.63	2.04 ± 0.67	2.37 ± 0.44	2.60 ± 0.36	5.73 ± 0.68	6.12 ± 0.80
Attachment loss (mm)	0.50 ± 0.50	0.57 ± 0.53	0.87 ± 0.37	0.88 ± 0.43	4.11 ± 1.06	4.41 ± 0.97
MMP-9 (ng/site)	0.20 ± 0.02	0.21 ± 0.08	0.30 ± 0.04	0.31 ± 0.04	0.76 ± 0.41	0.91 ± 0.45
TIMP-1 (ng/site)	0.18 ± 0.03	0.18 ± 0.07	0.29 ± 0.06	0.32 ± 0.08	0.46 ± 0.13	0.52 ± 0.16
GCF volume (µl/30 s)	0.17 ± 0.06	0.19 ± 0.06	0.34 ± 0.08	0.37 ± 0.09	0.51 ± 0.11	0.69 ± 0.16

MMP-9, matrix metalloproteinase-9; TIMP-1, tissue inhibitor of metalloproteinase-1; GCF, gingival crevice fluid.

of the periodontitis sites were significantly increased at the 6-month visits ($0.0001 < P < 0.05$).

The gingival crevice fluid MMP-9 and TIMP-1-values and gingival crevice fluid volume were expressed as absolute amounts (Table 2). The mean MMP-9, TIMP-1, and gingival crevice fluid volume values of periodontitis sites at baseline and 6 months were significantly higher than the mean MMP-9, TIMP-1, and gingival crevice fluid volume values of the gingivitis and healthy sites ($0.0001 < P < 0.01$). There were significant differences between the gingivitis and healthy sites with respect to the MMP-9, TIMP-1, and gingival crevice fluid volume values ($0.0001 < P < 0.01$). It was also noted that the mean baseline MMP-9, TIMP-1 and, gingival crevice fluid volume values of periodontitis sites were significantly increased at the 6-month visits ($0.0001 < P < 0.01$) (Table 2; Fig. 1). The correlation values between clinical measurements, age, smoking pack years, CD4, viral load, MMP-9, and TIMP-1-values at baseline and 6-month visits are shown in Table 3.

MMP-9 was correlated positively with TIMP-1, age, smoking pack years, viral load, probing depth, and attachment loss at baseline and 6-month visits ($0.001 < P < 0.05$). There was a negative correlation between MMP-9 and CD4 levels at the 6-month visits ($P < 0.001$). TIMP-1 was correlated significantly with CD4, viral load, attachment loss, and MMP-9 at baseline and 6-month visits ($0.001 < P < 0.05$). There was negative correlation between CD4 and MMP, probing depth and attachment loss at the 6-month visits ($r = 0.59$; $r = 0.48$ and $r = -0.40$, respectively; $0.001 < P < 0.05$).

Viral load was correlated positively with age, smoking pack years, probing depth, attachment loss, MMP-9, and TIMP-1 at baseline and 6-month visits ($0.001 < P < 0.05$). Smoking pack-years was positively correlated with viral load ($r = 0.65$ and 0.58), probing depth ($r = 0.70$ and 0.55), attachment loss ($r = 0.83$ and 0.65), and MMP-9 ($r = 0.72$ and 0.48) at the baseline and 6-month visits ($0.0001 < P < 0.01$). Cigarette smoking was negatively correlated with CD4 values at 6-month visits ($r = 0.43$; $P < 0.001$). Age was significantly associated with pack-years ($r = 0.78$ and 0.79), viral load ($r = 0.44$ and 0.41), probing depth ($r = 0.69$ and 0.48), attachment loss ($r = 0.64$ and 0.50), and MMP-9 ($r = 0.52$ and 0.32) at baseline and 6-month visits ($0.0001 < P < 0.01$).

The repeated measures analysis of variance demonstrated a significant effect when periodontitis, gingivitis and healthy sites were compared for their gingival crevice fluid MMP-9 and TIMP-1 values measured at baseline and 6-month visits (Table 4). There were significant differences in MMP-9 and TIMP-1 values between healthy, gingivitis, and periodontitis sites at baseline and 6-month visits ($P < 0.0001$).

A total of 11 sites in 11 patients showed 2 mm or more new attachment loss during the 6-month study period. The mean attachment loss, probing depth, MMP-9, and TIMP-1-values of these 11 active sites measured at

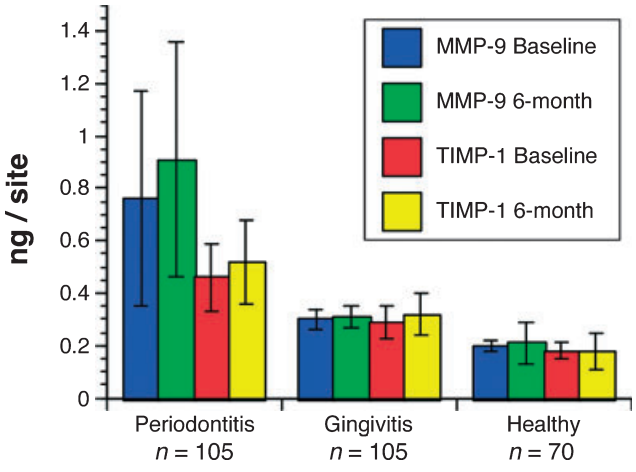

Fig. 1. Mean values of gingival crevice fluid matrix metalloproteinase-9 (MMP-9) and tissue inhibitor of metalloproteinase-1 (TIMP-1) with 95% confidence intervals at periodonititis, gingivitis, and healthy sites at baseline and 6-month visits.

Table 3. Pearson's correlation coefficient (r) values between dependent and independent variables

Parameters	Age	Pack-years	CD4	Virus load	Probing depth	Attachment loss	MMP-9	TIMP-1
Age								
Baseline	**1.0**	**0.78**	0.01	**0.44**	**0.69**	**0.64**	**0.52**	0.23
6-month	**1.0**	**0.79**	0.01	**0.41**	**0.48**	**0.50**	**0.32**	0.02
Pack-years								
Baseline	**0.78**	**1.0**	0.03	**0.65**	**0.70**	**0.83**	**0.72**	**0.38**
6-month	**0.79**	**1.0**	− 0.43	**0.58**	**0.55**	**0.65**	**0.48**	0.06
CD4								
Baseline	0.01	0.03	**1.0**	0.04	− 0.18	0.12	0.27	**0.43**
6-month	0.01	− **0.43**	**1.0**	− 0.72	− 0.48	− 0.40	− 0.59	**0.51**
Viral load								
Baseline	**0.44**	**0.65**	0.04	**1.0**	**0.54**	**0.53**	**0.76**	**0.55**
6-month	**0.41**	**0.58**	− 0.72	**1.0**	**0.57**	**0.50**	**0.67**	**0.55**
Probing depth								
Baseline	**0.69**	**0.70**	− 0.18	**0.54**	**1.0**	**0.67**	**0.46**	0.19
6-month	**0.48**	**0.55**	− 0.48	**0.57**	**1.0**	**0.77**	**0.39**	0.21
Attachment loss								
Baseline	**0.69**	**0.83**	0.12	**0.54**	**0.67**	**1.0**	**0.65**	**0.41**
6-month	**0.50**	**0.65**	− 0.40	**0.50**	**0.77**	**1.0**	**0.48**	**0.38**
MMP-9								
Baseline	**0.52**	**0.72**	0.27	**0.76**	**0.46**	**0.65**	**1.0**	**0.76**
6-month	**0.32**	**0.48**	− 0.59	**0.67**	**0.39**	**0.48**	**1.0**	**0.74**
TIMP-1								
Baseline	0.23	**0.38**	**0.43**	**0.55**	0.19	**0.41**	**0.76**	**1.0**
6-month	0.02	0.06	**0.51**	**0.55**	0.21	**0.38**	**0.74**	**1.0**

Reported correlations in bold text are positive at a significance level of $0.0001 < P < 0.05$. MMP-9, matrix metalloproteinase-9; TIMP-1, tissue inhibitor of metalloproteinase-1.

baseline and 6-month visits were higher than the mean attachment loss, probing depth, MMP-9, and TIMP-1-values of inactive sites (Table 5). It was also noted that the mean baseline attachment loss, probing depth, MMP-9, and TIMP-1-values of active sites were significantly increased at the 6-month visits ($0.001 < P < 0.05$).

Discussion

The activated inflammatory cells produce inflammatory mediators that stimulate the production of MMPs from mast cells, fibroblasts, epithelial cells, and polymorphonuclear leukocytes. Myint *et al*. (2) reported high numbers of mast cells in gingival tissues taken from patients with chronic periodontitis. The number of mast cells was found to be even higher in HIV-positive patients with chronic periodontitis compared to HIV-negative counterparts (2). The results of the present study support the hypothesis that the higher gingival crevice fluid levels of MMP-9 and TIMP-1 are associated with the periodontitis in HIV-positive patients. Increased levels of pro-MMP-9 in periodontitis-affected gingival tissues of systemically healthy subjects have been reported previously (14). Periodontal tissue breakdown has been associated with the conversion of pro-MMP-9 to its activated form in gingival crevice fluid (15). It has been reported that HIV infection can also increase MMP expression in polymorphonuclear leukocytes (4,5).

MMP activity is further modulated by TIMP-1, -2, and -3. Mellanen *et al*. (3) reported an increase in MMP-2, MMP-9, TIMP-1, and TIMP-2 levels in the saliva of HIV-positive patients. We have previously shown the increased gingival crevice fluid levels of MMP-3 and TIMP-1 in systemically healthy participants in a 6-month longitudinal study (9).

In the present study, 11 of 105 periodontitis sites (10.4%) had 2 mm or more attachment loss during the 6-month study period. The mean MMP-9 and TIMP-1 values of these 11 active sites at baseline and 6-month visits were significantly higher than the mean MMP-9 and TIMP-1 values of inactive periodontitis sites ($0.001 < P < 0.05$), indicating the role of MMP-9 and TIMP-1 in the progression of periodontitis sites. There was also a significant increase in the mean MMP-9 and TIMP-1 levels of active periodontitis sites at the 6-month visits compared to baseline ($P < 0.001$). The parallel increase in gingival crevice fluid TIMP-1 levels with the increase in gingival crevice fluid MMP-9 levels may not be enough to compensate for the up-regulation of MMP-9 and this may result in periodontal destruction. Other studies also detected an increase in TIMPs in systemically healthy patients with periodontitis (9,16,17). However, some studies reported a decrease in the levels

Table 4. Repeated measures analysis at baseline and 6-month visits

Parameters	H. site vs G. site	H. site vs P. site	G. site vs P. site
MMP-9			
Observed *t*-value baseline (CI interval)	− 12.60 (−0.12, − 0.08)	− 7.98 (−0.72, − 0.41)	− 6.67 (− 0.62, − 0.31)
Observed *t*-value 6-month (CI interval)	− 6.06 (−0.13, − 0.06)	− 9.32 (−0.87, − 0.53)	− 8.22 (− 0.77, − 0.044)
TIMP-1			
Observed *t*-value baseline (CI interval)	− 11.16 (−0.13, − 0.09)	− 14.85 (−0.34, − 0.25)	− 9.41 (− 0.22, − 0.14)
Observed *t*-value 6-month (CI interval)	− 7.29 (−0.17, − 0.09)	− 12.48 (−0.40, − 0.28)	− 7.74 (− 0.26, − 0.15)

There were significant differences in matrix metalloproteinase-9 (MMP-9) and tissue inhibitor of metalloproteinase-1 (TIMP-1) values between healthy (H), gingivitis (G), and periodontitis (P) sites at baseline and 6-month visits ($P < 0.0001$).

Table 5. Mean and SD of attachment loss, probing depth, matrix metalloproteinase-9 (MMP-9), and tissue inhibitor of metalloproteinase-1 (TIMP-1) values in active and inactive sites

	Active sites ($n = 11$)		Inactive sites ($n = 269$)	
	Baseline	6-month	Baseline	6-month
Attachment loss (mm)	4.72 ± 0.90	6.72 ± 0.90	1.88 ± 1.82	1.94 ± 1.81
Probing depth (mm)	6.27 ± 0.47	8.27 ± 0.47	3.39 ± 1.85	3.59 ± 1.84
MMP-9 (ng/site)	1.08 ± 0.47	1.55 ± 0.39	0.42 ± 0.34	0.46 ± 0.38
TIMP-1 (ng/site)	0.59 ± 0.09	0.80 ± 0.31	0.31 ± 0.14	0.34 ± 0.17

There were significant differences in mean attachment loss, probing depth, MMP-9, and TIMP-1 values between active and inactive sites at baseline and 6-month visits ($0.0001 < P < 0.001$). In active sites, the mean MMP-9 and TIMP-1-values were significantly higher at 6-month visits compared to baseline visits.

of TIMPs and an increase in MMP levels in diseased periodontal tissues (18,19). Altered balance between circulating MMP-9 and TIMP-1 during HIV infection may also play an important role in other diseases such as liver fibrosis progression in HIV-positive patients (20) and HIV-associated nephropathy (21).

With respect to the immunological variables, there was a positive correlation between viral load and probing depth and attachment loss at baseline and 6-month visits ($0.001 < P < 0.05$). CD4 was negatively correlated with probing depth, attachment loss, and MMP-9 at the 6-month visits ($0.001 < P < 0.01$), indicating the effect of immune status on the progression of established periodontitis sites in HIV-positive patients. We have previously shown the positive association between viral load and *Fusobacterium nucleatum* and *Prevotella intermedia* in HIV-positive patients, indicating that the subtle changes in immune system may allow proliferation of more virulent clones of periodontal pathogens (22).

The strong association between cigarette smoking and periodontal status was well established in the earlier studies (23–27). Cigarette smoking has been shown to alter host response mechanisms by adversely affecting polymorphonuclear leukocyte function, thereby depressing phagocyte-mediated protective responses to periodontopathic bacteria (28). In the present study, the measure of smoking pack-years was positively correlated with viral load, probing depth, attachment loss, and MMP-9 at baseline and 6-month visits. Cigarette smoking was negatively correlated with CD4 values at the 6-month visits, indicating the adverse effects of smoking on the host immune responses. Cigarette smoking increases the risk of lung colonization and has been documented to produce significant depression of the phagocytic function of alveolar macrophages in HIV-infected patients (29).

Age was significantly associated with pack-years, viral load, probing depth, attachment loss, and MMP-9, indicating the potential role of age as a risk factor for periodontal disease ($0.0001 < P < 0.01$). Higher prevalence and severity of periodontal disease with increasing age have been reported previously (9,22,25,30).

In summary, these data indicate that sites with high gingival crevice fluid levels of MMP-9 and TIMP-1 are at risk for progression of periodontitis in HIV-infected individuals. MMP-9 and TIMP-1 can be considered prognostic factors for periodontitis in HIV-positive patients.

Acknowledgements

This study was supported by NIDCR grant DE12417.

References

1. Birkedal-Hansen H, Moore WG, Bodden MK, *et al*. Matrix metalloproteinases: a review. *Crit Rev Oral Biol Med* 1993;**4:**197–250.
2. Myint M, Steinsvoll S, Yuan ZN, Johne B, Helgeland K, Schenck K. Highly increased numbers of leukocytes in inflamed gingiva from patients with HIV infection. *AIDS* 2002;**16:**235–243.
3. Fang KC, Raymond WW, Blount JL, Caughey GH. Dog mast cell alpha-chymase activates progelatinase b by cleaving the Phe88-Gln89 and Phe91-Glu92 bonds of the catalytic domain. *J Biol Chem* 1997;**272:**25628–25635.
4. Weeks BS, Klotman ME, Holloway E, Stetler-Stevenson WG, Kleinman HK, Klotman PE. HIV-1 infection stimulates T cell invasiveness and synthesis of the 92-kDa type IV collagenase. *AIDS Res Hum Retroviruses* 1993;**9:**513–518.
5. Chapel C, Camara V, Clayette P *et al*. Modulations of 92kDa gelatinase B and its inhibitors are associated with HIV-1 infection in human macrophage cultures. *Biochem Biophys Res Commun* 1994;**204:**1272–1278.
6. Mellanen L, Salo T, Ingman T, Konttinen YT, Lahdevirta J, Sorsa T. 72-kDa and 92-kDa gelatinases in saliva of patients with human immunodeficiency virus infection. *Acta Odontol Scand* 1998;**56:**135–142.
7. Tonetti MS, Freiburger K, Lang NP, Bickel M. Detection of interleukin-8 and matrix metalloproteinase transcripts in healthy and diseased gingival biopsies by RNA/PCR. *J Periodont Res* 1993;**28:**511–513.
8. Aiba T, Akeno N, Kawana T, Okamoto H, Horiuchi N. Matrix metalloproteinases-1 and -8 and TIMP-1 mRNA levels in normal and diseased human gingiva. *Eur J Oral Sci* 1996;**104:**562–569.
9. Alpagot T, Bell C, Lundergan W, Chambers DW, Rudin R. Longitudinal evaluation of GCF MMP-3 and TIMP-1 levels as prognostic factors for progression of periodontitis. *J Clin Periodontol* 2001;**28:**353–359.
10. Westerlund U, Ingman T, Lukinmaa PL, Salo T, Kjeldsen L, Borregaard N. Human neutrophil gelatinase and associated lipocalin in adult and localized juvenile periodontitis. *J Dent Res* 1996;**75:**1553–1563.
11. Unemori EN, Bair MJ, Bauer EA, Amento EP. Stromelysin expression regulates collagenase activation in human fibroblast. *J Biol Chem* 1991;**266:**23477–23482.
12. Silness J, Loe H. Periodontal disease in pregnancy (II). Correlation between oral hygiene and periodontal condition. *Acta Odontol Scand* 1964;**22:**121–135.
13. Loe H, Silness J. Periodontal disease in pregnancy (1). Prevalence and severity. *Acta Odontol Scand* 1963;**21:**533–551.
14. Smith PC, Munoz VC, Collados L, Oyarzun AD. In situ detection of matrix metalloproteinase-9 (MMP-9) in gingival epithelium in human periodontal disease. *J Periodont Res* 2004;**39:**87–92.

15. Teng YT, Sodek J, McCulloch CAG. Gingival crevicular fluid gelatinase and its relationship to periodontal disease in human subjects. *J Periodont Res* 1992;**27:** 544–552.
16. Haerian A, Adonogianaki E, Mooney J, Docherty JP, Kinane DF. Gingival crevicular stromelysin, collagenase and tissue inhibitor of metalloproteinases levels in healthy and diseased sites. *J Clin Periodontol* 1995;**22:**505–509.
17. Nomura T, Ishii A, Oishi Y, Kohma H, Hara K. Tissue inhibitors of metalloproteinases level and collagenase activity in gingival crevicular fluid: the relevance to periodontal diseases. *Oral Dis* 1998;**4:**231–240.
18. Soell M, Elkaim R, Tenenbaum H. Cathepsin C, matrix metalloproteinases, and their tissue inhibitors in gingival and gingival crevicular fluid from periodontitis-affected patients. *J Dent Res* 2002;**81:** 174–178.
19. Tuter G, Kurtis B, Serdar M. Effects of phase I periodontal treatment on gingival crevicular fluid levels of matrix metalloproteinase-1 and tissue inhibitor of metalloproteinase-1. *J Periodontol* 2002;**73:** 487–493.
20. Mastroianni CM, Liuzzi GM, D'Ettorre G, *et al*. Matrix metalloproteinase-9 and tissue inhibitors of matrix metalloproteinase-1 in plasma of patients co-infected with HCV and HIV. *HIV Clin Trials* 2002;**3:**310–315.
21. Ahuja TS, Gopalani A, Davies P, Ahuja H. Matrix metalloproteinase-9 expression in renal biopsies of patients with HIV-associated nephropathy. *Nephron Clin Pract*. 2003;**95:**c100–104.
22. Alpagot T, Duzgunes N, Wolff LF, Lee A. Risk factors for periodontitis in HIV^+ patients. *J Periodont Res* 2004;**39:**149–157.
23. Stoltenberg JL, Osborn JB, Pihlstrom B, *et al*. Association between cigarette smoking, bacterial pathogens, and periodontal status. *J Periodontol* 1993;**64:**1225–1230.
24. Bergstrom J, Preber H. Tobacco use as a risk factor. *J Periodontol* 1994;**65:**545–550.
25. Grossi SG, Zambon JJ, Ho AW, *et al*. Assessment of risk for periodontal disease. 1. Risk indicators for attachment loss. *J Periodontol* 1994;**65:**260–267.
26. Alpagot T, Wolff LF, Smith QT, Tran SD. Risk indicators for periodontal disease in a racially diverse population. *J Clin Periodontol* 1996;**23:**982–988.
27. Alpagot T, Font K, Lee A. Longitudinal evaluation of GCF IFN-γ levels and periodontal status in HIV^+ patients. *J Clin Periodontol* 2003;**30:**944–948.
28. MacFarlane GD, Herzberg MC, Wolff LF, Hardie NA. Refractory periodontitis associated with abnormal polymorphonuclear leukocyte phagocytosis and cigarette smoking. *J Periodontol* 2003;**30:**81–87.
29. Feldman C. Pneumonia associated with HIV infection. *Curr Opin Infect Dis* 2005;**18:**165–170.
30. Beck JD, Koch GG, Offenbacher S. Incidence of attachment loss over 3 years in older adults-new and progressing lesions. *Community Dent Oral Epidemiol* 1995;**23:** 291–296.