J Periodont Res 2004; 39; 149–157
Printed in the UK.

JOURNAL OF PERIODONTAL RESEARCH
doi: 10.1111/j.1600-0765.2004.00718.x

Risk factors for periodontitis in HIV$^+$ patients

Tamer Alpagot[1,2], Nejat Duzgunes[1], Larry F. Wolff[3], Aaaron Lee[1]
[1]University of the Pacific School of Dentistry, [2]University of California School of Dentistry, San Francisco and [3]University of Minnesota School of Dentistry, USA

Alpagot T, Duzgunes N, Wolff LF, Lee A. Risk factors for periodontitis in HIV$^+$ patients. J Periodont Res 2004; 39; 149–157. © Blackwell Munksgaard, 2004

Objective: The purpose of this study was to identify risk factors for periodontitis associated with human immunodeficiency virus (HIV) infection.

Methods: A total of 152 HIV$^+$ patients were recruited from the CARE clinic at the University of the Pacific School of Dentistry. Clinical measurements (gingival index, plaque index, bleeding index, probing depth, and attachment loss), gingival crevicular fluid (GCF) and subgingival plaque samples were taken from eight sites of each patient at baseline and 6-month visits. GCF neutrophil elastase was determined by measurement of *p*-nitroanalide resulting from hydrolysis of an elastase-specific peptide. GCF β-glucuronidase was determined by release of 4-methylumbelliferone from hydrolysis of a specific substrate. A bacterial concentration fluorescence immunoassay was used to detect periodontopathic bacteria in subgingival plaque samples.

Results: Viral load, age, smoking pack-years, *Fusobacterium nucleatum*, *Prevotella intermedia*, *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, neutrophil elastase, and β-glucuronidase were significantly correlated with clinical measurements ($0.0001 < p < 0.05$). Significantly higher levels of elastase, β-glucuronidase, *F. nucleatum*, *P. intermedia*, and *A. actinomycetemcomitans* were found at progressing sites than in non-progressing sites ($0.001 < p < 0.05$).

Conclusions: These data indicate that age, smoking pack-years, viral load, *F. nucleatum*, *P. intermedia*, *A. actinomycetemcomitans*, elastase, and β-glucuronidase are risk factors for periodontitis in HIV$^+$ patients.

Tamer Alpagot, DDS, PhD, Department of Periodontics, University of the Pacific School of Dentistry, 2155 Webster Street, San Francisco, CA 94115, USA
E-mail: talpagot@pacific.edu

Key words: age; β-glucuronidase; elastase; gingival crevicular fluid; human immunodeficiency virus; periodontitis; periodontopathic bacteria; risk factors; smoking; viral load

Accepted for publication October 20, 2003

Periodontal diseases are common in human immunodeficiency virus (HIV)-infected individuals and generally progress over an extended period of time with little or no pain or discomfort to the patient. This lack of signs and symptoms frequently masks the presence of periodontal disease until severe damage has occurred. On the other hand, periodontal lesions that result in pain usually represent more acute conditions such as necrotizing ulcerative gingivitis or periodontitis in HIV-infected individuals. Advanced periodontal disease with severe gingivitis, gingival recession, and alveolar bone loss in patients with HIV infection have been reported previously (1–9). It has been suggested that HIV-infected patients are at risk for severe periodontal diseases (1–9). Smith *et al.* reported that HIV$^+$ subjects had experienced more severe attachment loss localized to the lower incisors and upper posterior sextants compared to the non-HIV control subjects (10).

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 (11, 12). Most of the tissue damage is caused by the host response to infection (11). The etiology of periodontal disease in HIV$^+$ 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. Neutrophil plays an important role in

destruction of host tissues (11). Lytic enzyme release from polymorphonuclear leukocytes such as elastase and β-glucuronidase may contribute to the development of periodontal disease. Neutrophil elastase is one of the major enzymes of the azurophilic granules of human neutrophils, and its release in inflammation could contribute to tissue damage. Elastase degrades several proteins, including elastin, collagen, fibrinogen, hemoglobin, ground-substance components of the connective tissue and proteoglycans (13, 14). The increase in the amount of elastase in gingival crevicular fluid (GCF) with inflammation is due primarily to the parallel increase in the number of sulcular polymorphonuclear leukocytes. However, there is a lack of information related to the associations among elastase, the subgingival periodontopathic bacteria and periodontal disease occurrence in HIV-infected patients.

β-Glucuronidase is a lysosomal acid hydrolase which has a significant role in connective tissue ground substance degradation. β-Glucuronidase is involved in the degradation of glycosaminoglycans. Its action is on oligosaccharides generated by the action of hyaluronidase on the ground substance (15). Elevated levels of β-glucuronidase in GCF may be from local interaction of polymorphonuclear leukocytes with endotoxin and other factors released from subgingival microorganisms. Levels of this enzyme are associated significantly with inflammation, pocket depth, and alveolar bone loss (16, 17). There may be a significant relationship between the microbial profile of subgingival plaque and β-glucuronidase levels in GCF that could lead to loss of periodontal attachment in HIV^+ patients. In the subgingival plaque microflora of periodontal disease in HIV^+ patients, *Fusobacterium nucleatum*, *Prevotella intermedia*, *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, spirochetes, *Peptostreptococcus micros*, *Bacteroides fragilis*, *Campylobacter rectus* were prevalent periodontopathic bacteria (1, 2, 5, 18, 19). Recently, the elevated numbers of spirochetes of the genus *Treponema* have been reported in the subgingival plaque samples of HIV^+ individuals (6, 7). GCF contains potential markers derived not only from the host tissues and serum but also the subgingival microbial plaque. Correlating GCF enzyme levels with the presence of subgingival bacteria at specific sites could provide valuable information regarding the diagnosis and pathogenesis of periodontal disease in HIV-infected individuals.

$CD4^+$ T-helper lymphocytes are responsible for coordinating some important functions of the immune system. Dysfunction and/or depletion of $CD4^+$ T lymphocytes results in the immunologic defects found in HIV-infected individuals. $CD4^+$ T-lymphocyte count and viral load level serve as indicators for initiating treatment, and are used to monitor immunologic status and disease progression. In a longitudinal study, Barr *et al.* (3) reported an increase in attachment loss in immunodeficient individuals ($CD4^+$ T lymphocytes < 200 cells/mm^3). Glick *et al.* (20) reported that HIV-infected individuals presenting with a diagnosis of necrotizing ulcerative periodontitis were 20.8 times as likely to have $CD4^+$ T-lymphocyte counts below 200 cells/mm^3 compared to HIV-infected individuals presenting without necrotizing ulcerative periodontitis. Tomar *et al.* (21) reported that the medical stage of HIV disease and the presence of oral candidiasis significantly affected the likelihood of severe attachment loss. The purpose of the present study was to identify risk factors for periodontal disease associated with HIV infection.

Material and methods

Patient selection, sampling, and clinical evaluation

A total of 152 HIV-infected patients were recruited from the CARE clinic at the University of Pacific School of Dentistry. Subjects who were younger than 18 years of age, did not have at least one sextant with two premolars and two molars (excluding third molars) and four lower incisors, required premedication with antibiotics for a periodontal examination, received antibiotic therapy within the previous 3 months, had other systemic health problems, and had gone through periodontal therapy within the previous 6 months, did not have CD4 and viral load values determined within the previous 2 months were excluded. The protocol for all procedures was approved by the Institutional Review Board of California Pacific Medical Center. All study participants signed the committee-approved informed consent. Medical and demographic variables including medical history, age, race, cigarette smoking, alcohol consumption, oral hygiene practices, dental care utilization, level of education and income were obtained using a structured interview with the subject. Following extraoral and intraoral examination, the most diseased sextant (determined radiographically) in each subject was evaluated including the mesiobuccal sites of two premolars and two molars in that sextant excluding third molars. The plaque index (22), gingival index (23), probing depth, attachment level, and bleeding on probing were recorded for each experimental site including four posterior teeth and four lower anterior teeth by a calibrated examiner. The study sites in the lower anterior sextant were the mesiobuccal sites of two lower left incisors and the distobuccal sites of two lower right incisors. GCF 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 prior to transfer of the strip to a microfuge tube containing 350 µl of elution media (physiologic saline–0.1% Tween 20). The GCF samples were stored in a −80°C freezer. The subgingival plaque samples were collected by curet and transferred into a microfuge tube containing 350 µl of reduced transport fluid, then stored at −80°C until the day of analysis. 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:

(i) healthy sites (including sites with gingival recession): gingival index = 0, probing depth ≤ 3 mm and attachment loss ≤ 3 mm;
(ii) gingivitis sites: gingival index > 0, probing depth ≤ 3 mm, attachment loss = 0 mm;
(iii) periodontitis sites: gingival index > 0, probing depth ≥ 4 mm, attachment loss ≥ 2 mm.

Subjects were assigned to periodontitis, gingivitis or healthy groups based on each subject's most diseased study site. The subject, not the site, was the unit of analysis in this investigation. A progressing site was defined as a site which had 2 mm or more new attachment loss during the 6-month study period.

Evaluation of GCF neutrophil elastase and β-glucuronidase

Elastase was determined in enzyme-linked immunosorbent assay plates by measurement of nitroanalide resulting from the hydrolysis of the elastase-specific peptide, MeOSuc-Ala-Ala-Pro-Val-*p*-Nitroanalide (24). This method measures functionally active elastase, which includes free elastase and elastase bound to α2-macroglobulin. Procedures were described in detail elsewhere (25).

β-Glucuronidase was determined by the release of 4-methylumbelliferone from hydrolysis of 4-methylumberriferyl-β-D-glucuronide (4-MUGL). 4-MUGL is a fluorescent substrate for lysosomal β-glucuronidase. Procedures were described in detail elsewhere (16). Assay values for neutrophil elastase and β-glucuronidase were reported in total amount per site.

Determination of periodontopathic bacteria

A bacterial concentration fluorescence immunoassay was used to detect periodontopathic bacteria in subgingival plaque samples. The bacterial concentration fluorescence immunoassay utilizes fluorescently tagged bacterial specific monoclonal antibodies directed against the lipopolysaccharide of selective gram-negative bacteria. These bacterial specific monoclonal antibodies included antibodies specific for *P. gingivalis*, *P. intermedia*, *A. actinomycetemcomitans*, *F. nucleatum*, and *Eikenella corrodens*. The details of the method were described elsewhere (26, 27). Based on comparison to the standard, a plaque sample was considered negative when the relative number of bacterial cells was $< 10^3$. Plaque samples positive for a given bacteria were categorized into low positive ($\geq 10^3$ and $< 10^4$), medium positive ($\geq 10^4$ and $< 10^5$), and high positive ($\geq 10^5$).

Data analysis

Periodontitis, gingivitis, and healthy groups were compared by *t*-tests with respect to mean age and mean smoking pack-years. The differences in the mean GCF volumes of all sites, molars, premolars, anteriors and in the mean total amount of β-glucuronidase, and elastase between periodontitis, gingivitis, and healthy groups were tested by the *t*-tests. Enzyme values were subject to a square root transformation to render variances more homogenous and to reduce skewness. Since the distribution of bacterial sum values was skewed, the Wilcoxon rank sum test was used for the comparison of the median bacterial sum values in the periodontitis, gingivitis, and healthy groups. Chi-squared tests were used to test the associations with respect to categorical data. Age was dichotomized into older (> 35 years) and younger (≤35 years) age groups. History of smoking (including quitters and current smokers) was reported as pack-years (number of packs of cigarettes smoked per day multiplied by number of years smoked). Pack-years were then used to categorize participants as non-smoker, light smoker (0–5 pack-years), moderate smoker (> 5 and < 15 pack-years), heavy smokers (> 15 pack-years). To determine the associations between demographics, immunologic parameters, GCF enzymes, and microbial parameters with periodontal status, Spearman correlation analysis was performed between probing depth, attachment level, and other variables. Sum of the bacterial scores of the eight sites per subject was used for determination of correlation coefficients. The odds ratios were calculated to estimate the relative risk of developing attachment loss from 2×2 contingency tables. Repeated measures analysis was used to evaluate the changes in clinical measurements such as loss of attachment and increase in probing depth. Stepwise multiple regression analysis was performed to investigate whether a combination of risk factors could explain more of the variability in periodontal measures (probing depth and attachment loss) than any of the risk factor alone, and to develop risk assessment models for the estimation of probing depth and attachment loss in HIV-infected individuals. Adjustment of periodontal status in regression analysis was made by assigning the subjects to periodontitis, gingivitis, and healthy groups as described in 'Patient Selection, Sampling and Clinical Evaluation' section. Finally, all independent variables were entered into regression analysis to determine the contribution of each variable to the models for both probing depth and attachment loss (partial R^2 values). All analyses were subject based. A p-value ≤ 0.05 was considered indicative of a true difference. No adjustment for multiple testing was done.

Results

A total of 152 HIV^+ male subjects were enrolled comprising 88 whites (57.9%), 37 African Americans (24.3%), 22 Hispanics (14.5%), and 5 Asians (3.3%). At the time of examination, 96 (63.2%) study subjects were on a highly active antiretroviral therapy.

The mean clinical indices and the study groups are shown in Table 1. In the periodontitis group, 111 HIV^+ patients (73%) were evaluated. Subject mean value of attachment loss in the periodontitis group at baseline was indicative of moderate to severe chronic periodontitis (4.85 ± 0.62). The mean age of the study population was 34.1 years (range 18–64). The mean age of the periodontitis group

Table 1. Mean and SD of clinical measurements, pack-years, CD4, and viral load values in diagnostic groups at baseline and 6 months

	Periodontitis $n = 111$		Gingivitis $n = 29$		Healthy $n = 12$	
Variables	Baseline	6-month	Baseline	6-month	Baseline	6-month
Plaque index	1.79 ± 0.74*‡	1.81 ± 0.76†	1.01 ± 0.53*	1.04 ± 0.57†	0.56 ± 0.29*	0.58 ± 0.30†
Gingival index	1.39 ± 0.36*‡	1.44 ± 0.41†	1.16 ± 0.31*	1.18 ± 0.32†	0*	0†
Bleeding on probing	0.73 ± 0.42*‡	0.77 ± 0.54†	0.51 ± 0.36*	0.56 ± 0.39†	0*	0†
Probing depth (mm)	5.24 ± 0.66*‡	5.68 ± 0.72†	2.81 ± 0.53*	2.86 ± 0.55†	2.14 ± 0.49*	2.13 ± 0.47†
Attachment level (mm)	4.85 ± 0.62*‡	5.27 ± 0.71†	1.74 ± 0.49*	1.78 ± 0.51†	0.78 ± 0.39*	0.79 ± 0.40†
GCF (μl/30 s)	0.48 ± 0.12*‡	0.66 ± 0.18†	0.32 ± 0.08*	0.36 ± 0.09†	0.16 ± 0.06*	0.18 ± 0.07†
Pack-years	7.89 ± 6.47*‡	8.29 ± 6.82†	1.54 ± 1.23*	1.82 ± 1.26†	0.92 ± 0.66*	0.98 ± 0.69†
CD4 (per μl)	285 ± 147	292 ± 155	303 ± 172	311 ± 177	309 ± 145	301 ± 152
Viral load (per ml)	16367 ± 11562*‡	16849 ± 12315†	9372 ± 5429*	9758 ± 6149	5193 ± 4164*	3875 ± 3481†

*†There were significant differences between the study groups with respect to plaque index, gingival index, bleeding on probing, probing depth, attachment level, pack-years, viral load at baseline, and 6-month visits ($0.001 < p < 0.05$).
‡There were also significant differences between the baseline and 6-month values of probing depth, attachment level, and gingival crevicular fluid (GCF) in the periodontitis group ($0.01 < p < 0.05$).

Table 2. Odds ratios of the test variables as predictors of attachment loss at 6 months

Variables	Odds ratios	95% CI*	*p*-value
Age (> 35)	3.78	(1.93–5.62)	0.003
Smoking (> 15 pack-years)	4.92	(1.32–7.64)	0.001
Viral load (> 20,000 copies/ml)	2.75	(1.52–4.63)	0.017
CD4 (< 200 per μl)	1.61	(1.32–2.14)	0.068
Elastase (> 1.5 ng/site)	3.15	(1.73–5.26)	0.007
β-Glucuronidase (> 1.75 ng/site)	2.86	(1.62–4.96)	0.012
Fusobacterium nucleatum (medium or heavy/site)	3.24	(1.72–5.31)	0.005
Prevotella intermedia (medium or heavy/site)	2.93	1.67–5.18)	0.015
Actinobacillus actinomycetemcomitans (medium or heavy/site)	2.37	(1.43–4.74)	0.031
Porphyromonas gingivalis (medium or heavy/site)	2.06	(1.27–3.58)	0.047
Eikenella corrodens (medium or heavy/site)	1.27	(0.89–2.46)	0.369
Plaque index (2 or greater)	0.86	(0.63–1.19)	0.872
Gingival index (2 or greater)	2.31	(1.02–5.73)	0.042
Bleeding on probing	1.49	(1.06–1.85)	0.069
Probing depth (5 mm or greater)	1.28	(0.87–1.71)	0.375

*95% confidence intervals of the odds ratios.

was 39.4 ± 9.7 and significantly ($p < 0.004$) higher than that of the gingivitis group (32.2 ± 8.6) and healthy group (29.7 ± 6.9). The odds of individuals who were older than 35 years old of having periodontitis vs. gingivitis was 3.78 (95% CI = 1.93–5.62) times greater than the odds of individuals who were less than 35 years old of having periodontitis vs. gingivitis (Table 2). The mean pack-years of the periodontitis group was significantly higher ($p < 0.001$) than that of the gingivitis group (Table 1). Moreover, moderate smokers combined with heavy smokers had an odds ratio of 4.92 (95% CI = 1.32–7.64) of having periodontitis as opposed to non-smokers (Table 2). The mean viral load of periodontitis group at baseline was 16367 ± 11562 and significantly higher ($p < 0.001$) than the mean viral load of the gingivitis group (9372 ± 5429) (Table 1). On the other hand, there was no significant difference in mean CD4 levels of periodontitis and gingivitis groups.

The total amount of elastase and β-glucuronidase values in the study groups are shown in Fig. 1. Neutrophil elastase and β-glucuronidase values in the periodontitis group at baseline and 6 months were higher than in the gingivitis group and healthy group ($0.001 < p < 0.01$). There were also significant increases in neutrophil elastase and β-glucuronidase values at 6-month visits compared to baseline visits in the periodontitis group ($0.001 < p < 0.01$).

The odds ratios were calculated to estimate the relative risk of developing attachment loss from 2×2 contingency tables using the subject as the unit of analysis. The odds ratios of age (3.78), smoking pack-years (4.92), viral load (2.75), neutrophil elastase (3.15), β-glucuronidase (2.86), *F. nucleatum* (3.24), *P. intermedia* (2.93), and *A. actinomycetemcomitans* (2.37) were higher than the odds ratios of other variables in the study (Table 2). The odds ratios of *F. nucleatum*, *P. intermedia*, *A. actinomycetemcomitans*, *P. gingivalis*, and *E. corrodens* were based on the presence of medium to high positive bacteria ($\geq 10^4$) for a given subject.

The percentages of periodontopathic bacteria in each study group at baseline and 6-month visits are given in Table 3. *F. nucleatum* was the most prevalent bacteria in the periodontitis and gingivitis study groups. The percentages of periodontopathic bacteria in periodontitis and gingivitis groups at baseline and 6 months were significantly higher than the percentages of these bacteria in the healthy group ($0.001 < p < 0.01$). There was a significant difference ($0.001 < p < 0.05$) in median sum values for *F. nucleatum*, *P. intermedia*, *A. actinomycetemcomitans*, and *P. gingivalis* between gingivitis, periodontitis, and healthy groups. The percentages provided in Table 3 and Table 5 were based on the presence of a given bacteria in a given site ($\geq 10^3$). For example, 81.3% of the

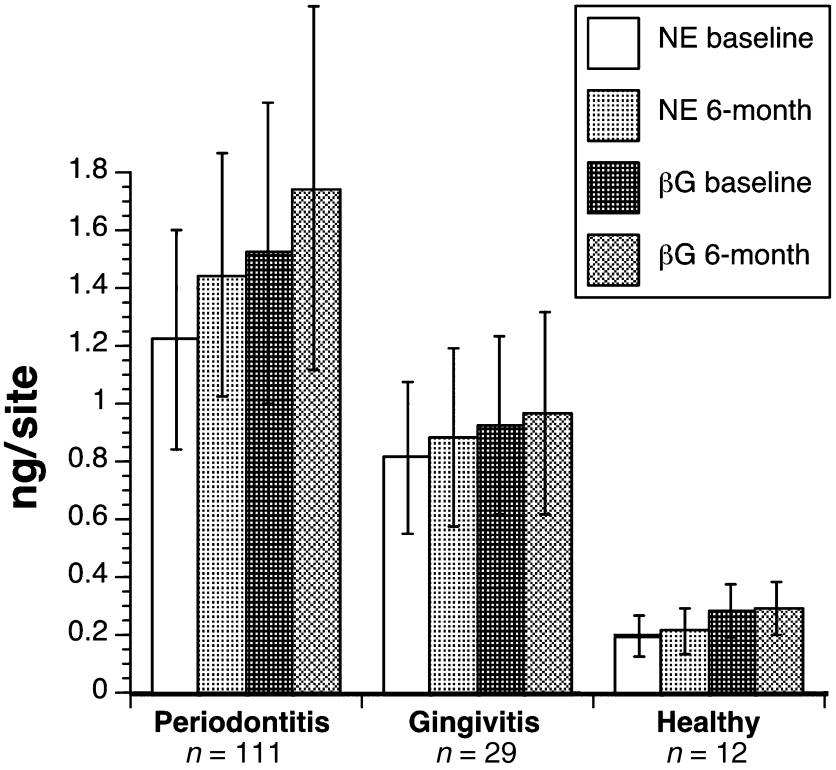

Fig. 1. Mean and SD of neutrophil elastase (NE) and β-glucuronidase (βG) in diagnostic groups at baseline and 6 months.

Table 3. Percentage of periodontopathic bacteria ($\geq 10^3$) in diagnostic groups at baseline and 6 months

	Periodontitis $n = 111$		Gingivitis $n = 29$		Healthy $n = 12$	
Variables	Baseline	6-month	Baseline	6-month	Baseline	6-month
F. nucleatum	81.3*‡	89.9†	70.1*	75.7†	9.5*	11.2†
P. intermedia	78.5*‡	84.6†	68.2*	72.8†	9.5*	9.9†
A. actinomycetemcomitans	72.8*‡	78.1†	63.6*	67.9†	4.8*	4.6†
P. gingivalis	70.9*	74.8†	58.4*	63.7†	5.9*	5.5†
*E. corrodens***	53.7	51.6	51.8	48.2	4.7	5.1

*†There were significant differences between the study groups with respect to *F. nucleatum*, *Prevotella intermedia*, *Actinobacillus actinomycetemcomitans*, and *Porphyromonas gingivalis* values at baseline and 6-month visits ($0.001 < p < 0.05$).

‡There were also significant differences between the baseline and 6-month values of *Fusobacterium nucleatum*, *P. intermedia*, and *A. actinomycetemcomitans* in the periodontitis group ($0.01 < p < 0.05$).

***Eikenella corrodens* values of the periodontitis and the gingivitis groups at baseline and 6-month visits were significantly higher than *E. corrodens* values of the healthy group ($p < 0.001$).

subjects in the periodontitis group had at least one site which had $\geq 10^3$ *F. nucleatum* at baseline.

The Spearman correlation coefficient (r) values between attachment loss, probing depth, age, pack-years, viral load, CD4, neutrophil elastase, β-glucuronidase, and periodontopathic bacteria in active subject group ($n = 41$) at baseline and 6-month visits are shown in Table 4. Age, pack-years, neutrophil elastase, β-glucuronidase, *F. nucleatum*, *P. intermedia*, *A. actinomycetemcomitans*, *P. gingivalis*, and viral load values were correlated significantly with attachment loss ($0.0001 < p < 0.05$). Smoking pack-years had the highest correlation value with attachment loss at baseline and 6-month ($r = 0.63$ and 0.65, respectively). There were also significant ($0.001 < p < 0.05$) correlations between *F. nucleatum*, *P. intermedia*, *A. actinomycetemcomitans*, *P. gingivalis* and probing depth and attachment loss at baseline and 6-month visits (Table 4).

A total of 65 sites in 43 patients in the periodontitis group showed 2 mm or more new attachment loss during the 6-month study period (Table 5) and were considered active. The mean neutrophil elastase and β-glucuronidase values, and the percentages of *F. nucleatum*, *P. intermedia*, and *A. actinomycetemcomitans* of these 65 active sites at 6-month visits were significantly higher than the mean neutrophil elastase and β-glucuronidase values and the percentages of *F. nucleatum*, *P. intermedia*, and *A. actinomycetemcomitans* of active sites at baseline visits ($0.001 < p < 0.01$). The baseline and 6-month values of neutrophil elastase, β-glucuronidase, *F. nucleatum*, *P. intermedia*, and *A. actinomycetemcomitans* in active sites were significantly higher than those in inactive sites ($0.001 < p < 0.01$).

To examine multiple associations between potential risk factors and probing depth or attachment loss, stepwise multiple regression analysis was performed. The R^2 values of the models from regression analysis of dependent variables (probing depth and attachment loss) with regressor variables (*F. nucleatum*, *P. intermedia*, neutrophil elastase, age, pack-years, viral load) are shown in Table 6. The R^2 value is the proportion of the variance explained by the model. Only variables which contributed significantly to each of the models for probing depth and attachment loss ($p < 0.05$) were included in the model. The model which had the best R^2 value from the regression analysis of mean probing depth with risk factors included *F. nucleatum*, neutrophil elastase, pack-years, and viral load. It was demonstrated that periodontal disease was more closely associated with a contribution of these factors combined than any of these parameters alone. This model explained 54% of the

Table 4. Spearman correlation coefficient (r) values between attachment loss, probing depth, age, pack-years, viral load, CD4, elastase, β-glucuronidase, and periodontopathic bacteria in active subject group (n = 41) at baseline and 6-month visits

Parameters	Pack-years (*r*)	Attachment loss (*r*)	Probing depth (*r*)	Elastase (*r*)	Viral load (*r*)
Age					
Baseline	**0.56**	**0.47**	0.15	− 0.17	0.16
6-month	**0.57**	**0.48**	0.14	− 0.19	0.18
Pack-years					
Baseline	**1.00**	**0.63**	**0.47**	**0.33**	**0.30**
6-month	**1.00**	**0.65**	**0.48**	**0.35**	**0.31**
Elastase					
Baseline	**0.33**	**0.39**	**0.54**	**1.00**	**0.25**
6-month	**0.35**	**0.46**	**0.58**	**1.00**	**0.28**
β-Glucuronidase					
Baseline	**0.30**	**0.36**	**0.52**	**0.79**	0.20
6-month	**0.32**	**0.41**	**0.47**	**0.82**	0.22
*Fusobacterium nucleatum**					
Baseline	**0.39**	**0.43**	**0.48**	**0.34**	**0.30**
6-month	**0.43**	**0.46**	**0.51**	**0.35**	**0.33**
*Prevotella intermedia**					
Baseline	**0.32**	**0.38**	**0.44**	**0.31**	**0.25**
6-month	0.34	0.41	0.46	0.32	0.26
*Actinobacillus actinomycetemcomitans**					
Baseline	**0.25**	**0.31**	**0.34**	**0.27**	0.22
6-month	**0.26**	**0.32**	**0.36**	**0.29**	0.23
*Porphyromonas gingivalis**					
Baseline	**0.24**	**0.30**	**0.32**	0.23	0.21
6-month	**0.26**	**0.33**	**0.35**	**0.24**	0.20
*Eikenella corrodens**					
Baseline	0.21	0.19	0.22	0.16	0.15
6-month	0.17	0.21	0.21	0.18	0.19
Viral load					
Baseline	**0.30**	**0.34**	**0.37**	**0.25**	**1.00**
6-month	**0.31**	**0.36**	**0.40**	**0.28**	**1.00**
CD4					
Baseline	0.15	0.21	0.19	0.17	0.19
6-month	0.18	0.22	0.23	0.17	0.21

Reported correlations in bold text are positive at a significance level of $0.0001 < p < 0.05$. *Sum of the bacterial scores of the eight sites per subject was used for determination of correlation coefficients.

variability in mean probing depth. The best regression model for the estimation of attachment loss included *F. nucleatum*, neutrophil elastase, age, pack-years, viral load. This model explained 59% of the variability in mean attachment loss.

Discussion

We have identified several risk factors for periodontitis in HIV^+ individuals, including age, smoking pack-years, viral load, *F. nucleatum*, *P. intermedia*, *A. actinomycetemcomitans*, neutrophil elastase, and β-glucuronidase. Age was significantly associated with attachment loss, suggesting the potential role of age as a risk factor of periodontal disease in HIV^+ subjects ($p < 0.007$). Higher prevalence and severity of periodontal disease with increasing age has been reported by previous investigators (16, 28–31). Our age findings demonstrated that age makes a significant contribution to the regression model for the estimation of attachment loss but not for probing depth.

Several factors may have contributed to the significant association we observed between cigarette smoking and greater probing depth and attachment loss in HIV^+ subjects ($p < 0.001$). 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 (32). In a recent study, cigarette smokers exhibited a less favorable healing outcome following periodontal surgery compared to non-smokers (33). In the present study, moderate smokers combined with heavy smokers had an odds ratio of 4.92 (95% CI = 1.32–7.64) of having periodontitis as opposed to non-smokers, suggesting the potential role of smoking in the etiology of periodontal disease. Smoking contributed significantly to the multifactorial risk profile of periodontal disease in HIV^+ subjects ($p < 0.05$). In regards to smoking, our data agrees with the findings of previous studies (16, 31, 34, 35). Beck *et al.* (36) reported that cigarette smokers were at higher risk for periodontal disease progression. In a recent longitudinal study, we reported a strong correlation between smoking pack-years and attachment loss in a systemically healthy study population ($r = 0.68$, $p < 0.0001$) (31).

In the present study, there was a significant association between GCF enzymes, neutrophil elastase, and β-glucuronidase, and clinical measurements, probing depth, and attachment loss. The positive associations between these polymorphonuclear leukocyte enzymes and clinical measurements may result from an increase in the number of sulcular polymorphonuclear leukocytes and/or activation of synthesis and release of enzymes from polymorphonuclear leukocytes. Only neutrophil elastase contributed significantly to the regression models of periodontitis in HIV^+ patients ($p < 0.05$). Our data support previous studies showing a significant association between GCF neutrophil elastase and β-glucuronidase levels and periodontal disease (16, 17, 37). Nakashima and coworkers (38) demonstrated a strong correlation between polymorphonuclear leukocyte numbers and β-glucuronidase activities in GCF samples ($p < 0.001$).

Elastase is one of the major enzymes of the azurophilic granules of polymorphonuclear leukocytes, and in inflammation its release into gingival tissue and/or into the gingival crevice during inflammation could contribute

Table 5. Mean and SD of neutrophil elastase, β-glucuronidase, *Fusobacterium nucleatum, Prevotella intermedia, Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Eikenella corrodens* in active and inactive sites of periodontitis group at baseline and 6-month visits

	Active sites $n = 65$		Inactive sites $n = 823$	
	Baseline	6-month	Baseline	6-month
Elastase (ng/site)	1.35 ± 0.39*‡	1.69 ± 0.51†	1.11 ± 0.34*	1.17 ± 0.36†
β-glucuronidase (ng/site)	1.63 ± 0.54*‡	1.88 ± 0.69†	1.42 ± 0.51*	1.61 ± 0.56†
F. nucleatum (%/site)	84.7% *‡	93.5%†	77.4%*	79.6%†
P. intermedia (%/site)	81.5%*‡	88.3%†	75.6%*	77.1%†
A. actinomycetemcomitans (%/site)	76.2% *‡	82.4%†	69.5%*	70.9%†
P. gingivalis (%/site)	71.8%	74.2%	67.7%	69.6.1%
E. corrodens (%/site)	55.3%	56.2%	52.7%	51.1%

*‡There were significant differences between the active sites and inactive sites with respect to neutrophil elastase, β-glucuronidase, *F. nucleatum*, *P. intermedia*, and *A. actinomycetemcomitans* values at baseline and 6-month visits ($0.001 < p < 0.05$).
†There were also significant differences between the baseline and 6-month values of neutrophil elastase, β-glucuronidase, *F. nucleatum*, *P. intermedia*, and *A. actinomycetemcomitans* in the active sites ($0.001 < p < 0.05$).

Table 6. R^2 values of models from regression analysis of dependent variables (probing depth and attachment loss) with independent variables such as *Fusobacterium nucleatum,* neutrophil elastase, age, pack-years, viral load in HIV^+ patients with active periodontitis sites

Variable	R^2 for probing depth	R^2 for attachment loss
Subject means	0.54 (*Fn*, NE, pack-years, viral load)	0.59 (*Fn*, NE, age, pack-years, viral load)

Fn, *F. nucleatum*; NE, neutrophil elastase.

to the development of periodontal disease. In the present study, there was a significant association between neutrophil elastase and probing depth and attachment loss ($p < 0.001$). In support of our findings, Armitage *et al.* (37) reported higher GCF neutrophil elastase levels at progressing sites than at non-progressing sites in a longitudinal clinical trial. In our study, neutrophil elastase was also associated with the presence of *F. nucleatum*, *P. intermedia*, and *A. actinomycetemcomitans* ($0.001 < p < 0.01$). This suggested a plausible interaction between neutrophil elastase releasing granulocytes, and periodontopathic bacteria.

Our findings with respect to the associations of *F. nucleatum*, *P. intermedia*, *A. actinomycetemcomitans*, and *P. gingivalis* with measures of periodontal disease, i.e. probing depth and attachment loss, support the observations made by numerous other investigators (1, 2, 5). In the present study, *F. nucleatum* was the most prevalent bacteria, followed by *P. intermedia* and *A. actinomycetemcomitans*. It is noteworthy that *F. nucleatum* contributed significantly to the multifactorial risk profile of periodontal disease in regression models ($p < 0.05$).

In the present study, 111 HIV^+ patients (73%) were evaluated in the periodontitis group. In our previous study performed in non-HIV population, 55% of the study subjects were evaluated in the periodontitis group (16). In the current study, the rate of disease progression in the entire study population including periodontitis, gingivitis, and healthy subject groups was 5.3%. The rate of disease progression in periodontitis group alone was 7.3%. To our knowledge, there is no data available with respect to the rate of periodontal disease progression in HIV^+ patients. In a recent longitudinal study, the progression of attachment loss in non-HIV patients with early onset periodontitis has been reported (39). In that study, the percentages of sites with 2 mm or more attachment loss were 3.2% at the end of the first year, and 7.5% at the end of the second year. In the present study, the increased rate of incidence and periodontal disease progression in HIV^+ patients may be due in part to an immunosuppressed health status, such as the existence of high viral load level and a diffuse invasion of opportunistic bacterial infections and fungi into the gingival tissue, leading to destructive inflammatory response in the periodontal tissues.

The baseline and 6-month values of neutrophil elastase, β-glucuronidase, *F. nucleatum*, *P. intermedia*, and *A. actinomycetemcomitans* in active sites were significantly higher than those in inactive sites ($0.001 < p < 0.01$). The mean neutrophil elastase and β-glucuronidase values, and the percentages of *F. nucleatum*, *P. intermedia*, and *A. actinomycetemcomitans* of these 65 active sites at 6-month visits were significantly higher than those of active sites at baseline visits ($0.001 < p < 0.01$). This finding suggests a potential role for GCF enzymes and periodontopathic bacteria in the progression of periodontal disease in HIV^+ patients. In a cross-sectional study, Scully *et al.* (5) reported a higher prevalence of *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia*, *F. nucleatum*, and *C. rectus* in HIV-infected individuals than in HIV-negative controls with similar periodontal status.

With respect to the immunologic variables, only viral load levels were correlated significantly ($0.01 < p < 0.05$) with *F. nucleatum* and *P. intermedia*, suggesting that the subtle changes in the immune system may allow proliferation of more virulent clones of periodontal pathogens. Viral load also contributed significantly to the regression models for the prediction of attachment loss and probing depth measurements ($p < 0.05$). On the other hand, CD4 T-lymphocyte counts were not associated significantly with the clinical measurements. In a 20-month longitudinal study published in 1992, Barr *et al.* (3) reported an

increase in attachment loss in immunodeficient individuals ($CD4^+$ T lymphocytes < 200 cells/mm^3) who were older than 35 years of age. This difference in findings between Barr's study and our study may be explained by the fact that protease inhibitors were not commonly used in the treatment of HIV^+ subjects prior to 1992.

In the present study, *F. nucleatum*, neutrophil elastase, age, pack-years, and viral load contributed significantly to the regression model for the estimation of the attachment loss. This model explained 59% of the variability in mean attachment loss. Other risk factors such as traumatic occlusion, other bacterial and host factors, genetic influences, race, low education, stress, inadequate oral hygiene and infrequent dental attendance may account for the remaining variability in attachment loss. HIV^+ individuals with several of these identified risk factors should be monitored closely to prevent future attachment loss. Preventive measures include oral hygiene instructions, scaling and root planing, initial treatment evaluation, supportive periodontal therapy with 3-month intervals, local administration of antibiotics, and periodontal surgeries to achieve pocket elimination. These preventive measures may have a lowering effect on the GCF levels of destructive neutrophils enzymes such as β-glucuronidase and elastase as well as subgingival plaque bacteria such as *F. nucleatum*, *P. intermedia*, and *A. actinomycetemcomitans*.

Conclusions

In summary, *F. nucleatum*, *P. intermedia*, *A. actinomycetemcomitans*, elastase, β-glucuronidase, smoking pack-years, viral load, and age are risk factors for periodontitis in HIV^+ patients. HIV^+ patients with these identified risk factors should be monitored closely and preventive measures should be taken. The results of this study should be further validated in an epidemiologic field study to assess the predictive potential of these regression models for increase in probing depth and attachment loss in a real world setting.

Acknowledgements

The authors wish to thank Dr David W. Chambers, Colline Bell, and Dr Gene Gowdey for their valuable contribution to this investigation. This study was supported by NIDCR grant DE 12417.

References

1. Murray PA, Grassi M, Winkler JR. The microbiology of HIV-associated periodontal lesions. *J Clin Periodontol* 1989;**16:**636–642.
2. Murray PA. HIV disease as a risk factor for periodontal disease. *Compendium* 1994;**15:**1052–1063.
3. Barr C, Lopez MR, Rua-Dobles A. Periodontal changes by HIV serostatus in a cohort of homosexual and bisexual men. *J Clin Periodontol* 1992;**19:**794–801.
4. Yeung SCH, Stewart GJ, Cooper DA, Sindhusake D. Progression of periodontal disease in HIV seropositive patients. *J Periodontol* 1993;**61:**699–704.
5. Scully C, Porter SR, Mutlu S, Epstein JB, Glover S, Kumar N. Periodontopathic bacteria in English HIV-seropositive persons. *AIDS Patient Care STDS* 1999;**13:**369–374.
6. Dewhirst FE, Tamer MA, Ericson RE, *et al*. The diversity of periodontal spirochetes by 16S rRNA analysis. *Oral Microbiol Immunol* 2000;**15:**196–202.
7. Paster BJ, Boches SK, Galvin JL, *et al*. Bacterial diversity in human subgingival plaque. *J Bacteriol* 2001;**183:**3770–3783.
8. Alpagot T, Duzgunes N, Lee A, Bell C, Chambers DW, Gowdey G. Risk factors for periodontitis in HIV^+ patients. *J Dent Res* 2002;**81:**A–369.
9. Paster JP, Russell MK, Alpagot T, *et al*. Bacterial diversity in necrotizing ulcerative periodontitis in HIV-positive subjects. *Ann Periodontol* 2002;**7:**8–16.
10. Smith GLF, Cross DL. Comparison of periodontal disease in HIV seropositive subjects and controls: (II) Microbiology, immunology and predictors of disease progression. *J Clin Periodontol* 1995;**22:** 569–577.
11. Van Dyke TE, Serhan CN. Resolution of inflammation: a new paradigm for the pathogenesis of periodontal diseases. *J Dent Res* 2003;**82:**82–90.
12. Taubman MA *et al*. Host response in experimental periodontal disease. *J Dent Res* 1984;**63:**455.
13. Virca GD, Schnebli HP. The elastase/a1-proteinase inhibitor balance in the lung. *Schweiz Med Wochenschr* 1984;**114:**895–899.
14. Cimasoni G, Kowashi Y. Proteinases of the gingival crevice and their inhibitors. In: Lehner, T, Cimasoni, G, eds. *Borderland between caries and periodontal diseases*, **Vol. II**, London**:** Academic Press, 1980: 31–39.
15. Podhrasky J, Jany Z, Velgos S. Beta glucuronidase activity in human gingiva in health and periodontal disease. *Arch Oral Biol* 1982;**27:**615–616.
16. Alpagot T, Wolff LF, Smith QT, Tran S. Risk indicators for periodontal disease in a racially diverse urban population. *J Clin Periodontol* 1996;**23:**982–988.
17. Lamster IB, Holmes LG, Gros KBW, *et al*. The relationship of β-glucuronidase activity in crevicular fluid to clinical parameters of periodontal disease. *J Clin Periodontol* 1994;**21:**118–127.
18. Zambon JJ, Reynolds HS, Genco RJ. Studies of the subgingival microflora in patients with acquired immunodeficiency syndrome. *J Periodontol* 1990;**61:**699–704.
19. Rams TE, Andriolo M Jr, Feik D. Microbiological study of HIV-related periodontitis. *J Periodontol* 1991;**62:**74–81.
20. Glick M, Muzyka BC, Salkin LM, Lurie D. Necrotizing ulcerative periodontitis. A marker for immune deterioration and a predictor for the diagnosis of AIDS. *J Periodontol* 1994;**65:**393–397.
21. Tomar SL, Swango PA, Kleinman DV, Burt BA. Loss of periodontal attachment in HIV-seropositive military personnel. *J Periodontol* 1995;**66:**421–428.
22. Silness J, Loe H. Periodontal disease in pregnancy (II). Correlation between oral hygiene and periodontal condition. *Acta Odontol Scand* 1964;**22:**121–135.
23. Loe H, Silness J. Periodontal disease in pregnancy (I). Prevalence and severity. *Acta Odontol Scand* 1963;**21:**533–551.
24. Nakajima K, Powers JC, Ashe BM, Zimmerman M. Mapping the extended substrate binding site of Cathepsin G and human leukocyte elastase. Studies and peptide substrates related to the a1-protease inhibitor reactive site. *J Biol Chem* 1979;**254:**4027–4032.
25. Alpagot T, Silverman S, Lundergan WP, Bell C, Chambers D. Crevicular fluid elastase levels in relation to periodontitis and metabolic control of diabetes. *J Periodont Res* 2001;**36:**169–174.
26. Wolff LF, Anderson L, Sandberg GP, Aeppli DM, Shelburne CE. Fluorescence immunoassay for detecting periodontal bacterial pathogens in plaque. *J Clin Microbiol* 1991;**29:**1645–1651.
27. Wolff LF, Anderson L, Sandberg GP, Reither L. Bacterial Concentration Fluorescence Immunoassay (BCFIA) for the detection of periodontopathogens in plaque. *J Periodontol* 1992;**63:**1093–1101.
28. Page RC, Beck JD. Risk assessment for periodontal diseases. *Int Dent J* 1997;**47:** 61–87.

29. Locker D, Leake JL. Risk indicators and risk markers for periodontal disease experience in older adults living independently in Ontario, Canada. *J Dent Res* 1993;**72:**9–17.
30. Grossi SG, Zambon JJ, Ho AW, *et al*. Assessment of risk for periodontal disease. I. Risk indicators for attachment loss. *J Periodontol* 1994;**65:**260–267.
31. 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.
32. MacFarlane GD, Herzberg MC, Wolff LF, Hardie NA. Refractory periodontitis associated with abnormal polymorphonuclear leukocyte phagocytosis and cigarette smoking. *J Periodontol* 1992;**63:**908–913.
33. Trombelli L, Cho K-S, Kim C-K, Scapoli S, Scabbia A. Impaired healing response of periodontal furcation defects following flap debridement surgery in smokers. *J Clin Periodontol* 2003;**30:**81–87.
34. Stoltenberg JL, Osborn JB, Pihlstrom BL, *et al*. Association between cigarette smoking, bacterial pathogens, and periodontal status. *J Periodontol* 1993;**64:**1225–1230.
35. Bergstrom J, Preber H. Tobacco use as a risk factor. *J Periodontol* 1994;**65:**545–550.
36. 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.
37. 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.
38. Nakashima K, Demeurisse C, Cimasoni G. The recovery efficiency of various materials for sampling enzymes and polymorphonuclear leukocytes from gingival crevices. *J Clin Periodontol* 1994;**31:**479–483.
39. Kamma JJ, Baehni PC. Five year maintenance follow-up of early onset periodontitis patients. *J Clin Periodontol* 2003; **30:**562–572.