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Effect of smoking on concentrations of receptor activator of nuclear factor κ B ligand and osteoprotegerin in human gingival crevicular fluid

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Abstract

Aim: To compare the levels of the soluble receptor activator of nuclear factor κ B ligand (sRANKL), osteoprotegerin (OPG) and their relative ratio in gingival crevicular fluid (GCF) among periodontitis patients with varying smoking histories.

Material and Methods: GCF samples were collected from 149 periodontitis patients who were never smokers ($n = 58$), former smokers ($n = 39$) and current smokers ($n = 52$). sRANKL and OPG concentrations in GCF were measured by enzyme-linked immunosorbent assays.

Results: sRANKL, OPG and their relative ratio were not statistically significant among the never smokers, former smokers and current smokers. However, OPG was significantly reduced and subsequently the sRANKL:OPG ratio was significantly increased in the high pack-years group as compared with never smokers. The positive correlation between pack-years and the sRANKL:OPG ratio remained statistically significant after adjusting for age and current smoking status.

Conclusion: Increased lifetime exposure to cigarette smoking above a minimum threshold suppresses OPG production and leads to increased sRANKL:OPG. This may partially explain increased bone loss in smoking-related periodontitis.

Key words: gingival crevicular fluid; OPG; RANKL; smoking

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Periodontitis is a chronic disease associated with degradation of the periodontal tissues leading to attachment loss, bone loss and possibly tooth loss if left untreated. Smoking is recognized as one of the major risk factors for chronic periodontitis, whereby the relative risk of developing periodontitis increases by up to six times in smokers (Linden & Mullally 1994, Bergstrom 2004). A clear dose–response relationship between periodontitis and smoking has also been reported in several studies (Martinez-Canut et al. 1995, Alpagot et al. 1996). Despite this, the mechanisms by which smoking contributes to the pathogenesis of periodontitis are poorly understood. Previous studies indicate that smoking may interfere with several reparative and destructive factors such as the function of inflammatory cells and production of immune mediators (Ryder et al. 1998, Gustafsson et al. 2000, Petropoulos et al. 2004).

One of the main diagnostic features of periodontitis is alveolar bone loss. Numerous studies have shown that smokers have more severe and greater progression of alveolar bone loss than non-smokers (Bergstrom et al. 2000a, Jansson & Lavstedt 2002). Smoking is also related to a higher incidence of localized alveolitis (Sweet & Butler 1979) and delayed alveolar healing in extraction sockets (Pinto et al. 2002).

Conflict of interest and source of funding statement

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Bone remodelling is a coupled process between bone formation and bone resorption. It is regulated by molecular interactions between the receptor activator of nuclear factor κ B ligand (RANKL), its cellular receptor, RANK, and the decoy receptor, osteoprotegerin (OPG) (Kong et al. 1999). This has been demonstrated in disease models such as rheumatoid arthritis and periodontitis (Crotti et al. 2003, Firestein 2003, Taubman et al. 2005). RANKL and OPG are expressed by multiple cells in periodontal tissues such as fibroblasts, endothelial cells and osteoblasts. Their expression is modulated by inflammatory cytokines such as interleukin-1 and tumour necrosis factor-α (Sakata et al. 1999).

In the presence of macrophage colony-stimulating factor, RANKL is required for osteoclast differentiation. RANKL binds directly to RANK on the surface of pre-osteoclasts and osteoclasts, stimulating both the differentiation of osteoclast progenitors and the activity of mature osteoclasts (Hsu et al. 1999). Conversely, OPG is the naturally occurring inhibitor of osteoclast differentiation. It binds to RANKL with high affinity and blocks RANKL from interacting with RANK (Yasuda et al. 1998). Overall, bone resorption occurs as a result of the uncoupled process in bone remodelling and it may be reflected in an increased RANKL:OPG ratio. The increased ratio can be due to increased RANKL, decreased OPG or a combination of both.

In gingival tissues, periodontitis patients exhibit higher expression of RANKL (Crotti et al. 2003, Vernal et al. 2004, Bostanci et al. 2007b) and lower expression of OPG (Liu et al. 2003) compared with healthy controls. Similar results have been reported for levels in gingival crevicular fluid (GCF) (Mogi et al. 2004, Vernal et al. 2004, Bostanci et al. 2007a). As a consequence, RANKL:OPG ratios are found to be increased in periodontitis patients compared with healthy controls (Liu et al. 2003, Mogi et al. 2004, Bostanci et al. 2007a, b). In a group of untreated periodontitis patients, Vernal et al. (2004) showed that higher levels of RANKL in GCF were associated with active sites compared with inactive sites. However, most of the mentioned studies failed to report the proportions of smokers within the study groups. Considering that smoking is one of the major risk factors in chronic periodontitis, more studies are warranted to determine the effect of smoking on the modulation of RANKL and OPG expression.

Because RANKL and OPG are key regulators in alveolar bone loss in periodontitis and the severity of periodontal breakdown is increased in smokers, we hypothesized that the RANKL:OPG ratio is higher in smokers compared with non-smokers, among periodontitis patients. In an effort to partially explain the greater amount of bone loss seen in smokers compared with non-smokers, we aimed to measure the levels of OPG, sRANKL and their relative ratio in GCF of periodontitis patients and to investigate the relationship between these biomarkers and cigarette smoking.

Material and Methods

Subject selection

Periodontitis patients attending clinics at the Adelaide Dental Hospital were invited to participate in the study. Written and informed consent were obtained from each subject before enrolment in the study. Complete medical histories were obtained after the enrolment. The exclusion criteria included pregnant women and sites with evidence of suppuration or clinical or radiographic evidence of endodontic pathology. This study was approved by the Human Ethics Research Committee of The University of Adelaide.

Smoking history was collected by means of self-reporting following a standardized questionnaire. Patients were then classified as current smokers (regular daily smokers), former smokers (previous regular smokers who had ceased the habit) or never smokers (never smoked cigarettes). The life-time smoking exposure of former and current smokers was expressed as pack-years: calculated as the number of cigarettes smoked per day multiplied by the number of years the patient had smoked, divided by 20 (a standard pack of cigarettes). The life-time exposure of these former and current smokers was subsequently categorized into $\geqslant 20$ pack-years, <20 pack-years.

All subjects underwent a clinical periodontal examination, and the presence and severity of periodontitis was ascertained from the documentation in each subject's dental records and assessment of past dental radiographs. Using the modified Hugoson & Jordan (1982) classification, subjects were divided based on the following criteria: no discernible radiographic evidence of bone loss (representing no periodontal disease, P0), proximal bone loss reaching at most one-third of normal bone height (mild periodontal disease, P1), proximal bone loss between one-third and two-thirds of normal bone height (moderate periodontitis, P2) or proximal bone loss more than two-thirds of normal bone height (severe periodontitis, P3).

Site selection and GCF sampling

Each subject had GCF samples taken from the two periodontal sites that had the deepest periodontal pockets. These sites were selected after full-mouth probing measurements of probing pocket depth (PPD) at six sites per tooth. The probing was carried out at the same visit during which GCF was sampled. The presence or absence of bleeding at the sampled site following probing was recorded. GCF was sampled by previously published methods (Offenbacher et al. 1986) with slight modifications. Briefly, all clinically detectable supragingival plaque was removed without touching the gingiva in order to minimize contamination of the paper strips by plaque. The sites under study were isolated with cotton rolls and gently dried with an air syringe. A saliva ejector was used to avoid salivary contamination if necessary. PerioPaper™ strips (Oraflow Inc., Plainview, NY, USA) were carefully inserted 1 mm into the gingival crevice for 1 min. The volume of GCF collected was measured with a Periotron 8000 (Harco, Tustin, CA, USA). The Periotron readings were then converted through a calibration curve to obtain the volume. One paper strip was used for each collection site. The paper strips from the individual sites were stored at -20°C until processed.

GCF was eluted from the paper strips with phosphate-buffered saline, pH 7.2, and collected following centrifugation as described previously (Fitzsimmons et al. 2009). The eluted samples from each strip were stored at -20°C until further analysis.

sRANKL and OPG analysis in GCF

The amount of sRANKL and OPG in GCF was determined using commercially available Enzyme Linked Immunosorbent Assay (ELISA) kits (R&D Systems, Minneapolis, MN, USA for

OPG and Biomedica, Vienna, Austria for sRANKL) in accordance with the manufacturers' instructions. The lower detection thresholds for the OPG and sRANKL ELISA assays were 50.8 and 1.6 pg/ml, respectively. Calculation of sRANKL and OPG concentrations in GCF was performed by dividing the total amount by the volume of the GCF collected. Data were reported as the amount of sRANKL or OPG/μl of GCF.

Statistical analysis

Descriptive statistics and frequency distribution curves were generated for sRANKL and OPG. The data were markedly skewed, particularly for OPG, where 74.8% of samples were below the detection threshold. For statistical analysis, the data were therefore log-transformed for individual mediators. Constants were used in these formulas so that a mathematically meaningful ratio of the transformed data could be computed that did not have negative values. The ratio of transformed variables represented the third dependent variable used in the analysis:

$$\text{sRANKL : OPG ratio} = \frac{\text{transformed sRANKL}}{\text{transformed OPG}}$$

Additional descriptive statistics were generated for smoking history and the demographic characteristics of the subjects.

Statistical analysis was undertaken to test hypotheses concerning the relationship between cigarette consumption and three dependant variables: transformed sRANKL, transformed OPG and the sRANKL:OPG ratio. It was expected that the mediators could be influenced separately by the qualitative experience of cigarette smoking (current, former, never) and the quantitative amount of exposure (pack-years); therefore, multivariate statistical models were constructed separately for each dependant variable. Age was expected to be associated with the mediators and with smoking, and so it was included as a covariate in the models. Because mediators were measured at two sites per subject, generalized estimating equations were used to adjust for clustering of observations within subjects. This was achieved in SAS Proc Genmod assuming an independent working correlation matrix. Each dependant variable was modelled as a linear variable using the identity link function. The statistical significance of parameter estimates was based on Wald's tests and 95% confidence intervals calculated using empirical standard error estimates, and $p \leqslant 0.05$ was considered to be statistically significant.

Results

Subject demographics

A total of 149 patients were recruited into the study with 56 males and 93 females, ranging in age from 26 to 86 years. Fifty-eight patients were classified as never smokers, 39 as former smokers and 52 as current smokers (Table 1). Overall, former smokers exhibited the deepest PPD, and the largest GCF volume collected, whereas current smokers showed the largest amount of gingival recession and the lowest volume of GCF collected. Current smokers had the highest percentage of patients suffering from moderate periodontitis, while former smokers had the highest percentage of people suffering from severe periodontitis. Although the mean number of cigarettes smoked per day was equal in former and current smokers, the mean duration of smoking and pack-years of smokers was higher among current smokers. The mean smoking-free time of former smokers since smoking cessation was 13.5 years (data not shown).

When subjects were stratified based on pack-years, there were 51 patients who smoked <20 pack-years, and 39 patients smoked $\geqslant 20$ pack-years (Table 2). The majority of former smokers were in the <20 pack-year group and current smokers were approximately equal in the two groups. The mean smoking-free time of former smokers who had smoked $\geqslant 20$ pack-years was calculated as 7.9 years, and for former smokers who had smoked <20 pack-years, it was 16.6 years (data not tabulated).

sRANKL and OPG concentrations in GCF

Out of the total of 298 sites analysed, sRANKL and OPG were detectable in GCF collected from 234 and 75 sites, respectively.

The log-transformed data were firstly analysed by stratification across three age groups and are shown in Table 3. The log-transformed OPG concentration was the highest in the oldest age group. The opposite was true for sRANKL, where the log-transformed sRANKL concentration was the highest in the youngest age group of <45 years. Neither of these observations was statistically significant. However, the log-transformed sRANKL:OPG ratio was significantly lower in the $\geqslant 65$-year age group compared with the <45-year age group ($p = 0.01$).

When subjects were categorized by smoking status, the log-transformed OPG concentration tended to be lower in the former and current smokers com-

Table 2. Subject demographics based on pack-years in the study group

	Never smoker	<20 pack-years	$\geqslant 20$ pack-years
Number of subjects	58	51	39
Mean age (years)	60	52	54
Former smokers	–	24	15
Current Smokers	–	27	24

Table 1. Subject demographics and clinical parameters of the sampling areas in the study group

	Never smoker	Former smoker	Current smoker
Number of subjects	58	39	52
Number of female:male	42:16	19:20	32:20
Age (years)	60 ± 15	57 ± 15	49 ± 10
PPD (mm)	4.9 ± 1.4	5.4 ± 1.4	5.1 ± 1.4
REC (mm)	1.1 ± 1.1	1.2 ± 1.3	1.3 ± 1.1
GCF volume (μl)	1.13 ± 0.59	1.16 ± 0.58	1.01 ± 0.62
P1	19 (33%)	8 (21%)	12 (23%)
P2	22 (38%)	14 (36%)	22 (42%)
P3	17 (29%)	17 (44%)	18 (35%)
Mean cigarettes/day	0	16.4 ± 11.7	16.5 ± 7.8)
Mean duration of smoking (years)	0	21.3 ± 13.3	28.0 ± 10.7
Pack-years	0	18.3 ± 16.1	22.8 ± 15.1

Values represent the means ± standard deviations or numbers of subjects (in parentheses % of subjects); PPD, probing pocket depth; REC, gingival recession; GCF, gingival crevicular fluid.

Table 3. sRANKL and OPG concentrations in GCF between different age groups (person-level analysis)

Age (years)	$N^{\dagger}$	Log-transformed OPG	Log-transformed sRANKL	Log-transformed sRANKL:OPG
<45	37	0.6	5.8	68.4
45–64	65	0.8	5.0	54.1
⩾65	46	1.7	5.2	42.9*

†Number of subjects.
*Significantly different compared with <45-year age group ($p = 0.01$) – Wald's test.

Table 4. sRANKL and OPG concentrations in GCF between smoking and pack-years groups (person-level analysis)

	$N^{\dagger}$	Log-transformed OPG	Log-transformed sRANKL	Log-transformed sRANKL:OPG
Never smoker	58	1.3	5.0	47.0
Former smoker	38	0.8	5.8	65.5
Current smoker	52	0.8	5.2	54.0
<20 pack-years	51	1.0	5.3	51.5
⩾20 pack-years	39	0.6*	5.5	68.4#

†Number of subjects.
*Significantly different compared with the never smoker group ($p = 0.05$) – Wald's test.
#Significantly different compared with the never smoker group ($p = 0.03$) – Wald's test.
sRANKL, soluble receptor activator of nuclear factor κ B ligand; OPG, osteoprotegerin; GCF, gingival crevicular fluid.

pared with the never smokers (Table 4). The opposite was observed with the log-transformed sRANKL concentration, where higher concentrations were measured in GCF of former and current smokers compared with the never smokers. As a consequence, the sRANKL:OPG ratio also appeared to be higher in the former and current smokers compared with never smokers. However, none of these relationships were statistically significant.

When the data were analysed based on smoking history in terms of pack-years, the log-transformed OPG concentration decreased with increasing pack-years, with the value being significantly lower in the high pack-years group (⩾20 pack-years) compared with the never-smoker group ($p = 0.05$) (Table 4). Although the log-transformed sRANKL appeared to be higher in the ⩾20 pack-years group, the difference was not statistically significant. Despite this, the log-transformed sRANKL:OPG ratio was significantly higher in the high pack-years group (⩾20 pack-years) compared with the never-smoker group ($p = 0.03$). In the multivariate analysis adjusting for age and smoking status, the log-transformed sRANKL:OPG ratio remained significantly higher in the high pack-years group compared with the never-smoker group ($p = 0.03$) (Table 4).

Discussion

Smokers with periodontitis show increased disease severity and alveolar bone loss. In order to partially explain these observations, the current study explored the inter-relationship between smoking and the RANKL:OPG axis in GCF of periodontitis patients. The concentrations of OPG, sRANKL in GCF and the sRANKL:OPG ratio were not significantly different between current smokers and former smokers when compared with never smokers. However, when pack-years were taken into consideration, significant differences could be found between the high pack-years group and the never smoker group. A negative correlation was observed between OPG concentration in GCF and pack-years. In addition, a positive correlation was found between the sRANKL:OPG ratio and pack-years. Furthermore, the high pack-year group showed a significantly higher sRANKL:OPG ratio compared with the never-smoker group even after adjusting for age and current smoking status.

Although OPG and RANKL have previously been measured in GCF of periodontitis patients, no consideration has been given to the smoking history of patients. Lower OPG concentrations, together with increased RANKL in GCF of periodontitis patients, have been reported compared with healthy controls (Mogi et al. 2004, Lu et al. 2006, Bostanci et al. 2007a). The non-significant changes measured in RANKL concentrations in the current study may be reflective of the large number of GCF samples that were below the level of detection of the assays. These results, however, are consistent with other studies that have also reported a significant proportion of GCF samples with undetectable levels of RANKL and OPG (Lu et al. 2006, Arikan et al. 2008). Nevertheless, the high number of samples below the detection threshold for OPG could be related to the low levels of OPG in GCF in our present periodontitis populations. Alternatively, the levels of OPG and RANKL in GCF may have been below the sensitivity of the ELISA assay used in this study.

Our results are consistent with studies that have assessed the impact of smoking on the concentration of these mediators in serum, saliva and GCF (Lappin et al. 2007, Buduneli et al. 2008, 2009). These studies showed decreased OPG concentrations and increased RANKL:OPG ratios in smokers compared with non-smokers. A negative correlation also existed between OPG concentration and pack-years. Another group measuring gene expression in gingival tissues for both mediators in periodontitis patients showed similar results in periodontitis patients who were smokers and non-smokers, but the influence of pack-years was not considered (Cesar-Neto et al. 2007).

These findings may indicate that greater alveolar bone loss among smokers in periodontitis patients could be related to the suppression of OPG expression by smoking cigarettes, leading to a higher sRANKL:OPG ratio compared with never smokers. In addition, the influence of smoking on the concentrations of the two mediators, particularly OPG, is dependent on the quantitative amount of cigarette exposure, and not the qualitative experience of smoking. Our data suggest that a minimum threshold of lifetime exposure is required before cigarette smoking starts to have an effect on OPG levels and the sRANKL:OPG ratio, which is in accordance with earlier studies (Martinez-Canut et al. 1995). A reduction in periodontal bone height is also shown to be significantly associated with lifetime exposure (Bergstrom et al. 2000b),

which may be partially a result of modulated RANKL:OPG ratios.

Based on the three age groups studied, the sRANKL:OPG ratio was significantly lower in older people compared with younger people. This finding may indicate that the progression of alveolar bone loss is more evident among the younger periodontitis patients as compared with the older periodontitis patients. While this may appear to be counter-intuitive as the prevalence of periodontitis naturally increases with age (Albandar et al. 1999, Albandar 2002), alveolar bone loss as a result of periodontitis is mostly irreversible, and so epidemiological measures of periodontitis mostly capture the cumulative experience of the disease. Nevertheless, age is an important factor in identifying individuals susceptible to periodontitis progression. The amount of bone loss in relation to a patient's age may be a good predictor of future disease progression. A young individual with aggressive periodontitis is considered to be at a higher risk of disease progression than an older individual with the same amount of disease (Heitz-Mayfield 2005). It can be proposed that the greater susceptibility to progression of alveolar bone loss in the younger group may be reflected in the sRANKL:OPG ratio. Interestingly, current smokers in this study were younger than former smokers and never smokers. However, the multivariate analysis showed age to be an independent factor influencing the sRANKL:OPG ratio. Therefore, further studies are warranted to study the relationships between the sRANKL:OPG ratio and periodontal disease susceptibility.

The mechanism by which OPG levels are reduced in GCF is yet to be elucidated. Several cell types within the periodontal tissue may contribute to the presence of OPG in GCF. These cells may be negatively affected by the direct and indirect effects of nicotine and other chemicals found in cigarettes leading to reduced OPG production. Indeed, gene and protein expression of OPG in gingival biopsies from smokers with periodontitis is decreased compared with non-smokers (Cesar-Neto et al. 2007). Furthermore, the combination of lipopolysaccharide and nicotine is shown to decrease OPG production in osteoblasts in a dose-dependent manner (Tanaka et al. 2006). Few other studies have investigated the direct effects of nicotine or other toxic components of cigarette smoke on OPG production by other cell types. However, periodontal ligament fibroblasts and epithelial cells directly exposed to nicotine decrease their overall protein synthesis (Giannopoulou et al. 2001, Chang et al. 2002). These studies may suggest a reason as to why OPG protein levels are decreased in GCF of smokers. The resultant increased sRANKL:OPG ratio may then lead to an imbalance in tissue homeostasis and tissue degradation seen in smokers with periodontitis.

In summary, the results of the present study show that reduced concentrations of OPG in GCF and a subsequent increase in sRANKL:OPG ratios occur in periodontitis patients with a history of cigarette smoking. The study found that an increased lifetime exposure to cigarette smoking above a minimum threshold is required for these patterns to be observed. These data build on previous studies that have not quantitatively considered the effect of smoking on the concentration of these mediators in GCF from patients suffering from periodontitis. Further studies are still required, however, to identify the mechanisms as to how the chemicals of cigarette smoke modulate these mediators. This may then allow a better understanding of the regulation of these mediators and their involvement in alveolar bone loss, a common characteristic of periodontitis patients who are also smokers.

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References

Albandar, J. M. (2002) Global risk factors and risk indicators for periodontal diseases. *Periodontology 2000* **29**, 177–206.

Albandar, J. M., Brunelle, J. A. & Kingman, A. (1999) Destructive periodontal disease in adults 30 years of age and older in the United States, 1988–1994. *Journal of Periodontology* **70**, 13–29.

Alpagot, T., Wolff, L. F., Smith, Q. T. & Tran, S. D. (1996) Risk indicators for periodontal disease in a racially diverse urban population. *Journal of Clinical Periodontology* **23**, 982–988.

Arikan, F., Buduneli, N. & Kutukculer, N. (2008) Osteoprotegerin levels in peri-implant crevicular fluid. *Clinical Oral Implants Research* **19**, 283–288.

Bergstrom, J. (2004) Tobacco smoking and chronic destructive periodontal disease. *Odontology* **92**, 1–8.

Bergstrom, J., Eliasson, S. & Dock, J. (2000a) A 10-year prospective study of tobacco smoking and periodontal health. *Journal of Periodontology* **71**, 1338–1347.

Bergstrom, J., Eliasson, S. & Dock, J. (2000b) Exposure to tobacco smoking and periodontal health. *Journal of Clinical Periodontology* **27**, 61–68.

Bostanci, N., Ilgenli, T., Emingil, G., Afacan, B., Han, B., Toz, H., Atilla, G., Hughes, F. J. & Belibasakis, G. N. (2007a) Gingival crevicular fluid levels of RANKL and OPG in periodontal diseases: implications of their relative ratio. *Journal of Clinical Periodontology* **34**, 370–376.

Bostanci, N., Ilgenli, T., Emingil, G., Afacan, B., Han, B., Toz, H., Berdeli, A., Atilla, G., McKay, I. J., Hughes, F. J. & Belibasakis, G. N. (2007b) Differential expression of receptor activator of nuclear factor-kappaB ligand and osteoprotegerin mRNA in periodontal diseases. *Journal of Periodontal Research* **42**, 287–293.

Buduneli, N., Biyikoglu, B., Sherrabeh, S. & Lappin, D. F. (2008) Saliva concentrations of RANKL and osteoprotegerin in smoker versus non-smoker chronic periodontitis patients. *Journal of Clinical Periodontology* **35**, 846–852.

Buduneli, N., Buduneli, E. & Kütükçüler, N. Interleukin-17, RANKL, Osteoprotegerin Levels in Gingival Crevicular Fluid from Smoker versus Non-smoker Chronic Periodontitis Patients during Initial Periodontal Treatment. *Journal of Periodontology* EPub before print, doi: 10.1902/jop.2009.090106.

Cesar-Neto, J. B., Duarte, P. M., de Oliveira, M. C., Tambeli, C. H., Sallum, E. A. & Nociti, F. H. Jr. (2007) Smoking modulates interleukin-6: interleukin-10 and RANKL:osteoprotegerin ratios in the periodontal tissues. *Journal of Periodontal Research* **42**, 184–191.

Chang, Y. C., Huang, F. M., Tai, K. W., Yang, L. C. & Chou, M. Y. (2002) Mechanisms of cytotoxicity of nicotine in human periodontal ligament fibroblast cultures in vitro. *Journal of Periodontal Research* **37**, 279–285.

Crotti, T., Smith, M. D., Hirsch, R., Soukoulis, S., Weedon, H., Capone, M., Ahern, M. J. & Haynes, D. (2003) Receptor activator NF kappaB ligand (RANKL) and osteoprotegerin (OPG) protein expression in periodontitis. *Journal of Periodontal Research* **38**, 380–387.

Firestein, G. S. (2003) Evolving concepts of rheumatoid arthritis. *Nature* **423**, 356–361.

Fitzsimmons, T. R., Sanders, A. E., Bartold, P. M. & Slade, G. D. (2009) Biomarkers of periodontal inflammation in the Australian adult population. *Australian Dental Journal*, **54**, 115–122.

Giannopoulou, C., Roehrich, N. & Mombelli, A. (2001) Effect of nicotine-treated epithelial cells on the proliferation and collagen production of gingival fibroblasts. *Journal of Clinical Periodontology* **28**, 769–775.

Gustafsson, A., Asman, B. & Bergstrom, K. (2000) Cigarette smoking as an aggravating factor in inflammatory tissue-destructive diseases. Increase in tumor necrosis factor-alpha priming of peripheral neutrophils measured as generation of oxygen radicals. *International Journal of Clinical Laboratory Research* **30**, 187–190.

Heitz-Mayfield, L. J. (2005) Disease progression: identification of high-risk groups and individuals for periodontitis. *Journal of Clinical Periodontology* **32** (Suppl. 6), 196–209.

Hsu, H., Lacey, D. L., Dunstan, C. R., Solovyev, I., Colombero, A., Timms, E., Tan, H. L., Elliott, G., Kelley, M. J., Sarosi, I., Wang, L., Xia, X. Z., Elliott, R., Chiu, L., Black, T., Scully, S., Capparelli, C., Morony, S., Shimamoto, G., Bass, M. B. & Boyle, W. J. (1999) Tumor necrosis factor receptor family member RANK mediates osteoclast differentiation and activation induced by osteoprotegerin ligand. *Proceedings of the National Academy of Sciences of the United States of America* **96**, 3540–3545.

Hugoson, A. & Jordan, T. (1982) Frequency distribution of individuals aged 20–70 years according to severity of periodontal disease. *Community Dentistry and Oral Epidemiology* **10**, 187–192.

Jansson, L. & Lavstedt, S. (2002) Influence of smoking on marginal bone loss and tooth loss – a prospective study over 20 years. *Journal of Clinical Periodontology* **29**, 750–756.

Kong, Y. Y., Yoshida, H., Sarosi, I., Tan, H. L., Timms, E., Capparelli, C., Morony, S., Oliveira-dos-Santos, A. J., Van, G., Itie, A., Khoo, W., Wakeham, A., Dunstan, C. R., Lacey, D. L., Mak, T. W., Boyle, W. J. & Penninger, J. M. (1999) OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. *Nature* **397**, 315–323.

Lappin, D. F., Sherrabeh, S., Jenkins, W. M. & Macpherson, L. M. (2007) Effect of smoking on serum RANKL and OPG in sex, age and clinically matched supportive-therapy periodontitis patients. *Journal of Clinical Periodontology* **34**, 271–277.

Linden, G. J. & Mullally, B. H. (1994) Cigarette smoking and periodontal destruction in young adults. *Journal of Periodontology* **65**, 718–723.

Liu, D., Xu, J. K., Figliomeni, L., Huang, L., Pavlos, N. J., Rogers, M., Tan, A., Price, P. & Zheng, M. H. (2003) Expression of RANKL and OPG mRNA in periodontal disease: possible involvement in bone destruction. *International Journal of Molecular Medicine* **11**, 17–21.

Lu, H. K., Chen, Y. L., Chang, H. C., Li, C. L. & Kuo, M. Y. (2006) Identification of the osteoprotegerin/receptor activator of nuclear factor-kappa B ligand system in gingival crevicular fluid and tissue of patients with chronic periodontitis. *Journal of Periodontal Research* **41**, 354–360.

Martinez-Canut, P., Lorca, A. & Magan, R. (1995) Smoking and periodontal disease severity. *Journal of Clinical Periodontology* **22**, 743–749.

Mogi, M., Otogoto, J., Ota, N. & Togari, A. (2004) Differential expression of RANKL and osteoprotegerin in gingival crevicular fluid of patients with periodontitis. *Journal of Dental Research* **83**, 166–169.

Offenbacher, S., Odle, B. M. & Van Dyke, T. E. (1986) The use of crevicular fluid prostaglandin E2 levels as a predictor of periodontal attachment loss. *Journal of Periodontal Research* **21**, 101–112.

Petropoulos, G., McKay, I. J. & Hughes, F. J. (2004) The association between neutrophil numbers and interleukin-1alpha concentrations in gingival crevicular fluid of smokers and non-smokers with periodontal disease. *Journal of Clinical Periodontology* **31**, 390–395.

Pinto, J. R., Bosco, A. F., Okamoto, T., Guerra, J. B. & Piza, I. G. (2002) Effects of nicotine on the healing of extraction sockets in rats. A histological study. *Brazilian Dental Journal* **13**, 3–9.

Ryder, M. I., Fujitaki, R., Johnson, G. & Hyun, W. (1998) Alterations of neutrophil oxidative burst by in vitro smoke exposure: implications for oral and systemic diseases. *Annals of Periodontology* **3**, 76–87.

Sakata, M., Shiba, H., Komatsuzawa, H., Fujita, T., Ohta, K., Sugai, M., Suginaka, H. & Kurihara, H. (1999) Expression of osteoprotegerin (osteoclastogenesis inhibitory factor) in cultures of human dental mesenchymal cells and epithelial cells. *Journal of Bone and Mineral Research* **14**, 1486–1492.

Sweet, J. B. & Butler, D. P. (1979) The relationship of smoking to localized osteitis. *Journal of Oral Surgery* **37**, 732–735.

Tanaka, H., Tanabe, N., Shoji, M., Suzuki, N., Katono, T., Sato, S., Motohashi, M. & Maeno, M. (2006) Nicotine and lipopolysaccharide stimulate the formation of osteoclast-like cells by increasing macrophage colony-stimulating factor and prostaglandin E2 production by osteoblasts. *Life Sciences* **78**, 1733–1740.

Taubman, M. A., Valverde, P., Han, X. & Kawai, T. (2005) Immune response: the key to bone resorption in periodontal disease. *Journal of Periodontology* **76**, 2033–2041.

Vernal, R., Chaparro, A., Graumann, R., Puente, J., Valenzuela, M. A. & Gamonal, J. (2004) Levels of cytokine receptor activator of nuclear factor kappaB ligand in gingival crevicular fluid in untreated chronic periodontitis patients. *Journal of Periodontology* **75**, 1586–1591.

Yasuda, H., Shima, N., Nakagawa, N., Yamaguchi, K., Kinosaki, M., Mochizuki, S., Tomoyasu, A., Yano, K., Goto, M., Murakami, A., Tsuda, E., Morinaga, T., Higashio, K., Udagawa, N., Takahashi, N. & Suda, T. (1998) Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. *Proceedings of the National Academy of Sciences of the United States of America* **95**, 3597–3602.

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Clinical Relevance

Scientific rationale for the study: sRANKL and OPG regulate bone resorption and may be modulated by cigarette smoking in periodontitis. We examined the relationship among sRANKL, OPG and their relative ratio in GCF with periodontitis patient smoking history.

Principal findings: A significant increase in the sRANKL:OPG ratios in GCF of periodontitis patients was positively correlated to smoking pack-years and remained significant after adjusting for age and smoking status.

Practical implications: Lifetime cigarette smoking exposure may contribute to periodontal tissue degradation by modulating OPG and sRANKL:OPG ratios in GCF. Pack-years smoking history may provide a better measure of the impact of smoking on bone resorption.