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Effect of smoking on serum RANKL and OPG in sex, age and clinically matched supportive-therapy periodontitis patients

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Abstract

Methods: The serum concentrations of receptor activator of nuclear factor κB ligand (RANKL) and osteoprotegerin (OPG) in age- and sex-matched groups of smokers and non-smokers with almost identical levels of periodontal disease were determined by an enzyme-linked immunosorbent assay (ELISA).

We ensured that the 35 smokers were gender, age and clinically matched with a group of 35 non-smokers (confirmed by cotinine immunoassay) from the same population of maintained patients with susceptibility to periodontitis.

Results: Cigarette smoker patients tended to have lower serum concentrations of RANKL and OPG than non-smoker patients. While no statistically significant difference was observed for RANKL, there were significant differences in the median serum concentration of OPG (smokers 23.76 pM, non-smokers 59.28 pM) and the ratio of serum concentrations of RANKL and OPG. Concentrations of OPG in the smoker patients also had a statistically significant negative correlation with tobacco consumption.

Conclusion: Bone loss in smoker-related periodontitis patients may be partially explained by suppression of OPG production.

Key words: health; OPG; periodontitis; RANKL; smoking

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Periodontitis is a chronic inflammatory disease generally considered to be caused by infection with Gram-negative bacteria and characterized by gingival inflammation and alveolar bone resorption (Page 1991). Bone resorption and bone formation are processes that are ''coupled'' even though there is evidence suggesting that each can take place independently (Corral et al. 1998). This coupling process requires that osteoblast cells are recruited to areas that are being degraded by osteoclast cells and are stimulated to replace bone that has been removed.

The function of osteoclasts, which cause resorption of alveolar bone in periodontal disease, is regulated by interaction with periodontal ligament cells. The periodontal ligament fibroblasts appear to be involved in both stimulatory and inhibitory processes. High levels of receptor activator of nuclear factor κB (NF-κB) ligand (RANKL) are expressed during root resorption of deciduous teeth and high levels of osteoprotegerin (OPG) are expressed by these cells where no root resorption is normally taking place, i.e. of permanent teeth (Fukushima et al. 2003). Gingival and periodontal ligament fibroblasts are involved in the development of osteoclasts and

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predominantly play a role in protecting teeth by producing OPG. In this respect, gingival fibroblasts have been shown to produce higher levels of OPG and may have a greater protective effect than the periodontal ligament fibroblasts (De Vries et al. 2006).

In addition, a large number of mediators associated with osteoclast differentiation and function are involved in this process. For example in periodontal disease, Th1 cytokines, such as interferon-γ and Th2 cytokines, including interleukin-4 and interleukin-10, appear to be associated with activation and suppression of bone resorption, respectively (Eastcott et al. 1994, Kawashima & Stashenko 1999, Kawai et al. 2000, Garlet et al. 2006).

An essential cytokine system for osteoclast biology has been characterized (Suda et al. 1999, Teitelbaum 2000) and consists of a ligand, RANKL (Anderson et al. 1997, Lacey et al. 1998, Yasuda et al. 1998a), a cellular receptor, receptor activator of NF-κB (RANK) (Anderson et al. 1997, Hsu et al. 1999) and a soluble decoy receptor, OPG (Simonet et al. 1997, Yasuda et al. 1998b). The function of the decoy receptor is to reduce interaction between RANKL and the cell surface receptor RANK such that while RANKL potentially stimulates osteoclasts to increase bone resorption, RANKL is blocked by OPG and this reduces the amount of bone resorption taking place (Fuller et al. 1998, Lacey et al. 1998).

It has been reported that a significantly increased concentration of RANKL and a decreased concentration of RANKL decoy receptor OPG occurs in the gingival crevicular fluid (GCF) of patients with periodontitis (Mogi et al. 2004). In addition, the RANKL/OPG ratio in the GCF of periodontitis patients has been shown to be higher than that for the synovial fluid of patients with rheumatoid arthritis (Kotake et al. 2001). The RANKL/OPG ratio is also increased and correlated with markers of bone resorption and markers of disease activity in multiple myeloma (Terpos et al. 2003).

The expression of OPG in periodontal diseases is decreased in both moderate periodontitis and advanced periodontitis stages, while RANKL mRNA is increased in periodontitis at the advanced stage (Liu et al. 2002). However, an increased ratio of RANKL to OPG mRNA in periodontitis may determine local osteoclastogenesis and osteoclastic bone resorption. In other words, it might play an important role in modulating the localized bone loss.

In a comparison of granulation tissue in chronic periodontitis with gingival tissue from patients without periodontitis, higher levels of RANKL and lower levels of OPG were expressed in the periodontitis tissue (Crotti et al. 2003).

In light of the above publications, a reasonable hypothesis is that the bone destruction seen in periodontitis is associated with a perturbation in the balance of RANKL and OPG, for example, elevated levels of RANKL and/or lower levels of OPG protein in tissue fluids and serum result in an excess amount of free RANKL capable of stimulating RANK, thereby increasing bone resorption by osteoclasts.

Evidence from cross-sectional and case–control studies in various populations and an abundant number of reviews on the subject have shown that adult smokers are about two to four times more likely than non-smokers to have periodontitis (Johnson 1994, Ainamo & Ainamo 1996, Johnson & Hill 2004). However, the influence of cigarette smoking on RANKL and OPG in periodontitis patients remains unknown.

The aim of this study was therefore to investigate the effects of cigarette smoking on the serum concentrations of RANKL and OPG in chronic periodontitis patients. However, to minimize the influence of other factors such as gender, age or the severity of periodontal disease, matched smoker and non-smoker patient groups were selected.

Methods

Ethics and informed consent

Before the start of this study, ethical approval was obtained from the Glasgow Dental Hospital and School Ethics Committee. All the patients who participated were informed of the nature of the study and provided with a copy of the protocol and a consent form, which, if they were willing to participate, they duly signed. They were also informed that they had the right to withdraw from the study at any time.

Patient recruitment

Patients were recruited to the study as follows: the patient databanks of the Periodontal Department of Glasgow Dental Hospital and School were searched to identify patients who had presented for treatment with a Community Periodontal Index of Treatment Need (CPITN) score of 4 in at least one sextant, had then completed a course of periodontal treatment in the Department and had been discharged for on-going maintenance by their general dental practitioner. Former patients who had been discharged between 3 and 5 years previously were contacted and invited to attend a screening clinic.

Inclusion criteria

At the screening clinic, patients were regarded as suitable for the study if they were affected by chronic periodontitis and had at least 16 teeth, including at least four molars, and had, in different quadrants, at least two periodontal pockets at least 5 mm in depth, with a minimum of 2 mm attachment loss.

Exclusion criteria

Patients were excluded if they presented with acute or advanced periodontal disease manifested by abscesses or tooth hypermobility; if there were oral conditions present that could interfere with examination procedures; if they presented with any other dental problem; if they were pregnant; if they suffered from cancer, cardiovascular disease, liver dysfunction, hepatitis or any disease requiring continuous medication; if they had received antibiotic therapy within the past 3 months; or if they had taken non-steroidal anti-inflammatory drugs in the past 6 weeks.

Smokers who smoked fewer than 10 cigarettes per day and non-smokers, if they were former smokers, were also excluded.

One hundred and fifty-eight patients (35 smokers and 123 non-smokers) met all the inclusion criteria and none of the exclusion criteria.

Clinical measurements

Both smokers and non-smokers were selected from the same treated and maintained patient population. At the start of the study, blood was collected and the patients underwent a detailed periodontal examination. A mean score was derived for the full-mouth clinical measurements (number of teeth, pocket depth, loss of clinical attachment, gingival

recession and % bleeding on probing) for each patient. In addition, the number of sites with pocket depths $\geqslant 5$ mm for each patient was recorded. Bleeding on probing was reported as the percentage of sites that bled on probing. Thirty-five of the patients recruited were cigarette smokers and 123 were non-smokers. A group of 35 non-smokers was then selected from this group, such that they matched the smokers in gender, age and in clinical status. On occasions, there was more than one possible match with each of the smokers regarding gender and age. With respect to the matching process, the non-smokers who most closely matched the smokers clinically were always selected.

For comparison with the treated and maintained patients, two control reference groups were included. These comprised 9 smokers and 10 non-smokers who were periodontally healthy (absence of periodontal pockets >3 mm and low incidence of bleeding on probing $<10\%$) with no history of periodontal disease. Both periodontally healthy groups were of similar age and gender mix, but were not carefully matched with one another, nor did they match the periodontitis groups, which were appreciably older. The control group of 19 subjects also met all the exclusion criteria.

Confirmation of smoking status and life-time exposure to tobacco

The smoking status of all the subjects investigated in the study was confirmed by assaying a 5 μl serum sample for cotinine using an enzyme-linked immunoassay (Cozart, Abingdon, UK) following the manufacturer's instructions. The life-time consumption of cigarettes by 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).

RANKL and OPG enzyme-linked immunosorbent assays (ELISAs)

The human sRANKL (hsRANKL) ELISA development kit (Peprotech EC, London, UK) and the hOPG ELISA development kit (R & D systems, Abingdon, UK) were used following the manufacturers' recommendations. Both ELISAs are able to detect both free and complexed RANKL and OPG. Furthermore, each assay can also be utilized to trap one of the antigens in the complex, and the presence of the other molecule in the complex can be detected by adding the appropriate detection antibody.

A 96-well Immunolon-4 microtitre plate (Dynex Technologies, Chantilly, VA, USA) was coated with 100 μl per well of the capture antibody (2 μg/ml in phosphate-buffered saline (PBS)). The plate was sealed and then incubated overnight at room temperature.

The plate was washed three times by filling each well with 400 μl of Washing buffer (PBS/0.01% (w/v) Tween 20).

The non-specific binding sites on the plate were blocked by adding 300 μl of blocking buffer (PBS/0.01% (w/v) Tween 20, 1% (w/v) bovine serum albumin) to each well, and the plate was incubated for 1 h at room temperature. The plate was washed three times as described above.

The standard solution was diluted in doubling dilutions from 1000 to 31.25 pg/ml in reagent diluent. The human sera, which were collected from the study patients, were diluted 1/2 (OPG) or 1/4 (RANKL) using reagent diluent. Assay standards or samples (100 μl) were added to duplicate wells in the plate, which was incubated for 2 h at room temperature.

The detection antibody diluted in reagent diluent (100 μl) was added to each well at a concentration of 50 ng/ml. The plate was incubated for two hours at room temperature. Thereafter, it was washed three times as described above. Avidin peroxidase (100 μl) diluted 1:2000 in reagent diluent was added to each well. The plate was then incubated at room temperature for 20 min. The plate was washed three times and the substrate solution (100 μl TMB) was added to each well and the plate was incubated at room temperature until the colour developed. The reaction was stopped by adding 50 μl of 0.12 M of HCl to each well. The optical density of each well was determined immediately using a microplate reader (Dynex Technologies MRII) set at 450 nm with a 630 nm reference wavelength. The concentration of RANKL and OPG in each of the samples was then determined by comparing the average sample optical density readings with the concentrations from the assay standard curve.

The relative serum concentrations of OPG and RANKL (picomoles per litre) were determined from the molecular weights of each protein: OPG 55 kDa and RANKL 27.5 kDa, respectively.

Statistical analyses of the RANKL and OPG ELISA data

Non-parametric distribution-free statistical tests were used to analyse the data as the concentrations of RANKL and OPG and the ratio of RANKL:OPG were not normally distributed and the data contained a number of outliers. Statistical power calculations show that using a power of 80% and an $\alpha = 0.0167$ (multiple testing), 35 smokers and 35 non-smokers are required to observe a statistically significant difference when non-smoker serum concentrations of OPG and RANKL exceed smoker concentrations by 2.00:1.00.

The relationship between tobacco consumption and [RANKL] and [OPG] was also determined using the Spearman correlation coefficient, correcting for multiple testing by applying a Bonferroni correction.

Results

Cigarette consumption and cotinine assay results

Eleven of the non-smoker patient samples fell below the low cut-off point for the assay (20 ng/ml). However, 24 non-smoker patients had slightly higher than expected values (maximum 49 ng/ml), but by no means did they approach the levels encountered in the smoker samples, which yielded levels close to 400 ng/ml. Smokers had high levels of cotinine in their sera (406.9 ± 37.8 ng/ml) compared with non-smokers (29.5 ± 9.5 ng/ml), confirming a large difference in tobacco exposure between these groups (Table 1). The healthy control group of non-smokers all had low cotinine levels below the cut-off of 20 ng/ml (13.2 ± 3.2) and the healthy smoker group had high levels (452 ± 56 ng/ml).

Table 1. Verification of smoking status by cotinine assay

Group	M/F	Cotinine (ng/ml)
Smoker	12/23	406.9 ± 37.8*
Non-smoker	12/23	29.5 ± 9.5

*Mean and standard deviation.

Table 2. Effect of smoking on clinical indices (summary)

$N = 70$	Median (Q1–Q3)		p
	smokers (12M/23F)	non-smokers (12M/23F)	
Age (years)	43.0 (40.0–51.5)	43.0 (41.0–50.5)	0.7986
N of teeth	24.0 (23.0–25.5)	24.0 (22.0–26.0)	0.8645
N of $p \geqslant 5$ mm	10.00 (5.00–15.00)	9.00 (3.00–13.00)	0.4651
PD score (mm)	2.80 (2.68–3.01)	2.76 (2.57–2.98)	0.1834
CAL score (mm)	3.40 (3.11–3.97)	3.23 (3.03–3.70)	0.2965
GR score(mm)	0.63 (0.34–0.90)	0.54 (0.26–0.97)	0.5350
%BOP	0.30 (0.21–0.37)	0.28 (0.19–0.37)	0.6321

Clinical scores and cigarette consumption by 35 smokers and 35 sex-aged and clinically matched non-smokers.

PD, pocket depth; CAL, clinical attachment level; GR, gingival recessions; BOP, bleeding on probing.

Table 3. Effect of Smoking on serum RANKL and OPG concentrations

$N = 70$	Median (Q1–Q3)		S *versus* NS	Correlation with pack-year	
	smokers 12M/23F	non-smokers 12M/23F	p	ρ	p
Pack-year	22 (20–31)	NA	NA	NA	NA
Age (years)	43.0 (40.0–51.5)	43.0 (41.0–50.5)	0.6692	NA	NA
RANKL (pM)	41.47 (13.91–58.06)	48.23 (27.64–110.32)	0.0942	−0.1714	0.1560
OPG (pM)	23.76 (18.62–40.84)	59.28 (28.83–75.56)	**0.0006**	**−0.3398**	**0.0040**
RANKL/OPG	1.16 (0.56–2.47)	0.95 (0.55–1.78)	**0.0161**	0.0891	0.4631
XsRANKL (pM)	8.43 (0.00–25.71)	0.00 (0.00–23.18)	0.2222	0.0572	0.6382
XsOPG (pM)	0.00 (0.00–10.07)	3.45 (0.00–28.21)	0.0746	−0.1892	0.1167

ELISA assay results for the OPG and RANKL assays in 70 periodontitis patients with similar levels of disease.

RANKL/OPG is the ratio of RANKL to OPG in the patients' serum samples

XsRANKL is the excess amount (pM) of RANKL left over after deducting the OPG concentration.

XsOPG is the excess amount (pM) of OPG left over after deducting the RANKL concentration.

ELISA, enzyme-linked immunosorbent assay; OPG, osteoprotegerin; RANKL, receptor activator of nuclear factor κB ligand.

Text in bold highlights the statistically significant correlations and probabilities.

Clinical indices in smokers and non-smokers

The medians and interquartile ranges of the clinical measurements taken from the 35 smokers and 35 age- and sex-matched non-smoker patients, which were used to match the patient groups clinically, are shown in Table 2. According to the Mann–Whitney *U*-test, there were no statistically significant differences in the median number of teeth, or pockets $\geqslant 5$ mm, or the median scores for pocket depth, loss of clinical attachment, gingival recession and percentage bleeding on probing ($p = 0.8645$, 0.4651, 0.1834, 0.2965, = 0.5350 and 0.6321, respectively).

Comparison of serum RANKL and OPG concentrations in smokers and non-smokers

The 35 smoker patients had a lower median value for their serum RANKL concentrations (41.47 pM) and OPG concentrations (23.76 pM) than the 35 non-smoker patients (48.23 and 59.28 pM), respectively. Despite the finding that smoker patients had lower RANKL concentrations than non-smoker patients, the difference in RANKL concentrations was not statistically significant ($p = 0.1560$). However, there was a statistically significant (after correcting for multiple testing) lower concentration of OPG in the sera of smoker patients compared with non-smoker patients ($p = 0.0040$; Table 3).

Smoker patients had a higher ratio of RANKL to OPG (1.16) than non-smoker patients (0.95) and the difference was statistically significant ($p = 0.0161$) Table 3.

Smoker patients demonstrated an excess of free RANKL in the serum, with a median concentration of 843 pM of the protein, whereas non-smoker patients tended to have an excess of OPG, 3.45 pM. These differences were not statistically significant (Fig. 1).

Correlation between cigarette consumption and RANKL and OPG concentrations and ratios

The median life-time exposure to tobacco by the smoker patients was 22 pack-years and the range was 10–45 years; the mode was 27 pack-years (Table 3). There were no statistically significant correlations between pack-years and serum concentrations of RANKL or the ratio of RANKL:OPG ($p = 0.1560$ and 0.4631, respectively). In contrast, there was a statistically significant (after correcting for multiple testing) inverse relationship between the use of tobacco (pack-years or cigarettes per day) and the serum concentrations of OPG in the smoker patients $\rho = -0.3398$, and -0.3158, $p = 0.0066$ (Fig. 2; Table 3; data shown only for pack-years).

Periodontally healthy controls

The group of periodontally healthy controls gave a similar result regarding OPG concentrations; the median level of 28.5 pM (range: 26.4–35.4 pM) in the serum of healthy smoker was statistically significantly lower than the median level of 44.7 pM (range: 34–48 pM) in the serum of healthy non-smoker controls ($p = 0.0133$). There were no statistically significant differences in the excess amounts of RANKL in the serum of healthy smokers or in the excess of OPG in healthy non-smokers (Table 4).

Discussion

The RANK system contributes to bone resorption by inducing production of a cysteine proteinase, cathepsin K, by activated osteoclasts and this enzyme is involved in bone matrix solubilization (Crotti et al. 2003). In periodontal disease, increased concentrations of RANKL are found in diseased tissues, and the upset balance with OPG concentrations is associated with disease severity (Liu et al. 2002, Crotti et al. 2003, Garlet et al. 2004). OPG is a member of the TNF receptor family that is expressed by osteoblasts. It inhibits bone resorption by high-affinity binding to RANKL and prevents RANKL from coupling with the RANK receptor (Hofbauer et al. 2000). It is known that some factors involved in the pathogenesis of periodontal disease, such as prostaglandin-E_2 (PGE_2), increase RANKL expression and decrease OPG expression (Feldmann et al. 1996, Suda et al. 1999). Other mediators, including

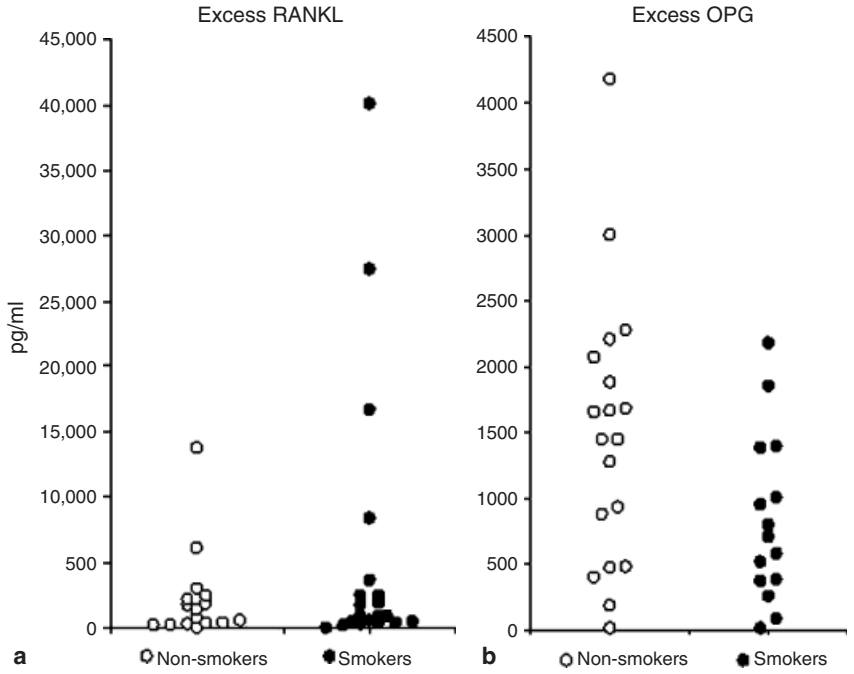

Fig. 1. Excess RANKL and OPG in the serum of smokers and non-smokers.

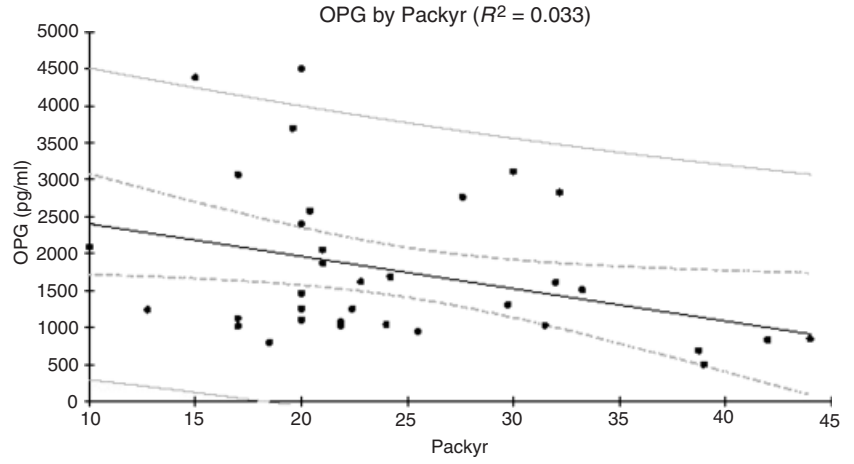

Fig. 2. Osteoprotegerin (OPG) by pack-year (Packyr; $R^2 = 0.133$).

Table 4. Effect of smoking on serum RANKL and OPG concentrations in healthy controls

$N = 19$	Median (Q1–Q3)		S *versus* NS
	smokers 4M/5F	non-smokers 4M/6F	*p*
Pack-year	23.0 (17.0–31.0)	NA	NA
Age (years)	31 (24–45)	33 (26–41)	0.7197
RANKL (pM)	31.0 (24.8–38.5)	39.0 (33.0–41.0)	0.1128
OPG (pM)	28.5 (26.4–35.4)	44.7 (33.8–47.6)	**0.0133**
RANKL/OPG	1.1 (0.9–1.6)	0.9 (0.8–1.1)	0.2775
XsRANKL (pM)	1.5 (0.0–13.4)	0.0 (0.0–0.0)	0.2110
XsOPG (pM)	0.0 (0.0–4.2)	5.7 (0.7–7.8)	0.1560

ELISA assay results for the OPG and RANKL assays in 19 healthy controls.
RANKL/OPG is the ratio of RANKL to OPG in the serum samples
XsRANKL is the excess amount (pM) of RANKL left over after deducting the OPG concentration.
XsOPG is the excess amount (pM) of OPG left over after deducting the RANKL concentration.
ELISA, enzyme-linked immunosorbent assay; OPG, osteoprotegerin; RANKL, receptor activator of nuclear factor κB ligand.
Text in bold highlights the statistically significant correlations and probabilities.

cytokines, are also involved in this process and Th1 (interferon-γ) and Th2 cytokines (interleukin 4 and interleukin-10) appear to be associated with activation and suppression of bone resorption, respectively (Eastcott et al. 1994, Kawashima & Stashenko 1999, Kawai et al. 2000, Garlet et al. 2006).

Both smoker and non-smoker patients were selected from the same treated and maintained population. It had been our original intention to conduct a longitudinal study and treated patients were chosen because ethical considerations would have precluded the recruitment of untreated patients. However, because of the strict exclusion criteria, however, it proved impossible to recruit a sufficient number of smokers to make a longitudinal study viable and so we are presenting the cross-sectional data, which would have been our baseline findings. Our matching process ensured that any differences in the concentrations of RANKL and OPG were most likely to due mainly to cigarette smoking and not to any differences in age, gender or clinical status. The cotinine levels in many of the non-smoker patients appeared to be higher than might be expected for this group. The reasons for this are unclear, but would most probably be explained by passive smoking: almost all patients were recruited from the Glasgow area before the recent Scottish Executive legislation (26/3/2006) concerning smoking in public houses, restaurants and public transport, etc. Many of those recruited also worked within a smoky environment and had family members who smoked. However, despite the finding that some of our non-smoker patients had appreciable cotinine levels, the assay results clearly demonstrate that there was a clear distinction between the smokers and those who reported that they had never smoked.

The effect of disease on RANKL and OPG concentrations cannot be entirely excluded, but, as stated above, every attempt was made to minimize the impact of disease by selecting groups that were clinically very similar: for example, bleeding on probing was almost identical for both groups, indicating that they probably had similar levels of gingival inflammation.

The finding that OPG levels were significantly reduced in smoker patients indicated that these individuals were possibly at a greater risk of bone destruction than the non-smokers. We also

observed slightly reduced concentrations of RANKL in the smoker patients, but this reduction was not statistically significant. However, the ratio of RANKL to OPG was statistically significantly higher in the smokers. There was also a negative correlation between the amount of tobacco consumed and the levels of OPG in the circulation.

Although the mechanism by which OPG levels in serum were reduced is yet to be elucidated, co-treatment of osteoblasts with nicotine and lipopolysaccharide (LPS) has been shown to reduce OPG production by these cells (Tanaka et al. 2006).

Furthermore, the concomitant addition of nicotine and LPS has been shown to stimulate the formation of osteoclast-like cells by increasing macrophage colony-stimulating factor (MCSF) and PGE_2 production by osteoblasts (Tanaka et al. 2006). The finding that PGE_2 reduces OPG synthesis by osteoblasts (Feldmann et al. 1996, Suda et al. 1999) suggests the importance of the interaction between these cells and osteoclast precursors in bone remodelling and that this probably operates via the RANKL-RANK- and NF-κB signalling system and the facilitation of osteoclast formation (Kikuchi et al. 2001, Sato et al. 2004).

One of the consequences of LPS stimulation is increased bone resorption by osteoclasts (Amano et al. 1997), partly by stimulating NF-κB via Toll like receptor-4 activation (LPS receptor) and presumably by increasing RANKL stimulation of NF-κB (Yamashita et al. 2006, Yang et al. 2006).

Despite taking care in selecting our patient groups to be clinically very similar, we cannot exclude the possibility that subtle differences were present, in particular differences in the composition of the subgingival microflora, and in Gram-negative bacterial LPS. However, any such differences could be a characteristic of the smokers and in conjunction with tobacco products, responsible for reduced OPG concentrations in the smokers compared with the non-smokers.

There are numerous theories as to how smoking is implicated in the causation of periodontitis. This study, in which sex, age and clinically matched subjects were compared, suggests that one mechanism could be suppression of OPG production and increased risk of bone resorption. Prolonged cigarette smoking has already been linked to an increase in osteoporosis and bone fractures in both male and female patients, and tobacco exposure has been implicated as a risk factor for decreased bone density (Benson & Shulman 2005, Lamichhane 2005, White et al. 2006).

As it is already well established that smoking greatly increases the risk for progressive periodontitis, it may be argued that there is no requirement for a predictive laboratory test. However, some smokers are relatively resistant to periodontitis and, indeed, some non-smokers are highly susceptible (Bergström 2003, Van Dyke & Sheilesh 2005). It would be interesting to know whether reduced OPG levels *per se* could predict for periodontitis both in smokers and in non-smokers, but only a carefully controlled longitudinal study of sufficient duration would allow this hypothesis to be tested.

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Clinical Relevance

Scientific rationale for the study: Osteoclasts, which cause resorption of alveolar bone in periodontal disease, are regulated by RANKL. OPG reduces interactions between RANKL and the osteoclast surface receptor RANK to reduce bone resorption. Increased RANKL and decreased OPG concentrations occur in GCF of periodontitis patients. The influence of cigarette smoking on RANKL and OPG was hitherto unknown.

Principal findings: This study found lower serum OPG concentrations and increased RANKL/OPG ratios in smokers compared with sex, age and clinically matched non-smoker periodontitis patients.

Practical implications: Bone loss in smoker-related periodontitis patients may be partially explained by suppression of OPG production.