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Markers of bone destruction and formation and periodontitis in type 1 diabetes mellitus

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Abstract

Aim: To determine plasma concentrations of bone metabolism markers in type 1 diabetes mellitus patients and non-diabetic and to evaluate the influence of periodontitis on biomarkers of bone formation in these patient groups.

Methods: Plasma concentrations of receptor activator of nuclear factor-κB ligand (RANKL), osteoprotegerin (OPG), C-terminal telopeptide of type 1 collagen and osteocalcin were measured in type 1 diabetes mellitus patients ($n = 63$) and non-diabetics ($n = 38$) who were also subdivided on the basis of their periodontal status.

Results: Diabetics had significantly lower osteocalcin concentrations, lower RANKL to OPG ratios and higher OPG concentrations (as shown by other researchers) than non-diabetics. The ratio of RANKL to OPG was altered by the periodontal status. Osteocalcin had a negative correlation and OPG a positive correlation with the percentage of glycated haemoglobin in the blood.

Conclusion: Because, osteocalcin, a biomarker of bone formation, is lower in patients with periodontitis and in patients with type 1 diabetes mellitus with and without periodontitis than in non-diabetics without periodontitis, this might indicate that diabetics are less able to replace bone lost during active bursts of periodontitis and explain the greater severity of disease seen in studies of patients with diabetes.

Key words: bone-markers; diabetes; periodontitis

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Periodontitis is recognized as a complication of diabetes. It is a chronic inflammatory disease of the supporting tissues of the teeth, generally considered to be caused by infection with Gram-negative bacteria and characterized by gingival inflammation and bone resorption (Page 1991). Bone resorption and bone formation are processes that are ''coupled'' but can take place independently (Corral et al. 1998). The function of bone resorbing osteoclasts is regulated by interaction with fibroblasts in the ligament between the tooth and the bone (Kanzaki et al. 2002). These periodontal ligament fibroblasts are involved in both stimulatory and inhibitory processes. Receptor activator of nuclear factor-κB ligand (RANKL) interacts with receptors on the surface of osteoclasts to stimulate bone resorption. Osteoprotegerin (OPG) also produced by periodontal ligament fibroblasts (Kanzaki et al. 2002) blocks the activity of RANKL by binding to RANK. High levels of RANKL are expressed during root resorption of deciduous teeth and raised levels of OPG are expressed where no root resorption is normally taking place, i.e. adjacent to permanent teeth (Fukushima et al. 2003).

The expression of OPG in periodontal tissues is decreased in both moderate periodontitis and advanced periodontitis, while RANKL mRNA is increased in periodontitis at the advanced stage (Liu et al. 2002). Higher circulating levels of OPG are found in type 1 diabetics than in healthy individuals (Kim et al. 2005). Plasma OPG concentrations have been shown to be implicated in the regulation of vascular calcification, atherogenesis, coronary artery disease (Dhore et al. 2001, Jono et al. 2002) and associated with progression of coronary artery calcification in type 2 diabetes mellitus (Anand et al. 2007).

An increased ratio of RANKL to OPG mRNA in periodontitis may determine local bone resorption. C-terminal

Conflict of interests and source of funding statement

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telopeptide of type 1 collagen (ICTP) is the carboxyterminal telopeptide of type 1 collagen, the only collagen type found in mineralized bone (Risteli & Risteli 1993). ICTP is released into the circulation during bone resorption (Abildgaard et al. 1997). Increased amounts of this product in serum or plasma indicate an increase in osteoclast activity. Osteocalcin is a major non-collagenous protein of the bone matrix. Osteocalcin is produced by osteoblasts. Serum osteocalcin levels have been used as biomarkers for bone formation because this protein is released into the circulation during this process (Bullon et al. 2005). A number of studies have measured osteocalcin levels in relation to periodontitis and in diabetic patients (Alexopoulou et al. 2006) but there are no studies examining diabetic patients with periodontitis. Higher serum levels of osteocalcin were measured in rats, with experimentally induced periodontitis, treated with doxycycline and alendronate indicating higher bone formation (Buduneli et al. 2005).

Bone loss seen in periodontitis is associated with a perturbation in the balance of RANKL and OPG and may be reflected in alterations in osteocalcin and/or ICTP levels. Our hypothesis is that higher levels of OPG reported for type 1 diabetes mellitus patients modify the level of destruction seen in those patients also suffering from periodontitis. The aim of this study was to measure the circulating levels of the markers of bone metabolism in diabetics with and without periodontitis. We also aimed to determine whether the presence of periodontitis had an influence on the plasma concentrations of markers of bone metabolism in diabetics and systemically healthy individuals.

Methods

Ethics and informed consent

Ethical approval was obtained from the Glasgow Royal Infirmary Ethics Committee. Informed consent was obtained from all the participants. Patients were told that they had the right to withdraw from the study at any time.

Inclusion criteria

All patients were non-smokers recruited from out-patient clinics at Glasgow Royal Infirmary, Stobhill Hospital, Glasgow, and Glasgow Dental Hospital and School. The age range of the subjects was 22–56 years and included both male and female Caucasians, who were generally healthy and had at least 20 teeth.

Exclusion criteria

Smoking within the past 5 years, pregnancy, immunosuppression, medication which predisposes to gingival hyperplasia, hormone replacement therapy; patients prescribed systemic antibiotics within the previous 6 weeks of the oral examination and patients who required prophylactic antibiotic cover before a periodontal examination.

Clinical measurements

The percentage of glycated haemoglobin (HbA1c%) was measured by high-performance liquid chromatography using an automated analyser Menarini HA-8160 analyzer (Menarini Diagnostics Firenze, Italy) according to the method described by Schnedl et al. (2005).

Sixty-three of the patients recruited were diabetics and 38 were non-diabetics. The patients underwent a detailed periodontal examination and a blood sample was taken into a tube containing an anticoagulant. A full-mouth six-point periodontal charting was successfully carried out for 99 of the 101 patients recording the following: gingival recession, clinical probing depth (CPD), clinical attachment loss (CAL), measured from the amelocemental junction, but only 96 of the 101 patients had complete records of bleeding on gentle probing.

To be included in the periodontitis groups patients had to have a minimum of two sites with CPD andCAL of >4 mm. Non-periodontitis control subjects had no sites withCAL or CPD of >4 mm. Once the study population was divided according to these criteria, 13 had to be excluded from the dataset because they did not fit into either the periodontitis or the non-peridontitis groups (two because the clinical data were not complete and 11 because they would not fit either category). Two non-diabetics which did not have full-mouth bleeding on probing (BOP) recorded were retained because full-mouth records of CPD and CAL were available.

RANKL, OPG, ICTP and osteocalcin enzyme-linked immunosorbent assays (ELISAs)

The plasma was separated from the blood by centrifugation and stored in aliquots at -70°C and thawed immediately before assay. The human soluble RANKL (hsRANKL) ELISA development kit (Peprotech EC, London, UK), the human OPG (hOPG) ELISA development kit (R & D Systems, Abingdon, UK) the UniQ ICTP EIA kit (Orion Dianostica, Espoo, Finland) and the osteocalcin immunoassay kit (BioSource Europe, Nivelles, Belgium) were purchased. ICTP assays were performed using a 1/3 dilution of plasma and osteocalcin assays were performed using a 1/5 dilution of plasma.

The method used for each assay followed the manufacturers' guidelines. The concentration of sRANKL 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 relevant assays for these proteins are capable of detecting both the free and RANKL–OPG complex (Lappin et al. 2007). The relative plasma concentrations of OPG and sRANKL and osteocalcin and ICTP (nanograms per millilitre) were calculated.

Statistical analyses of the ELISA data

A pilot experiment, where OPG levels were measured and a twofold difference obtained, was utilized in statistical power calculations. With a power of 80% and an $\alpha = 0.0125$ the minimum number of patients required for the comparisons was 68. Non-parametric distribution-free statistical tests were used to analyse the data. The postulated null hypothesis was ''no statistically significant change'' and the maximum number of comparisons was four.

The relationship between the percentage of glycated haemoglobin in the blood (HbA1c%) and (RANKL), (OPG), (osteocalcin) and (ICTP) was determined using the Spearman correlation coefficient, correcting for multiple testing by applying a Bonferroni correction. The correlation analysis was also controlled for confounding variables such as gender, age and presence of periodontitis.

Results

Demographic and clinical parameters

The non-diabetic group consisted of 16 male and 22 female subjects and the diabetic group consisted of 30 male and 33 female patients. The low HbA1c group consisted of 12 male and 18 female patients and the high HbA1c group of 17

Table 1. Number of sites and teeth with clinical probing depths >4 mm, sites and teeth with clinical attachment loss >4 mm and proportion of sites bleeding on probing (mean ± standard deviation)

	Number of sites CPD>4 mm	Number of teeth CPD>4 mm	Number of sites CAL>4 mm	Number of teeth CAL>4 mm	Proportion of sites BOP
Diabetic status (*n*)					
Non-diabetic (38)*(36)	4.13 ± 5.61	2.16 ± 1.39	2.82 ± 6.00	1.39 ± 2.57	0.33 ± 0.17
Low HbA1c (<8.5%) (30)* (29)	3.96 ± 8.85	2.14 ± 4.62	2.89 ± 6.13	1.82 ± 3.51	0.46 ± 0.19
High HbA1c (>8.5%) (33)† (31)	3.88 ± 5.61	2.36 ± 2.19	4.33 ± 5.99	2.39 ± 2.40	0.51 ± 0.17
Periodontal status (*n*)‡					
Non-diabetic control (19)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.35 ± 0.14
Non-diabetic periodontitis (17) * (15)	8.71 ± 6.09	4.57 ± 2.68	6.16 ± 7.84	2.39 ± 2.68	0.32 ± 0.21
Diabetic (26)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.40 ± 0.16
Diabetic periodontitis (26)	8.86 ± 10.45	5.00 ± 4.89	9.05 ± 7.70	5.10 ± 3.63	0.55 ± 0.18

*Two of the non-diabetic (periodontitis) patients do not have full-mouth BOP data and one diabetic has this information missing, but full charting of CAL and CPD are available.

†Two patients do not have full CAL or BOP data.

‡Two non-diabetics and eleven diabetics were omitted from the periodontal status analysis as they failed to meet the inclusion criteria.

CPD, clinical probing depth; CAL, clinical attachment loss; BOP, bleeding on probing.

male and 16 female patients. The median ages of the patients in the non-diabetic and the diabetic groups (low-HbA1c and high-HbA1c groups) were very similar; non-diabetics 40 (range 21–56) years, diabetics with low HbA1c% 34 (20–53) years and diabetics with high HbA1c% 40 (20–54) years. Clinical data are presented in Table 1. The number of sites and teeth with CPD and CAL>4 mm and the number of sites BOP are indicated. The major differences between the diabetic patient groups and the healthy patient groups with periodontitis were as follows: The healthy patients appeared to have lower average levels of BOP than the diabetics, but contrary to what we expected the non-diabetics without periodontitis had a slightly higher level of BOP than the non-diabetics with periodontitis. The diabetics with low HbA1c% appeared to have lower numbers of teeth and sites with CAL>4 mm and CPD>4 mm than the diabetics with high HbA1c, but there was no statistically significant difference in these parameters between these two groups. The Kruskal–Wallis test results comparing the three groups were as follows: CPD>4 mm, $p = 0.394$; number of teeth with CPD>4 mm, $p = 0.299$; CAL>4 mm, $p = 0.304$ and number of teeth with CPD>4 mm, $p = 0.171$.

Although, the number of teeth and sites with CPD>4 mm was similar in both diabetic and non-diabetic periodontitis groups there were more sites and teeth with CAL>4 mm in the diabetic periodontitis group. None of these differences were statistically significant.

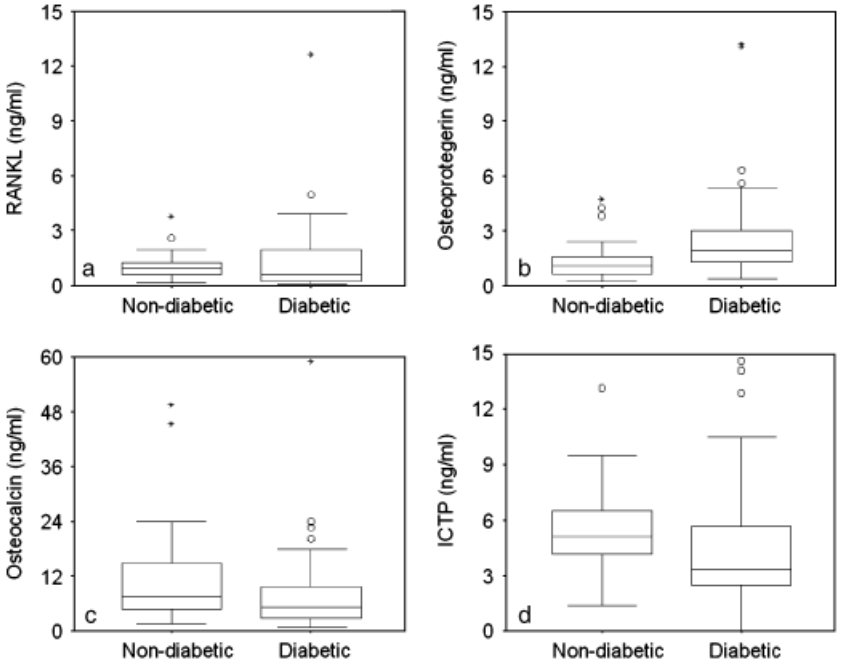

Fig. 1. The box plot with quartiles, showing outliers (open diamonds) and extreme values (asterisks), represents the plasma concentration of (a) RANKL, (b) osteoprotegerin, (c) osteocalcin and (d) ICTP in 38 patients without diabetes (non-diabetic) and 63 patients with type 1 diabetes mellitus (diabetic). ICTP, C-terminal telopeptide of type 1 collagen; RANKL, receptor activator of nuclear factor-κB ligand.

Plasma concentrations of RANKL, OPG, ICTP and osteocalcin

Diabetic patients had a higher concentration of OPG and a lower concentration of osteocalcin than non-diabetic patients. The Mann–Whitney *U*-test indicated that both findings were statistically significantly different (OPG, $p < 0.001$; osteocalcin, $p = 0.010$). The differences between the groups in RANKL and ICTP levels were not statistically significant (Fig. 1).

Influence of glycated haemoglobin on plasma RANKL, OPG, ICTP and osteocalcin

Diabetics were divided into two groups high and low depending on their mean

Table 2. Comparison of median (Quartile 1–3) plasma osteocalcin, ICTP, RANKL, OPG levels and RANKL:OPG ratios in the patient groups

	Osteocalcin (ng/ml)	ICTP (ng/ml)	RANKL (ng/ml)	OPG (ng/ml)	RANKL:OPG (mol:mol)
Diabetic status (*n*)					
Non-diabetic (38)	4.78 (3.20–6.24) *	7.18 (4.62–14.60)	0.83 (0.36–1.26)	1.11 (0.65–1.55)	1.45 (0.57–2.83)
HbA1c (<8.5%) (30)	3.34 (2.04–5.55)	5.40 (2.98–10.44)	0.66 (0.19–2.27)	2.20 (1.54–3.02)	0.65 (0.24–1.11)
High HbA1c (>8.5%) (33)	3.30 (2.52–5.81)	5.29 (2.71–7.82)	0.66 (0.23–1.92)	1.88 (1.31–3.11)	0.90 (0.26–1.44)
Periodontal status (*n*)†					
Non-diabetic (19)	4.80 (3.22–5.86)	5.00 (3.59–6.98)	0.95 (0.71–1.23)	0.65 (0.61–1.15)	1.09 (0.46–1.70)
Non-diabetic Periodontitis (17)	5.22 (4.28–6.50)	6.23 (4.18–10.20)	0.51 (0.15–2.00)	1.82 (1.37–2.72)	2.22 (2.02–3.06)
Diabetic (26)	3.15 (2.26–5.40)	5.32 (3.63–9.95)	0.63 (0.25–1.68)	2.16 (1.28–2.89)	0.68 (0.21–1.91)
Diabetic Periodontitis (26)	3.71 (2.26–5.57)	5.00 (3.59–6.98)	0.95 (0.71–1.23)	0.65 (0.61–1.15)	0.84 (0.34–1.14)

*Statistically significant differences between the groups are shown by the linkages.
†Two non-diabetics and eleven diabetics were omitted from the periodontal status analysis as they failed to meet the inclusion criteria.
Because the data were not normally distributed, the Mann–Whitney *U*-test was used to compare the differences between the patient groups and Bonferroni correction was applied to adjust the statistic for multiple comparisons.
ICTP, C-terminal telopeptide of type 1 collagen; OPG, osteoprotegerin; RANKL, receptor activator of nuclear factor-κB ligand.

HbA1c% over the previous 2 years. The median HbA1c% (8.60%) was taken as the cut-off point and the two groups were compared with each other and the non-diabetic group (Table 2, Fig. 2). The results obtained for OPG indicated a statistically significant difference between some of the groups according to the Kruskal–Wallis test (Fig. 2b). When the three groups were compared and analysed post hoc with the Mann–Whitney *U*-test and a Bonferroni correction for multiple comparisons, statistically significant differences were found for the following comparisons: non-diabetic group and low HbA1c group ($p < 0.001$) and the non-diabetic group and high HbA1c group ($p < 0.001$). Diabetics with high HbA1c% showed the lowest concentrations of osteocalcin (Fig. 2c; median = 5.91 ng/ml). The results showed a statistically significant difference between the groups according to the Kruskal–Wallis test ($p = 0.006$) When the three groups were analysed by the Mann–Whitney *U*-test with Bonferroni's correction a statistically significant difference in plasma osteocalcin concentrations was observed when comparing the non-diabetic group and the high HbA1c group ($p = 0.011$) and between the low- and high-HbA1c groups ($p = 0.0163$). There were no statistically significant differences in RANKL or ICTP concentrations between any of the groups ($p = 0.649$ and 0.092, respectively) (Fig. 2a and d).

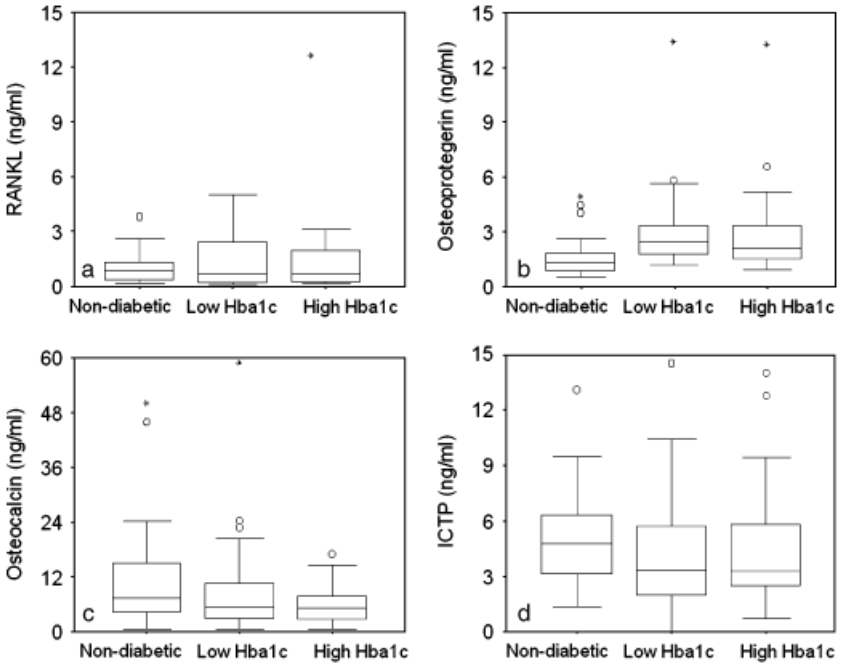

Fig. 2. The box plot with quartiles, showing outliers open diamonds and extreme values (asterisks), represents the plasma concentration of (a) RANKL, (b) Osteoprotegerin, (c) Osteocalcin and (d) ICTP in 38 non-diabetic patients and 63 patients with diabetes split into two groups based on their HbA1c%: 30 with low HbA1c% (less than the median) and 33 with high HbA1c% (median HbA1c% and above). ICTP, C-terminal telopeptide of type 1 collagen; RANKL, receptor activator of nuclear factor-κB ligand.

There was a statistically significant difference in the RANKL:OPG ratio (Table 2) between the non-diabetic and the diabetic patient groups according to the Kruskal–Wallis test ($p = 0.0023$). In the post hoc analysis: both high- and low-HbA1c groups were statistically significantly different from the non-diabetics ($p = 0.0015$ and 0.0016, respectively), but not from each other ($p = 0.080$).

Influence of periodontitis on plasma RANKL, OPG, ICTP and osteocalcin

There was no difference in the extent of periodontitis between the non-diabetic and the diabetic patients. Thirteen patients (11 diabetics and 2 non-diabetics) were excluded from the rest of the study because of difficulty in placing them in either the periodontitis group or the non-periodontitis control group. Therefore the groups consisted of diabetic non-periodontitis $n = 26$; diabetic with periodontitis $n = 26$; non-diabetic non-periodontitis $n = 19$; and non-diabetic with periodontitis $n = 17$.

When the patients were allocated according to their disease status, diabetics with periodontitis had the highest plasma concentration of OPG (Table 2, Fig. 3b). The Kruskal–Wallis test indicated that the results were statistically significantly different ($p < 0.001$). The Mann–Whitney *U*-test with a Bonferroni correction indicated statistically significant differences in plasma OPG (Fig. 3b) in the following comparisons: non-diabetics and diabetics without periodontitis ($p = 0.0193$); non-diabetic patients with periodontitis and diabetics with periodontitis ($p < 0.0001$) and; non-diabetics without periodontitis and non-diabetics with periodontitis ($p = 0.0002$).

The highest osteocalcin levels were in the non-diabetics without periodontitis and the lowest in the non-diabetics with periodontitis but the levels in this non-diabetics without periodontitis were similar to those in both diabetic patient groups, i.e. the diabetic patients with and without periodontitis. There were statistically significant differences in osteocalcin (Table 2, Fig. 3c) concentrations ($p = 0.0033$) and the post hoc test showed statistically significant differences between the non-diabetics without periodontitis and the non-diabetics with periodontitis groups ($p = 0.038$); the non-diabetic group without periodontitis and diabetics without periodontitis ($p = 0.0082$). There were no statistically significant differences in RANKL or ICTP concentrations (Table 2, Fig. 3a and d). Statistically significant differences in the ratio of RANKL:OPG (Table 2) were observed between the following patient groups: the non-diabetic patients without periodontitis and the non-diabetic patients with periodontitis ($p = 0.0018$) and the diabetic patients with periodontitis and the non-diabetic patients with periodontitis ($p < 0.0001$).

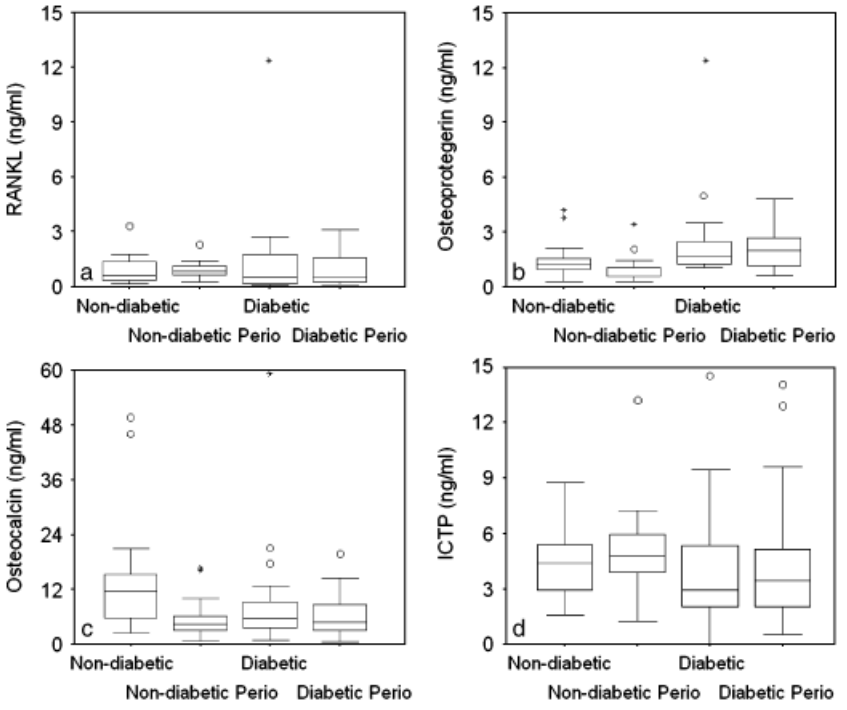

Fig. 3. The box plot with quartiles, showing outliers (open diamonds) and extreme values (asterisks), represents the plasma concentration of (a) RANKL, (b) osteoprotegerin, (c) osteocalcin and (d) ICTP in 36 patients without type 1 diabetes mellitus (non-diabetic) and 52 patients with diabetes each split into two groups based on the presence of periodontitis. Seventeen of the non-diabetics had periodontitis and 19 did not and 26 diabetics had periodontitis and 26 did not. Two control patients and 11 diabetics were excluded from the analysis because they did not fit the inclusion criteria to be included in either the periodontitis or the control (non-periodontitis) groups. ICTP, C-terminal telopeptide of type 1 collagen; RANKL, receptor activator of nuclear factor-κB ligand.

Correlations

The plasma concentrations of RANKL did not show any statistically significant correlation with HbA1c%; Spearman's correlation coefficient (ρ) = -0.059 ($p = 0.560$).

The plasma concentration of OPG indicated a statistically significant positive correlation (rho = 0.339, $p = 0.0005$) with the HbA1c% in blood. The plasma concentration of osteocalcin indicated a statistically significant negative correlation (rho = -0.324; $p = 0.0018$) with HbA1c%. These relationships remained statistically significant when the data were controlled for the presence of periodontitis (HbA1c% and OPG, $p = 0.008$ and HbA1c% and osteocalcin, $p = 0.009$) the gender ($p = 0.008$ and 0.009) or the age ($p = 0.006$ and 0.004) of the patients.

Osteocalcin concentrations also correlated with the extent of periodontitis ($\rho = -0.285$, $p = 0.004$) and appeared to be statistically significant when controlling for the presence of diabetes ($p = 0.041$), but this was before the data were corrected for multiple comparisons.

Plasma ICTP concentrations did not correlate with HbA1c% ($\rho = -0.146$; $p = 0.147$) or OPG levels, but there was a positive correlation between ICTP and RANKL ($\rho = 0.280$; $p = 0.0050$ and the RANKL:OPG ratio ($\rho = 0.323$; $p = 0.0011$).

BOP correlated with the number of deep sites ($p < 0.001$) and with the HbA1c% ($p = 0.002$).

Discussion

Activation of the transcription factor nuclear factor-κB following ligation of RANKL to the cell surface receptor RANK an event that promotes osteoclast formation (Hofbauer et al. 2000) and also induces the production of a cysteine proteinase, cathepsin K, in osteoclasts. Cathepsin K is involved in bone matrix solubilization (Crotti et al. 2003). OPG is a member of the TNF receptor family that is expressed by osteoblasts and other cell types, notably insulin secreting β cells of the pancreas (Schrader et al. 2007). OPG inhibits osteoclast formation

by high affinity binding to RANKL and prevents RANKL from coupling with the RANK receptor (Hofbauer et al. 2000). This tends to result in reduced bone resorption.

In periodontitis, increased concentrations of RANKL are found in diseased tissues, and the disruption to the balance between RANKL and OPG concentrations is associated with disease severity (Liu et al. 2002, Crotti et al. 2003, Garlet et al. 2006). Although diabetes has been established as a risk factor for periodontitis and the osteocalcin levels in serum, gingival tissues and saliva correlate to periodontal status in non-diabetics (Bullon et al. 2005), the osteocalcin concentrations in diabetics and their relationship to periodontitis have not been established.

In this study in order to avoid possible confounding between the periodontitis and the non-periodontitis groups we defined the level of disease before the analysis, i.e. a diagnosis of periodontitis was confirmed by a minimum of two sites with >4 mm CAL and CPD. Therefore 13 patients with only one site of >4 mm CAL and/or CPD were excluded. Statistically significant differences were not observed in RANKL concentrations, but in contrast statistically significant differences in OPG and osteocalcin and RANKL:OPG ratios were observed. Serum osteocalcin is presently considered to be a valid marker of bone turnover when resorption and formation are coupled, and a specific marker of bone formation when formation and resorption are uncoupled (Giannobile et al. 2003).

In this study both diabetic and periodontitis patients have lower levels of osteocalcin than non-diabetic patients without periodontitis, suggesting lower bone formation. Reduced osteocalcin levels in the presence of periodontitis have been reported before by other researchers (Bullon et al. 2005 and Buduneli et al. 2005) and the present data show a statistically significant inverse relationship between the extent of periodontitis and the osteocalcin levels. The data also suggest that the failure to adequately control diabetes leads to a reduction in plasma osteocalcin and that the elevated blood glucose acts independently of the presence of other factors such as age gender or periodontitis to influence the expression of osteocalcin.

There are several mechanisms that might explain the greater incidence of periodontitis in diabetes: a greater susceptibility to infection as a result of diminished neutrophil function, the formation of advanced glycation end products which increase oxidative stress in the tissues; and the binding of advanced glycation end products to cell surface receptors which stimulates the increased production of inflammatory cytokines and causes delayed wound healing. These mechanisms would induce periodontal destruction through osteoclastogenesis. However, there is also evidence that diabetes suppresses osteoblastogenesis after bacterial challenge and the net bone loss could be due to reduced bone formation because of apoptosis of bone lining cells (He et al. 2004). Advanced glycation end products have also been shown to induce apoptosis of osteoblasts in diabetics via the mitogen-activated protein (MAP) kinase and cytosolic apoptotic pathway (Alikhani et al. 2007). The reduced plasma osteocalcin concentrations in the diabetic patients in the present study could be explained by a reduction in the number of osteocalcin secreting osteoblasts.

Numerous studies have established lower concentrations of OPG and lower expression of OPG in periodontitis patients compared with normal healthy individuals (Belibasakis et al. 2007, Bostanci et al. 2007, Lappin et al. 2007), but in contrast diabetes patients have higher levels of OPG than healthy individuals (Kim et al. 2005). The results of the present study confirmed that higher OPG concentrations are detected in diabetics, and indicated that there is a positive correlation between OPG and HbA1c%. It is particularly intriguing that the diabetic patients have higher circulating levels of OPG than healthy individuals because they have a greater severity of periodontitis than healthy subjects (Khader et al. 2006). OPG is considered to be a bone protective protein as it essentially reduces the activity of RANKL. High plasma levels of OPG are also linked to endothelial cell dysfunction (Secchiero et al. 2006) and the early onset of diabetes mellitus and have implications in the higher susceptibility of diabetics to coronary artery disease (Anand et al. 2007) and myocardial infarction (Avignon et al. 2007). The finding that the level of BOP was greater in the diabetics could also be partly explained if there was a dysfunction of the endothelial cells in the gingival blood vessel walls. Further studies are required to confirm this. We expected to see a significantly higher level of BOP in the periodontitis groups compared with those without periodontitis but in the non-diabetics this was not the case. This may be due to the poor level of oral hygiene seen in the general population in the United Kingdom (Kelly et al. 1998). Although the number of teeth and sites with >4 mm CPD were similar between the diabetic and non-diabetic periodontitis groups the diabetic periodontitis group had more teeth and sites affected by CAL >4 mm. These differences were accounted for by a greater prevalence of gingival recession in the diabetics with periodontitis.

Hyperglycaemia does not appear to be directly responsible for elevated plasma OPG in diabetics (Knudsen et al. 2007). However, our results suggest a strong relationship between HbA1c% and increased OPG concentrations acting independently of other factors such as age, gender or periodontitis. A question that has yet to be resolved is whether high levels of OPG are directly involved in the pathogenic process or are indicative of the process taking place. The pathogenesis of type 1 diabetes mellitus is caused by immune-mediated pancreatic β cell destruction. Schrader et al. (2007) indicated that cytokine induced OPG production may protect pancreatic β cells from further damage and may be partially mediated through inhibition of p38 MAP kinase phosphorylation and suggested that OPG may be elevated to protect the pancreatic β cells. In diabetics increased levels of OPG should in theory reduce bone resorption; however, diabetic patients have been shown in a recent meta analysis to have a similar extent of periodontitis to non-diabetics, but more severe disease (Khader et al. 2006).

Bone remodelling is a coupled process involving bone formation and bone resorption. As bone formation (indicated by osteocalcin levels) is lower in diabetic patients, regardless of periodontal status, cross-linked ICTP a specific biochemical marker of bone resorption was measured (Bacovsky et al. 2002). Although lower levels of ICTP were observed in the plasma of the diabetic patients there was no statistically significant difference in ICTP levels between non-diabetic and diabetic patients. Increased levels of this bone marker have been observed in the GCF of periodontitis patients (Giannobile 1999, Giannobile et al. 2003). The current results would tend to agree since higher median plasma concentrations of

ICTP were observed in the groups with periodontitis in this study but the differences failed to reach statistical significance. This failure to see a statistically significant difference may be due to measuring ICTP in plasma rather than GCF or to the lack of sufficient statistical power for determining differences in this particular marker. The lower levels of ICTP that were observed possibly reflect the influence of OPG at reducing bone resorption.

In conclusion diabetics do not appear to be more susceptible to bone destruction, but may have a defect in bone formation. The reduced osteocalcin levels in type 1 diabetics suggests that these patients have a reduction in their intrinsic ability to replace bone, such as that which has been destroyed during ''acute bursts'' of periodontitis. This may make them more susceptible to progression of this disease. In support of this other researchers have not indicated a significant change in bone density in diabetics but have indicated that bone formation may be lower in diabetic patients (Bridges et al. 2005, Oz et al. 2006). Future studies in type 1 diabetics should investigate markers of bone metabolism in combination with bone density measurements.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. The following patients were excluded because they met neither the criteria for the control group (no sites >4 mm Clinical Probing Depths or Clinical Attachment Loss attributable to periodontal disease) nor the periodontitis group (at least 2 sites with >4 mm Clinical attachment loss and increased Clinical Probing Depths): Study codes 4, 10, 18, 22, 32, 37, 42, 61, 66, 80, 102, 124, 131.

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Clinical Relevance

Scientific rationale for study: RANKL interacts with receptors (RANK) on the surface of osteoclasts to stimulate bone resorption. OPG decays RANKL reducing RANK activation. OPG levels in diabetics are reported to be high, therefore it should follow that the amount of bone loss in patients with periodontitis would be less in diabetics than non-diabetics. However without a measure of RANKL and an indicator of bone formation (osteocalcin) and destruction (ICTP) it is difficult to assess the impact of increased OPG levels on net bone turnover.

Principal findings: We confirmed that higher plasma OPG and lower RANKL:OPG ratios are found in diabetics and that the presence of periodontitis altered the RANKL:OPG ratio. Lower osteocalcin levels were found in diabetics and in periodontitis. However, there was no statistically significant change in ICTP levels in the diabetic groups or in the groups with periodontitis.

Practical implications: Biomarkers indicating bone formation are lower in type 1 diabetes mellitus patients. Inflammatory signals that would tend to increase bone destruction are possibly counteracted by high OPG levels in diabetics. It therefore appears that overall bone turnover may be reduced in type 1 diabetes possibly due to reduced new bone formation as indicated by a reduction of plasma osteocalcin.