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Plasma vitamin D and cytokines in periodontal disease and postmenopausal osteoporosis

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Background and Objective: Osteoporosis and periodontal disease are chronic diseases, in the pathogenesis of which plasma osteoprotogerin (OPG) and RANKL are important. The study aimed to investigate the relationship between periodontal disease and plasma cytokines, vitamin D and bone mineral density in postmenopausal women with and without osteoporosis.

Material and Methods: One hundred and eighty-five postmenopausal women with osteoporosis and 185 age- and sex-matched control subjects were recruited. Periodontal disease was subdivided into active or past periodontal disease. Osteoprotegerin, RANKL, 25-hydroxyvitamin D_3 (25OHD), biochemical markers of bone turnover (serum C-terminal telopeptide, CTX), anthropometry and bone mineral density were measured.

Results: A significantly higher proportion of the women with osteoporosis had active or past periodontal disease or both compared with control subjects (87.6 vs. 37.8%, $p < 0.001$). Plasma 25OHD was significantly lower ($p < 0.001$) and RANKL and OPG significantly higher in the women with osteoporosis than in control subjects ($p < 0.0001$). RANKL, OPG and CTX were significantly higher in women with active periodontal disease than in those without ($p < 0.001$), as were OPG and CTX in past periodontal disease ($p < 0.001$). In active and past periodontal disease, 25OHD was significantly lower ($p < 0.001$). Multiple logistic regression analysis showed that periodontal disease was best predicted by RANKL, 25OHD, C-terminal telopeptide and weight, $r^2 = 10.4\%$.

Conclusion: Periodontal disease is more common in women with osteoporosis and is associated with lower vitamin D and higher concentrations of RANKL and OPG. Raised cytokines may provide the underlying mechanism that links these two conditions.

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Periodontal disease is a chronic inflammatory disorder of bacterial origin, which results in alveolar bone loss, tooth loosening and subsequent tooth loss (1,2). The primary event in periodontal disease is gingivitis, caused by poor oral hygiene with subsequent bacterial infection, but smoking, genetic, hormonal and dietary factors may all contribute to its development. Osteoporosis is also a chronic multifactorial disease, which may share genetic, hormonal, dietary and other lifestyle risk factors with periodontal disease. Osteoporosis may therefore be a risk factor for periodontal disease, so treatments and lifestyle modifications that are beneficial in the management of osteoporosis may also be useful in the prevention of periodontal bone loss (3–9).

Receptor activator of nuclear factor κB ligand (RANKL), its cellular receptor RANK and its decoy receptor osteoprotegerin (OPG) constitute a cytokine system that plays a critical role in osteoclastogenesis. RANKL is produced by cells of osteoblastic lineage and by activated T lymphocytes and is the essential factor for osteoclast formation, fusion, activation and survival. The stimulatory effects of RANKL on bone resorption are counteracted by OPG, which acts as a soluble neutralizing receptor. RANKL and OPG are regulated by various hormones (e.g. glucocorticoids, vitamin D and estrogen) and cytokines (10–15).

The relationship between systemic bone loss (i.e. bone mineral density) and oral bone loss, e.g. alveolar bone loss and tooth loss, has already been established (16–22). Both cross-sectional (16–25) and longitudinal studies (26,27) have demonstrated the relationship between worsening dental health and generalized loss of bone density. Many factors have been investigated in attempts to explain the observed link between periodontal disease and both localized and systemic bone loss. These factors have included estrogen deficiency, genetic polymorphisms [vitamin D receptor, estrogen receptor-α, pro-collagen (α1) type I and osteoprotegerin], vitamin D deficiency and a number of different cytokines (28–52).

Vitamin D and calcium insufficiency are risk factors for multiple chronic diseases, including osteoporosis and periodontal disease. Low vitamin D status and inadequate calcium nutrition are common and are particularly prevalent amongst the institutionalized elderly, in certain geographical areas, in ethnic minorities and in people of low socio-economic status (39). Furthermore, studies have shown that calcium and vitamin D supplementation can improve periodontal health, as well as increasing bone mineral density in the jaw and inhibiting alveolar bone resorption (40,52).

Several studies have attempted to assess the role of OPG and RANKL in periodontal disease, but these studies have focused on the local *in situ* production of these cytokines and have produced conflicting findings (53–65). There is growing evidence in postmenopausal women that elevated concentrations of RANKL and OPG are important for the development of generalized osteoporosis (66–68). Given that periodontal disease is more common in women with osteoporosis, it is possible that the two conditions may be linked by the inflammatory component of periodontal disease causing elevations in RANKL and altered RANKL/OPG ratio. As far as the authors are aware, no studies have investigated the role of serum vitamin D, RANKL and OPG in the pathogenesis of periodontal disease and postmenopausal osteoporosis. Therefore, the aim of this study was to investigate the relationship between periodontal disease and plasma OPG, RANKL, 25-hydroxyvitamin D_3 (25OHD), biochemical markers of bone turnover, anthropometry and bone mineral density in postmenopausal women with and without osteoporosis.

Material and methods

Subjects

Ethics committee approval was obtained, and all subjects gave their written informed consent. All new postmenopausal women referred to the direct access dual energy X-ray absorptiometry service at The James Cook University Hospital were asked to participate. Women who had stopped menstruation for at least 1 year were considered postmenopausal. A total of 772 women agreed to take part, of whom 185 women had a diagnosis of osteoporosis (T score ≤ -2.5 at the lumbar spine and/or femoral neck). A further 185 control subjects were identified from the remaining 587 women without osteoporosis (T score > -2.5 at the lumbar spine and femoral neck). The control subjects were identified from each decade using a table of random numbers. All subjects were Caucasian and were born in northern England.

Methods

Equal numbers were recruited across the year, to avoid the confounding effects of seasonal variation in vitamin D status. Each subject was given a unique identifier. An independent observer held the key, so that the information was anonymized to the researcher.

Underlying secondary causes of osteoporosis were identified by questionnaires, medical history, physical examination and blood tests, including parathyroid hormone, C-reactive protein (CRP), sex hormones and 25OHD. The subjects' medical history and medications were also assessed using a questionnaire. A standardized interview based on the European Vertebral Osteoporosis Study (EVOS) questionnaire (69) was conducted with each patient to assess medical history, including risk factors for osteoporosis and reproductive history.

Subjects were asked about episodes of back pain, height loss, immobilization (for a minimum of 8 wk), physical exercise (regular exercise being defined as more than 30 min continuous activity at least once per week), family history of osteoporosis (either a definite diagnosis or a low-trauma hip fracture in a first-degree relative), medications and medical conditions. Subjects were asked specifically if they took corticosteroids or anticonvulsants. Subjects were also asked if they suffered from kidney disease, hyperthyroidism, eating disorders, coeliac disease, inflammatory arthritis (including rheumatoid arthritis), liver disease, inflammatory bowel disease or other illnesses or had undergone gastrectomy. Putative secondary causes of osteoporosis were classified as the use of oral corticosteroids, smoking, excess alcohol consumption (>14 units/wk), long-term immobilization, gastrectomy, liver disease, inflammatory bowel disease, coeliac disease, inflammatory arthritis, anorexia nervosa and hypogonadism.

A standardized interview questionnaire was conducted with each patient to assess their current and past dental history (see below). We identified gingivitis and periodontal disease progression based on a validated written questionnaire (70–76). Gingivitis status was determined from the answer to the questions, 'Have your gums bled recently?' or 'Do your gums usually bleed?' over the previous 12 mo. The severity of periodontal disease was

identified by asking participants if they had been diagnosed by a dentist or hygienist with periodontal disease or gum disease, if they had loose or wobbly teeth (self assessed) or they think they see more of the roots of their teeth than in the past. The women were also asked if they had full or partial dentures before the age of 50 years and, if so, what the reasons were. Subjects were asked if they knew that the dentures were due to periodontal disease as diagnosed by their dentist or for other reasons, e.g. extensive carries lesions or trauma. Periodontal disease was then subdivided into none, active or past periodontal disease. Active periodontal disease was defined as the presence of one symptom from recent gum bleeding, usual gum bleeding or gum recession. Any subject with one of these symptoms was classified as having active periodontal disease, even if they had dentures (full or partial) and was not included in other subsets. Past periodontal disease was indicated by the presence of full or partial dentures before the age of 50 years and the absence of current active periodontal disease. There were no reports of tooth mobility, so we did not include this in any definitions.

Anthropometric measurements

Standardized measurements of height in centimetres and weight in kilograms were taken in light clothing with shoes removed. A Stadiometer calibrated to 1 cm and scales calibrated to 0.1 kg were used for this purpose. Body mass index was calculated in kilograms per square metre.

Bone mineral density measurements

Dual energy X-ray absorptiometry was used to determine scan area (in centimetres squared), bone mineral content (in grams) and areal bone mineral density (in grams per square centimetre) at the lumbar spine (L2–L4) and femoral neck, using a Hologic QDR 2000 machine (Hologic Instruments, Waltham, MA, USA). This had a daily calibration check using the Hologic spine phantom and had a coefficient of variation of 0.5% throughout the period of study. *In vivo* precision for measurement with this system is 1.0% at the lumbar spine and 1.5% for the femoral neck.

Biochemistry

All subjects had fasting blood taken in the morning. The blood samples were collected and centrifuged at 3395 *g* for 3 min at room temperature, within 1 h of collection. This separated the plasma from the blood samples, and the plasma samples were then stored in a freezer at −40°C. The samples were allocated a study number to enable their identification. These samples were removed from the freezer and defrosted at room temperature prior to the assays.

All assays were performed in the Pathology laboratory at South Tees NHS Hospitals Trust. All the laboratory tests were subject to validation using National External Quality Assurance Schemes. Parathyroid hormone was measured by Roche Elecsys immunometric assay. Plasma calcium, phosphate, alkaline phosphatase and albumin were measured using an Olympus AU2700. C-reactive protein was determined by using a fixed rate turbidimetric method (Olympus Diagnostica GmbH, Hambourg, Europe). An IMMULITE Analysis method was used to determine sex hormone binding globulin and interleukin-6 (IL-6). Estradiol, testosterone, C-terminal telopeptide (CTX) and osteocalcin were determined by electrochemiluminescence immunoassay implemented on the Roche Elecsys 1010/2010 and MODULAR ANALYTICS E170 (Elecsys module) immunoassay analysers. The 25OHD concentration was determined by ELISA from the IDS OCTEA (Immuno Diagnostic Systems, Fountain, AZ, USA).

A Biomedica Gruppe ELISA kit (Vienna, Austria) for determining human soluble RANKL and OPG was used in this study. This kit directly determines soluble, uncomplexed human RANKL and OPG in biological fluids. Measurement of RANKL and OPG was performed using two-site sandwich ELISAs specifically designed to quantify RANKL and OPG in biological fluids. In the OPG ELISA, the capture antibody is a monoclonal anti-OPG antibody. In the RANKL ELISA, the detection antibody is a biotinylated polyclonal anti-RANKL antibody, and in the OPG ELISA, it is a biotinylated polyclonal anti-OPG antibody. In both ELISAs, the conjugate is streptavidin–horseradish peroxidase and the substrate is tetramethylbenzidine solution. The amount of colour developed is directly proportional to the amount of OPG or RANKL in the sample. Intra- and interassay coefficients of variation were $< 10\%$ for all assays.

Statistical analysis

Statistical analysis was performed using SPSS for Windows (version 11.5, SPSS Inc. Chicago, IL, USA). Descriptive statistics were obtained, and data were tested for normality using the Kolmogorov–Smirnov test for Gaussian distribution. Data that conformed to a normal distribution were analysed using Student's unpaired *t*-tests. Data that showed considerable deviation from the normal distribution were analysed using the Mann–Whitney *U*-test. Correlation coefficients (Pearson and Spearman) and correlation matrices were produced to investigate whether there were correlations between anthropometric or clinical data and biochemical measurements. The chi-squared test was used to determine the significance of differences in proportions.

Multiple logistic regression analysis was performed, and a stepwise approach was chosen in order to develop the best predictive model for periodontal disease. Variables were entered into the model if their probability value was ≤ 0.05 and excluded if this was ≥ 0.1. In order to do this, the data from both the women with osteoporosis and the control subjects were combined. All anthropometric, bone mineral density and biochemical data were entered. Both forward and backward stepwise logistic regression was undertaken in order to develop the best model. A p value of 0.05 or less was considered significant for all analyses.

Results

There was no significant difference in mean ± SD age between the women with osteoporosis and the control

subjects. Women with osteoporosis weighed less, had lower body mass index and were shorter than the control subjects (Table 1). Among the women with osteoporosis, 57 had putative secondary causes of osteoporosis (oral corticosteroid users $n = 30$, rheumatoid arthritis $n = 18$ and inflammatory bowel disease $n = 9$), compared with 12 in the control group (oral corticosteroid users $n = 8$, rheumatoid arthritis $n = 3$ and inflammatory bowel disease $n = 1$). There was no significant difference in smoking or alcohol consumption and no difference in menopausal status. As expected, the mean lumbar spine and femoral neck bone mineral density was significantly lower in the group with osteoporosis than in the control subjects (Table 1). Women with osteoporosis had a lower serum estradiol and 25OHD and higher parathyroid hormone, CRP, IL-6, RANKL, OPG and biochemical markers of bone turnover than the control subjects (Table 1). A significantly higher proportion of the women with osteoporosis had periodontal disease, as follows: active periodontal disease was 11.9 vs. 0.5% ($p < 0.0001$), past periodontal disease was 75.7 vs. 37.3% ($p < 0.0001$) and active or past periodontal disease was 87.6 vs. 37.8% ($p < 0.0001$) compared with the control subjects.

Comparisons were made between women with and without active periodontal disease and between those with or without past periodontal disease (Tables 2 and 3). Results showed that 209 (56.48%) postmenopausal women had past periodontal disease, of whom 82 had partial dentures and 127 had complete dentures by the age of 50 years. Furthermore, 23 (6.2%) postmenopausal women had active periodontal disease. There were no significant differences in mean age, but the mean spine and femoral neck bone mineral density were significantly lower in the women with active or past periodontal disease compared with those without. Significant differences were seen in the measurements of circulating OPG, RANKL, 25OHD, biochemical markers of bone turnover, anthropometry and bone mineral density in the women with active and past periodontal disease compared with those without. The concentrations of 25OHD, estradiol and testosterone were significantly lower in women with either active or past periodontal disease compared with women without. The C-reactive protein, RANKL, OPG and CTX were significantly higher in women with active periodontal disease compared with those without. The C-reactive protein, OPG and CTX were also significantly higher in women with past periodontal disease. In particular, RANKL (0.79 vs. 0.43 pmol/L), OPG (12.21 vs. 9.33 pmol/L) and CTX (0.37 vs. 0.30 ng/mL) were higher in women with active periodontal disease compared with those with past periodontal

Table 1. Comparison of anthropometric bone mineral density and plasma measurements in women with osteoporosis and control subjects

Parameter	Patients ($n = 185$)	Control subjects ($n = 185$)
Age (years)	62.06 ± 14.53	62.56 ± 13.24
Weight (kg)	60.92 ± 14.03	69.17 ± 15.06**
Height (cm)	155.6 ± 8.53	158.81 ± 6.51*
Body mass index (kg/m^2)	25.28 ± 6.46	27.39 ± 5.66**
Lumbar spine bone mineral density (g/cm^2)	0.86 ± 0.202	1.08 ± 0.195*
Femoral neck bone mineral density (g/cm^2)	0.71 ± 0.161	0.87 ± 0.181*
Parathyroid hormone (ng/L)	58.55 ± 43.10	44.60 ± 29.77*
25-Hydroxyvitamin D_3 (nmol/L)	66.62 ± 43.76	97.21 ± 45.76*
Estradiol (pmol/L)	156.46 ± 97.45	172.05 ± 77.71*
C-reactive protein (mg/L)	6.42 ± 7.72	4.03 ± 4.24*
Interleukin-6 (pg/mL)	5.83 ± 5.52	5.40 ± 0.40*
RANKL (pmol/L)	0.66 ± 0.67	0.37 ± 0.38**
Osteoprotegerin (pmol/L)	18.70 ± 9.70	10.44 ± 5.85**
Osteocalcin (ng/mL)	26.13 ± 15.35	24.08 ± 16.08*
C-terminal telopeptide (ng/mL)	0.33 ± 0.23	0.22 ± 0.19**
Inflammatory bowel disease	4.9%	0.5%*
Steroid use	16.8%	3.8%*
Rheumatoid arthritis	9.7%	1.6%*
Physical inactivity	85.9%	23.8%*
Weight loss	48.1%	7.6%*
Active periodontal disease	11.9%	0.5**
Past periodontal disease	75.7%	37.3**
Active or past periodontal disease	87.6%	37.8%**

Values given are the means ± SD except for percentages.
*$p < 0.001$, **$p < 0.0001$.

Table 2. Differences between those women with past periodontal disease as indicated by the presence of dentures (82 with partial dentures and 127 with full dentures, none with active disease) and without past periodontal disease (no dentures)

Parameter	Women with dentures ($n = 209$, 56.49%)	Women without dentures ($n = 161$, 43.51%)
Age (years)	64.31 ± 11.603	62.01 ± 14.19
Lumbar spine bone mineral density (g/cm^2)	0.93 ± 0.21	1.01 ± 0.22**
Femoral neck bone mineral density (g/cm^2)	0.76 ± 0.18	0.81 ± 0.18*
RANKL (pmol/L)	0.43 ± 0.61	0.46 ± 0.48
Osteoprotegerin (pmol/L)	9.33 ± 9.17	7.33 ± 7.95*
C-terminal telopeptide (ng/mL)	0.30 ± 0.23	0.25 ± 0.19*
25-Hydroxyvitamin D_3 (nmol/L)	78.86 ± 48.74	85.88 ± 45.11*
C-reactive protein (mg/L)	6.00 ± 6.75	4.22 ± 5.61*
Estradiol (pmol/L)	106.58 ± 87.21	152.82 ± 96.01*
Testosterone (pmol/L)	0.52 ± 0.46	0.65 ± 0.53*

Values given are the means ± SD except for percentages.
*$p < 0.001$, **$p < 0.0001$.

Table 3. Differences between those women with active periodontal disease (symptomatic) and those without

Parameter	Women with active periodontal disease (n = 23, 6.2%)	Women without active periodontal disease (n = 347, 93.79%)
Age	63.25 ± 12.56	62.24 ± 13.98
Lumbar spine bone mineral density (g/cm^2)	0.89 ± 0.17	0.97 ± 0. 22*
Femoral neck bone mineral density (g/cm^2)	0.70 ± 0.13	0.79 ± 0.19*
RANKL (pmol/L)	0.79 ± 1.59	0.42 ± 0.40*
Osteoprotegerin (pmol/L)	12.21 ± 10.64	8.21 ± 8.52*
C-terminal telopeptide (ng/mL)	0.37 ± 0.28	0.27 ± 0.21*
25-Hydroxyvitamin D_3 (nmol/L)	64.48 ± 38.38	83.07 ± 47.61*
C-reactive protein (mg/L)	9.62 ± 9.07	4.93 ± 6.01*
Estradiol (pmol/L)	102.47 ± 61.36	168.35 ± 96.01*
Testosterone (pmol/L)	0.37 ± 0.35	0.59 ± 0.50*

Values given are the means ± SD.
*$p < 0.001$.

Table 4. Multiple stepwise regression analysis with or without current and past periodontal disease as dependent variable and all anthropometric, bone mineral density and biochemical data entered

Predictor variable	SEM	β-value
Vitamin D	0.058	−0.208**
C-terminal telopeptide	0.037	0.111*
RANKL	0.055	0.133*
Weight	0.001	−0.101*

Adjusted $r^2 = 0.104$, $F = 11.104$, $p < 0.0001$. Significant variables are shown in the table. Osteoprotegerin was not a significant predictor in this model. The β-value represents the degree to which each predictor affects the outcome if the effects of all other predictors are held constant.
*$p < 0.001$, **$p < 0.0001$.

disease. Both plasma RANKL and OPG showed a significant correlation with each other ($r = 0.285$, $p < 0.0001$). A positive association was found between circulating levels of OPG and CTX ($r = 0.215, p < 0001$).

Logistic regression was performed to test how well each variable could account for the presence of periodontal disease. This identified a large number of variables that could account for a proportion of the variance in periodontal disease. Stepwise multiple logistic regression analysis was therefore performed to determine the combination of variables that accounted for the greatest proportion of variance in periodontal disease. All anthropometric, bone mineral density and biochemical parameters were included (Table 4). Periodontal disease was best predicted by a combination of RANKL, CTX, vitamin D and weight, giving an r^2 value of 10.4%.

Discussion

This study has shown a significantly higher prevalence of periodontal disease in postmenopausal women with osteoporosis compared with age-matched subjects without osteoporosis, including active or past periodontal disease and both combined (Table 1); this confirms the results of previous work (3–9). This association of osteoporosis with periodontal disease may be explained by the fact that both conditions have common predisposing factors of bone loss. However, in the case of osteoporosis, bone loss is generalized, whereas in periodontal disease it may be localized to the alveolar bone of the jaw. Osteoporosis and periodontal disease may share genetic, hormonal, dietary and other lifestyle risk factors. Previous studies have identified risk factors for the development of periodontal disease, and these include advancing age, early menopause, smoking and high alcohol consumption (3–9).

Raised inflammatory markers (CRP), RANKL and OPG were seen in women with osteoporosis, active and past periodontal disease (Tables 1, 2 and 3) and may also account for some of the link between the two conditions. The more marked differences observed between those with past periodontal disease and those with active periodontal disease suggest that it is the activity of the disease that may be important. This may account for the raised CRP, RANKL and OPG, which would then stimulate osteoclast activity and cause the observed significant elevations in CTX. Another possible explanation for the raised CRP could be the general ill health associated with both periodontal disease and osteoporosis. Periodontal disease was best predicted by RANKL, serum CTX, 25OHD and weight. Lower weight is commonly observed in men and women with osteoporosis compared with control subjects. This finding is generally thought to be due to the increased ill health and/or co-morbidities amongst osteoporotic subjects, such as those observed in this study. These would also tend to increase the risk of periodontal disease.

The lower concentrations of serum 25OHD seen in women with osteoporosis and periodontal disease and the fact that it is included in the multiple regression model are interesting. This is because of the recent discovery of an association between lowered serum vitamin D and increased cytokines, such as RANKL, IL-6 and tumour necrosis factor α, that are all involved in stimulating osteoclastogenesis (77–79). In animal studies, it has been shown that serum 25OHD levels < 80 nmol/L gave rise to osteopenia as a result of increased osteoclastogenesis, suggesting that levels of 25OHD > 80 nmol/L are needed for optimal bone volume (79). The threshold of 25OHD required to suppress cytokine induction and for the maintenance of bone health is not known.

Osteoprotegerin was significantly higher in postmenopausal women with periodontal disease than in those

without (Tables 2 and 3). In contrast, studies on periodontal disease and the role of OPG by Duarte *et al*., Buduneli *et al*. and Tang *et al*. (61,63–65) reported reduced expression of OPG in periodontitis specimens compared with the control nonperiodontitis group, whereas Lu *et al*. (58) found that the OPG level did not change in diseased sites of patients with periodontitis.

Data analysis on RANKL and its role in periodontal disease are also inconsistent. Several animal model studies have provided evidence that elevated RANKL may be important in the pathogenesis of the periodontal disease (57,59). Duarte *et al*. and Shu *et al*. (61,80) found an increased production of RANKL in gingival tissues obtained from periodontitis specimens compared with control subjects, whereas Buduneli *et al*. (63) found that RANKL levels in saliva samples did not differ between the two groups. It should be noted that the sample sizes for these studies were small (between 25 and 95 participants). However, there do not, as far as the authors are aware, appear to be any *in vivo* measurements of serum RANKL in human subjects until the present study. Our results showed a significant increase in plasma RANKL in postmenopausal women with periodontal disease compared with those without.

RANKL, RANK and OPG are known to be key molecules that regulate osteoclast recruitment, differentiation and activation (81). This has led to new concepts in the pathogenesis of periodontitis that have implicated inflammation triggered by the host immune response to periodontal biofilm micro-organisms in the disease process (81). The host response to bacteria involves the activation of T and B cells in the inflammatory infiltrate. This results in raised levels of RANKL that in turn promote osteoclastic bone resorption. Periodontal tissue destruction can be ameliorated by immunobiological interference with immune cell RANKL expression or function. The new disease concepts provide a foundation to build biological approaches to target RANKL production in periodontal lesions.

Women with osteoporosis, past and active periodontal disease all had significantly lower estradiol levels compared with those without. Low estradiol is certainly one possible explanation for the lower observed bone mineral density in these groups. The lower estradiol could be explained if there were differences in hormone replacement therapy use between the two groups, but the numbers on hormone replacement therapy were small (22 women with past periodontal disease and nine without dentures, five with active periodontal disease and nine without) and so seem unlikely to account for the observed difference. Furthermore, estradiol dropped out of the multiple logistic regression, suggesting that it was not the underlying mechanism. A more plausible explanation may be the greater ill health amongst those with osteoporosis, resulting in lower body weight and higher levels of inflammation and cytokines.

Our study has a number of strengths, including its large number of participants whose skeletal status was well characterized. The number of participants in this study was comparatively high, which should strengthen the precision of the results and the prediction models. Furthermore, this study was able to include in the regression models cytokines as well as other endogenous and environmental factors known or suspected to be related to age-related bone loss and periodontal disease.

The study has a number of potential limitations that need to be considered. Firstly, the study consisted of white Caucasian postmenopausal women only, and therefore the results cannot be extrapolated to other races or to men without further research. Secondly, the validity of the results is also dependent upon the correct answers being given to the questionnaire rather than measurements to determine the presence or absence of periodontal disease. The use of questionnaires has been validated by a number of studies (70–76). In addition, cross-sectional studies, although useful, have inherent limitations, including the investigators' inability to determine temporality, which can be better established through prospective longitudinal studies together with radiographic measures of periodontal disease.

In conclusion, periodontal disease is more common in women with osteoporosis and is associated with higher concentrations of CRP, RANKL and OPG, resulting in increased bone turnover, as well as lowered vitamin D. These raised levels of cytokines, perhaps partly driven by lowered vitamin D concentrations, could stimulate osteoclast activity, accounting for the observed raised serum CTX, and may provide the underlying mechanism that links these two conditions. Although factors such as hormonal status, smoking, alcohol use and diet may place women at risk for both periodontal disease and osteoporosis, our data suggest that body weight, circulating levels of RANKL, OPG, 25OHD and biochemical markers of bone turnover are also important.

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