

# Serum, Saliva, and Gingival Crevicular Fluid Osteocalcin: Their Relation to Periodontal Status and Bone Mineral Density in Postmenopausal Women

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**Background:** Periodontitis and osteoporosis are characterized by the loss of bone mass. Osteocalcin levels have been postulated as a marker of inhibition of bone formation. The aim of the present study was to assess plasma, saliva, and gingival crevicular fluid (GCF) levels of osteocalcin and correlate them with periodontitis and osteoporosis.

**Methods:** Seventy-three postmenopausal women, over 35 years old, were recruited for the study. Serum, saliva, and GCF osteocalcin were measured. Vertebral bone mineral density was measured by dual-energy x-ray absorptiometry. Differences between groups were assessed by analysis of variance (ANOVA), chi-square test, and non-parametric Kruskal-Wallis test.

**Results:** Thirty-four (46.6%) were classified in the normal healthy bone group, 11 women (15.1%) in the osteopenic group, and 28 women (38.4%) in the osteoporotic group. No statistically significant differences between these densitometric groups were observed in probing depth ( $P = 0.24$ ); clinical attachment level ( $P = 0.11$ ); or mean osteocalcin concentrations in serum, saliva, and GCF. Twenty-seven (37.0%) of the women were classified without periodontitis (NPG) and 63.0% ( $N = 46$ ) with periodontal disease (PG). There were no statistical differences in serum and saliva osteocalcin concentrations between these two groups. GCF osteocalcin concentrations were significantly higher in the PG women than in the NPG group ( $P = 0.008$ ). Mean probing depth correlated significantly with GCF osteocalcin concentrations ( $r = 0.35$ ;  $P = 0.002$ ).

**Conclusion:** The results further support the concept that osteocalcin levels in GCF correlates with periodontal but not with osteoporosis status. *J Periodontol 2005;76:513-519.*

## KEY WORDS

Bone resorption/prevention and control; gingival crevicular fluid/analysis; osteocalcin; osteopenia; osteoporosis; periodontitis; plasma/analysis; saliva/analysis.

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Osteoporosis is a skeletal disorder characterized by compromised bone strength predisposing it to increase risk of fracture, with bone strength determined by both bone density and bone quality.<sup>1</sup> Periodontitis is an inflammatory disease usually leading to bone loss. In periodontitis, there is an increased turnover of alveolar bone although there may be a dominance of bone resorption over bone formation, leading to alveolar bone loss and loss of attachment.<sup>2</sup> Based upon knowledge of the etiology and the common risk factors involved, some authors<sup>3-5</sup> have studied the association between both processes. The majority of studies<sup>3-5</sup> have shown low bone mass to be independently associated with loss of alveolar crestal height and tooth loss. However, studies that focus on the relation of clinical attachment loss and osteoporosis are less consistent.<sup>3</sup> Many factors have been identified which contribute to bone mass loss, including natural or surgically created menopause at an early age.<sup>4</sup> Increase in bone resorption due to gonadal reasons and accelerated bone loss in the first decade after menopause appear to be the main pathogenic factors in women.<sup>5</sup> Therefore, postmenopausal women are one of the risk groups for periodontitis and osteoporosis.

Biochemical methods of measuring specific markers are used routinely to provide information on bone resorption and formation. While many substances

are being investigated for their potential to aid in the assessment and monitoring of tissue loss in periodontitis, few are so directly related to bone metabolism. Osteocalcin, a 49 amino acid non-collagenous matrix protein of calcified tissue, is synthesized by osteoblasts. It has been postulated to have a role in both bone resorption and mineralization,<sup>6</sup> is currently described as the most specific marker of osteoblast function,<sup>7</sup> and has been identified as the current best marker in plasma of spontaneous bone loss in untreated postmenopausal women.<sup>8</sup> The serum level of this protein is considered to be a marker of bone formation<sup>9,10</sup> and is routinely assayed in investigation and monitoring of metabolic bone disorders.<sup>7</sup> Serum osteocalcin is presently considered 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.<sup>11</sup>

Gingival crevicular fluid, which is an exudate that can be harvested from the sulcus or periodontal pocket, has been regarded as a promising medium for the detection of periodontal disease activity.<sup>12,13</sup> The majority of new diagnostic tests for periodontal disease use GCF.<sup>14,15</sup> However, of the more than 50 different components in GCF evaluated to date, the majority lack specificity to alveolar bone destruction and essentially constitute soft-tissue inflammatory events.<sup>16,17</sup> Several investigations on osteocalcin levels in GCF from periodontal patients<sup>2,16,18-21</sup> have been reported, suggesting that osteocalcin levels in GCF may reflect inflammation at diseased sites, and there has been interest in osteocalcin as a potential marker of bone turnover in periodontal disease.<sup>2,10,11</sup> However, the role of osteocalcin in periodontal disease progression is still unclear.

The aim of the present study was to assess plasma, saliva, and GCF levels of osteocalcin and correlate them with periodontitis and osteoporosis.

## MATERIALS AND METHODS

### *Study Population and Clinical Examination*

For 3 years, all the postmenopausal women >35 years old who were patients at our Dental School were invited to participate in the study, and 73 patients were recruited. Our protocol and consent forms were approved by the Committee of Ethics and Research of the University of Seville Hospital Virgen Macarena. All the women met the following inclusion criteria: at least 12 months since the last menstrual period; at least seven teeth; no hormone therapy; no medical treatment influencing bone metabolism; and no metabolic diseases.

General data were recorded: age, age at menopause, years since menopause, and history of cigarette smoking. One clinician examined all the patients, recording the following measurements for all teeth: 1) O'Leary plaque index;<sup>22</sup> 2) van der Velden bleeding on probing

(BOP) index;<sup>23</sup> 3) probing depth (PD), measured from the gingival margin to the most apical penetration of the probe; and 4) recession measured from the cemento-enamel junction to the gingival margin. Clinical attachment level (CAL) was calculated by adding recession to PD. PD and CAL were assessed at six sites per tooth: mesial edge, midtooth, and distal edge on both facial and lingual sides. According to the criteria established by Machtei et al.,<sup>24</sup> the clinical entity of periodontitis is based on the presence of CAL  $\geq 6$  mm in two or more teeth and one or more sites with PD  $\geq 5$  mm.

### *Sample Collection and Analytical Determinations*

Blood was centrifuged to obtain plasma. GCF sampling was performed based on the method previously described by Nomura et al.<sup>25</sup> Each patient was monitored at four sites in the upper incisor teeth. In the periodontitis group the sites were always PD  $\geq 5$  mm. After isolation from saliva, the area around each site sampled was dried and supragingival plaque removed. GCF was collected for 2 minutes using four strips (20  $\times$  4 mm) of paper.<sup>§</sup> Each strip was placed gently into the gingival crevice until slight resistance was felt, and GCF volume was collected. The GCF absorbed ( $\mu$ l)/wet area ( $\text{mm}^2$ ) in the strip paper was calculated by comparing it to the amount of a standard plasma containing a low concentration of <sup>125</sup>I used as a tracer that the strip can absorb, similar to what has been described for blood specimens.<sup>26,27</sup> A saliva sample was also collected from the sublingual zone with the same paper strips. The plasma, saliva, and GCF samples were placed in a microcentrifuge tube and vortexed vigorously in 200  $\mu$ l of extraction buffer consisting of 0.5 NaCl, 50 mM Tris-HCL (pH 7.4), 5 mM CaCl<sub>2</sub>, and 0.05% Brij 35 (polyoxyethylene<sup>23</sup> lauryl ether<sup>||</sup>) by a modification of the method described by Lee et al.<sup>28</sup>

### *Osteocalcin Assay*

Osteocalcin measurements in serum, saliva, and GCF were made using an electrochemiluminescence technique.<sup>¶</sup> Results were obtained from a calibration curve performed in the system by means of a two-point calibration and a master curve included into the reactive bar code. The linearity defined by the detection limit and the calibration curve maximum concentration is established in 0.500 to 300 ng/ml. The detection limit, the lowest measurable osteocalcin concentration, is calculated as the concentration situated two standard deviations above the minor concentration calibrator value of the master curve is 0.5 ng/ml. The minimum volume used by the analyst to carry out a determination was 20  $\mu$ l sample.

§ Whatmann Lab Sales Ltd., Maidstone, Kent, U.K.

|| Nacalai Tesque Inc., Kyoto, Japan.

¶ Immulite 2000, Diagnostic Products Corporation, Los Angeles, CA.

### Bone Mineral Density Measurements

Vertebral bone mineral density (BMD) was measured in each woman by dual-energy x-ray absorptiometry.<sup>#</sup> We used antero-posterior projections of L2-L4. BMD was calculated as the average of density between L2 to L4, and expressed as g/cm<sup>2</sup>. The World Health Organization<sup>29</sup> defines osteoporosis by a BMD measured as two-and-one-half standard deviations ( $-2.5$  SD) below average peak bone density achieved in young adults, matched by gender and race. Based on the values obtained, three groups were established: normal healthy group (BMD  $\geq -1$  SD), osteopenic group (BMD between  $< -1$  SD and  $> -2.5$  SD), and osteoporotic group (BMD  $\leq -2.5$  SD).

### Statistical Analysis

Data are expressed as mean  $\pm$  standard deviation. Differences between groups in normally continuous variables were assessed by analysis of variance (ANOVA), using the Bonferroni test to establish differences between groups. Discrete variables were analyzed by means of frequency distribution. The comparative analysis of discrete variables was carried out using the chi-square test. Non-parametric Kruskal-Wallis test was used to analyze non-normally distributed variables. The Pearson correlation coefficient was used to analyze correlation between variables.  $P < 0.05$  was considered significant.

### RESULTS

The mean age of the 73 women was  $57.3 \pm 7.7$  years (range 38 to 76 years). Only four were smokers, one smoked  $>20$  cigarettes/day and three  $<20$  cigarettes/day. Mean menopausal age was  $48.1 \pm 5.0$  (range 38 to 60 years). Mean number of years since menopause was  $9.15 \pm 7.5$  (range 1 to 29 years).

According to the criteria established by Machtei et al.,<sup>24</sup> 37.0% ( $N = 27$ ) of the women were classified without periodontitis (NPG) and 63.0% ( $N = 46$ ) with periodontal disease (PG) (Table 1). Mean PD, mean CAL, and BOP were statistically higher in PG than in the NPG group ( $P < 0.05$ ). There were no significant differences in the percentage of sites with plaque between both groups ( $P = 0.48$ ).

According to bone mineral density assessed by densitometry, 34 women (46.6%) were classified in the normal (healthy) bone group (NBG), 11 (15.1%) in the osteopenic group (OEG), and 28 (38.4%) in the osteoporotic group (OPG) (Table 2). There was no significant difference between NPG and PG groups in the distribution of women according to their densitometric results ( $P = 0.36$ ). No statistically significant differences between groups were observed in probing depth ( $P = 0.24$ ) or in CAL ( $P = 0.11$ ). Women in osteopenic and osteoporotic groups showed more BOP than those in the healthy bone group, but differences were not

Table 1.

### Clinical Characteristics by Periodontal Disease Status (mean $\pm$ SD)

	Non-Periodontitis Group	Periodontitis Group
N women (%)	27 (37.0)	46 (63.0)
PD (mm)	$2.0 \pm 0.5$	$2.6 \pm 0.7$
CAL (mm)	$2.1 \pm 0.3$	$3.6 \pm 1.1$
BOP (%)	$64.9 \pm 18.3$	$74.5 \pm 17.5$
Plaque (%)	$72.4 \pm 25.8$	$67.5 \pm 30.1$

Table 2.

### Periodontal Status in Relation With Bone Mineral Density (mean $\pm$ SD)

	NBG	OEG	OPG	P
N women (%)	34 (46.6)	11 (15.1)	28 (38.4)	
NPG (%)	14 (51.9)	2 (7.4)	11 (40.7)	
PG (%)	20 (43.5)	9 (19.6)	17 (37.0)	0.36
PD (mm)	$2.4 \pm 0.5$	$2.6 \pm 0.5$	$2.3 \pm 0.5$	0.24
CAL (mm)	$2.9 \pm 1.1$	$3.7 \pm 1.6$	$3.0 \pm 0.9$	0.11
BOP (%)	$65.6 \pm 16.9$	$79.3 \pm 18.4$	$74.3 \pm 18.4$	0.04
Plaque (%)	$63.0 \pm 32.6$	$59.0 \pm 35.8$	$81.2 \pm 12.5$	0.01

statistically significant ( $P = 0.04$ ; non-significant Bonferroni test). On the contrary, the mean percentage of sites with plaque in osteoporotic women ( $81.2\% \pm 12.5\%$ ) was significantly higher than that in the osteopenic and normal healthy bone groups ( $63.0\% \pm 32.6\%$  and  $59.0\% \pm 35.8\%$ , respectively) ( $P = 0.01$ ; with significant differences between the normal healthy bone group and osteoporotic group in Bonferroni test).

Mean osteocalcin concentrations in serum, saliva, and GCF in correlation with periodontal status of the women are shown in Table 3. Serum and saliva osteocalcin concentrations did not show statistical differences between the two groups ( $P > 0.05$ ). On the contrary, GCF osteocalcin concentrations were significantly higher in the periodontally diseased group ( $35.0 \pm 30.3$  ng/ml) than in the healthy group ( $18.1 \pm 15.2$  ng/ml) ( $P = 0.008$ ).

Mean osteocalcin concentrations in serum, saliva, and GCF correlated with bone mineral density are

<sup>#</sup> QDR 1000, Hologic Inc., Waltham, MA.

**Table 3.****Correlations Between Serum, Saliva, and Gingival Crevicular Fluid Osteocalcin Concentrations (ng/ml) and Periodontal Status (mean ± SD)**

	NPG	PG	P
Serum OC	8.1 ± 3.7	6.8 ± 3.6	0.18
Saliva OC	4.9 ± 3.1	5.5 ± 3.1	0.41
GCF OC	18.1 ± 15.2	35.0 ± 30.3	0.008

**Table 4.****Correlation Between Serum, Saliva, and Gingival Crevicular Fluid Osteocalcin Concentrations (ng/ml) and Bone Mineral Density (mean ± SD)**

	Total	NBG	OEG	OPG	P
Serum OC	7.3 ± 3.6	7.1 ± 3.2	5.6 ± 3.4	8.2 ± 4.1	0.12
Saliva OC	5.4 ± 3.1	5.2 ± 3.9	5.7 ± 2.8	5.3 ± 2.2	0.88
GCF OC	27.5 ± 26.2	27.5 ± 24.7	43.5 ± 47.3	24.4 ± 15.9	0.13

shown in Table 4. No statistically significant differences between densitometric groups were observed ( $P > 0.05$ ).

Women were classified according to their serum osteocalcin concentration into two groups: normal osteocalcin concentration group (osteocalcin concentration between 3.5 to 10 ng/ml) and increased osteocalcin concentration group (osteocalcin concentration  $\geq 10$  ng/ml). The correlation between serum osteocalcin concentrations and periodontal clinical parameters was examined (Table 5). No statistically significant differences were observed ( $P > 0.05$ ). Using the Pearson lineal correlation test, serum osteocalcin concentration did not correlate with any periodontal clinical parameter.

To study the correlation between saliva osteocalcin concentrations and periodontal clinical parameters, women were classified according to their serum osteocalcin concentration in three groups: 1) osteocalcin concentration  $< 3$  ng/ml; 2) osteocalcin concentration ranging from 3 to 7 ng/ml; and 3) osteocalcin concentration  $> 7$  ng/ml (Table 6). No statistically significant differences in probing depth, bleeding on probing, or percentage of dental plaque between the three groups were observed ( $P > 0.05$ ). On the contrary, there were significant differences between the three groups in clinical attachment loss ( $P = 0.01$ ; with significant

**Table 5.****Correlation Between Serum Osteocalcin Concentrations and Periodontal Clinical Parameters (mean ± SD)**

	Normal OC	Increased OC	P
PD (mm)	2.4 ± 0.5	2.5 ± 0.7	0.73
CAL (mm)	3.0 ± 1.0	3.5 ± 2.0	0.43
BOP (%)	69.0 ± 18.3	81.1 ± 16.0	0.21
Plaque (%)	70.4 ± 27.2	84.8 ± 30.7	0.73

**Table 6.****Correlation Between Saliva Osteocalcin Concentrations (ng/ml) and Periodontal Clinical Parameters (mean ± SD)**

	OC $< 3$	OC 3-7	OC $> 7$	P
PD (mm)	2.3 ± 0.5	2.6 ± 0.5	2.3 ± 0.5	0.11
CAL (mm)	2.6 ± 0.7	3.5 ± 1.3	2.9 ± 1.0	0.01
BOP (%)	67.0 ± 18.0	75.2 ± 19.2	66.7 ± 16.5	0.19
Plaque (%)	68.8 ± 26.8	84.5 ± 32.6	73.3 ± 25.6	0.41

**Table 7.****Correlation Between Gingival Crevicular Fluid Osteocalcin Concentrations (ng/ml) and Periodontal Clinical Parameters (mean ± SD)**

	OC $< 12$	OC 12-30	OC $\geq 30$	P
PD (mm)	2.2 ± 0.3	2.4 ± 0.5	2.6 ± 0.5	0.003
CAL (mm)	2.7 ± 1.1	3.1 ± 1.1	3.4 ± 1.1	0.008
BOP (%)	63.8 ± 19.8	69.6 ± 16.1	78.0 ± 16.5	0.01
Plaque (%)	64.3 ± 30.9	72.7 ± 29.6	71.0 ± 26.0	0.57

differences between the group  $< 3$  OC and  $> 7$  OC in Bonferroni test). Using the Pearson lineal correlation test, saliva osteocalcin concentrations did not correlate with any periodontal clinical parameter.

To study the correlation between GCF osteocalcin concentrations and periodontal clinical parameters, women were classified in three groups according to their GCF osteocalcin concentration: 1) osteocalcin concentration  $< 12$  ng/ml; 2) osteocalcin concentration ranging from 12 to 30 ng/ml; and 3) osteocalcin concentration  $> 30$  ng/ml (Table 7). Mean probing depth

correlated significantly with GCF osteocalcin concentrations ( $r = 0.35$ ;  $P = 0.002$ ). Women with low GCF osteocalcin concentrations showed a significantly lesser probing depth ( $2.2 \pm 0.3$  ng/ml) than those with higher GCF osteocalcin concentrations ( $P = 0.03$ ). Bonferroni test also showed significant differences between the group with 12 to 30 ng/ml and the group with  $>30$  ng/ml. Mean CAL did not correlate with GCF osteocalcin concentrations ( $r = 0.18$ ;  $P = 0.119$ ) but CAL was significantly higher in the group with  $>30$  ng/ml osteocalcin concentration ( $P = 0.08$ ). Bleeding on probing percentage increased in correlation with GCF osteocalcin concentration ( $r = 0.25$ ;  $P = 0.02$ ). Bonferroni test showed statistical differences between the group with  $<12$  ng/ml GCF osteocalcin and the group with  $>30$  ng/ml ( $P = 0.01$ ). No statistically significant differences in percentage of dental plaque between the three groups were observed ( $P > 0.05$ ).

## DISCUSSION

Osteocalcin levels in serum, saliva, and gingival crevicular fluid were determined in the present cross-sectional study. To our knowledge, this is the first study to measure osteocalcin concentrations in these three fluids in both healthy and periodontally diseased subjects. We showed that OC in GCF correlated significantly ( $P = 0.008$ ) with the periodontal status of the patients. Moreover, in the present study, OC in GCF correlated significantly with probing depth, clinical attachment level, and bleeding on probing, three main clinical parameters of periodontal disease. Additionally, saliva OC has been shown to correlate significantly with clinical attachment level.

It is unclear where osteocalcin in GCF originates. Some of this protein will be derived from circulating levels. However, it is also possible that osteocalcin will be produced locally, either alveolar bone is resorbed or from active local osteoblast synthesis.<sup>2,30</sup> The reservoir of osteocalcin locked into the alveolar bone and released into the GCF by osteoblast activity will, most likely, be correlated with the local osteoblast activity. The localization of osteocalcin in the entire human periodontium including gingiva, periodontal ligament, cementum, and alveolar bone has been studied.<sup>31</sup> The authors demonstrated its presence within the alveolar bone, cementum, and periodontal ligament and a high expression of osteocalcin in periodontal ligament fibroblasts. Since osteocalcin may play important roles in cementogenesis, osteoblast differentiation, and bone mineralization, it may express hard tissue metabolism. Furthermore, the finding of increased bone density in osteocalcin null mutant mice<sup>32</sup> implicates osteocalcin as a potential inhibitor of bone formation.

GCF was thought to be an inflammatory exudate capable of detecting episodes of periodontitis,<sup>16</sup> but it has not been determined whether GCF markers in

humans directly correlate with those at the underlying bone. There are clear diagnostic advantages in identifying an unambiguous marker of periodontal disease. We therefore investigated GCF osteocalcin levels as a marker of periodontitis. Osteocalcin is a specific osteoblast product and raised levels in adult periodontitis may be related to the severity of breakdown and/or repair of alveolar bone. During active bone resorption, osteocalcin and osteocalcin fragments are likely to be released from the extracellular matrix into the GCF.<sup>33</sup> The fact that bone remodeling occurs simultaneously in multiple skeletal location is generally viewed as evidence that it is locally controlled, through autocrine and/or paracrine mechanisms.<sup>30</sup> So, osteocalcin levels in GCF are more informative than serum or saliva levels regarding bone turnover in the periodontium.

In the present study, patients with the highest OC in GCF had significantly more probing depth, clinical attachment level, and bleeding on probing, all clinical parameters that involve periodontal disease. Moreover, periodontally diseased women showed 2-fold higher OC in GCF than healthy women ( $P = 0.008$ ). These results agree with previous data reported by other investigators who studied the relationship between GCF osteocalcin levels and periodontal disease.<sup>10,18,19</sup> Kunimatsu et al.<sup>18</sup> reported a positive correlation between GCF osteocalcin N-terminal peptide levels and clinical parameters in a cross-sectional study of 14 patients with periodontitis and five with gingivitis.

Nakashima et al.<sup>19</sup> reported that OC in GCF correlated significantly with the gingival index, but not with probing depth. A longitudinal study using an experimental periodontitis model in beagle dogs<sup>10</sup> reported a strong correlation between GCF osteocalcin levels and active bone turnover as assessed by bone-seeking radiopharmaceutical uptake. The results of the present study, together with those of previous studies,<sup>10,18,19</sup> support the concept that OC in GCF can be used as a marker of periodontal disease.

On the other hand, several studies have found that OC in GCF did not correlate with periodontal status.<sup>2,14,16,20,21</sup> Treatment of adult periodontitis patients with subantimicrobial doxycycline failed to reduce GCF osteocalcin levels.<sup>16</sup> A cross-sectional study of 20 periodontitis patients reported no differences in GCF osteocalcin levels between deep and shallow sites in the same patients.<sup>2</sup> Moreover, in a longitudinal study of untreated periodontitis patients with  $\geq 1.5$  mm attachment loss during the monitoring period,<sup>20</sup> GCF osteocalcin levels alone were unable to distinguish between active and inactive sites. In another study, osteocalcin levels in the GCF during orthodontic tooth movement were highly variable between subjects and lacked a consistent pattern related to the stages of tooth movement.<sup>14</sup> Recently, Wilson et al.<sup>21</sup> did not detect

osteocalcin in GCF in 14 subjects with untreated periodontitis. Several studies<sup>2,16,20</sup> were performed in periodontally diseased patients and did not compare healthy and diseased subjects, but compared osteocalcin levels in diseased and healthy sites in patients with adult periodontitis.

One study concluded that a potential role exists for whole osteocalcin as a bone-specific marker of bone turnover but not as a predictive indicator of periodontal disease.<sup>11</sup> However, the results of the present study demonstrated that OC in GCF is 2-fold higher in postmenopausal periodontally diseased women in comparison with healthy women, strongly suggesting that osteocalcin levels in GCF can also be used as a marker of periodontal status.

Osteocalcin is predominantly synthesized by osteoblasts and it plays an important role in both bone resorption and mineralization.<sup>30</sup> The serum levels of this protein are considered to be a marker of bone remodeling and are routinely assayed in the investigation and monitoring of metabolic bone disorders, especially in osteoporosis.<sup>7</sup> Elevated serum osteocalcin levels have been shown in periods of rapid bone turnover such as osteoporosis, multiple myeloma, and fracture repair.<sup>34</sup> However, in the present study no correlation between serum, saliva, and gingival crevicular fluid osteocalcin concentrations and bone mineral density assessed by densitometry was found. The finding that serum OC did not correlate with bone mineral density could be explained because of the age of the study population ( $57.3 \pm 7.7$  years) and the relatively high number of years that passed since menopause ( $9.15 \pm 7.5$  years). In agreement with these results, a previous report on elderly women has shown that values of serum total osteocalcin were similar among patients with and without subsequent hip fracture.<sup>35</sup> The utility of serum OC to predict osteoporosis in women over 67 has been reported as limited.<sup>36</sup> According to the study by Looker et al., biochemical markers of bone remodeling are neither surrogates nor substitutes for measurements of bone mineral density in elderly women.<sup>37</sup>

In the present study no correlation between serum OC and periodontal status was found. Osteocalcin is produced by osteoblasts in bone remodeling sites and only a small fraction is directly secreted to blood.<sup>38</sup> Serum levels of osteocalcin are unlikely to generate data that reflect periodontal disease, as circulating levels reflect bone turnover in the whole skeleton and any contribution from bone remodeling would be masked.<sup>11</sup>

In conclusion, the results of the present study, together with previous reports,<sup>10,18,19</sup> further support the concept that osteocalcin levels in GCF are related with the patient periodontal status. It could be deduced that an inhibition of bone formation could take place, and this would facilitate the periodontal bone resorp-

tion. Additional longitudinal studies may be warranted to elucidate more fully the usefulness of osteocalcin as a diagnostic aid of periodontal disease activity.

## REFERENCES

1. Garnero P. Markers of bone turnover for the prediction of fracture risk. *Osteoporos Int* 2000;11(Suppl. 6):S55-S65.
2. Lee AJ, Walsh TF, Hodges SJ, Rawlinson A. Gingival crevicular fluid osteocalcin in adult periodontitis. *J Clin Periodontol* 1999;26:252-256.
3. Wactawski-Wende J. Periodontal diseases and osteoporosis: Association and mechanisms. *Ann Periodontol* 2001;6:197-208.
4. Hreshchychshn MM, Hopkins A, Zylstra S, Anbar M. Effects of natural menopause, hysterectomy and oophorectomy on lumbar spine and femoral neck bone densities. *Obstet Gynecol* 1988;72:631-638.
5. Riggs BL, Melton LJ. Evidence for two distinct syndromes of involutional osteoporosis. *Am J Med* 1986;75:899-901.
6. Roach HI. Why does bone matrix contain non-collagenous proteins? The possible roles of osteocalcin, osteonectin, osteopontin and bone sialoprotein in bone mineralisation and resorption. *Cell Biol Int* 1994;18:617-628.
7. Hauschka PV, Lian JB, Cole DE, Gundberg CM. Osteocalcin and matrix Gla protein: Vitamin K-dependent proteins in bone. *Physiol Rev* 1989;69:990-1047.
8. Delmas PD. Clinical use of biochemical markers of bone remodeling in osteoporosis. *Bone* 1992;13(Suppl. 1):S17-S21.
9. Christenson RH. Biochemical markers of bone metabolism: An overview. *Clin Biochem* 1997;30:573-593.
10. Giannobile WV, Lynch SE, Denmark RG, Paquette DW, Fiorellini JP, Williams RC. Crevicular fluid osteocalcin and pyridinoline cross-linked carboxyterminal telopeptide of type I collagen (ICTP) as markers of rapid bone turnover in periodontitis: A pilot study in beagle dogs. *J Clin Periodontol* 1995;22:903-910.
11. Giannobile WV, Al-Shammari KF, Sarment DP. Matrix molecules and growth factors as indicators of periodontal disease activity. *Periodontology 2000* 2003;31:125-134.
12. Curtis MA, Gillett IR, Griffiths GS, et al. Detection of high-risk groups and individuals for periodontal diseases: Laboratory markers from analysis of gingival crevicular fluid. *J Clin Periodontol* 1989;16:1-11.
13. Page RC. Host response tests for diagnosing periodontal diseases. *J Periodontol* 1992;63:356-366.
14. Griffiths GS. Formation, collection and significance of gingival crevice fluid. *Periodontology 2000* 2003;31:32-42.
15. Iitto V-J, Overall CM, McCulloch C. Proteolytic host cell enzymes in gingival crevice fluid. *Periodontology 2000* 2002;31:77-104.
16. Golub LM, Lee HM, Greenwald RA, et al. A matrix metalloproteinase inhibitor reduces bone-type collagen degradation fragments and specific collagenases in gingival crevicular fluid during adult periodontitis. *Inflamm Res* 1997;46:310-319.
17. Nomura T, Ishii A, Oishi Y, Kohma H, Hara K. Tissue inhibitors of metalloproteinases level and collagenase activity in gingival crevicular fluid: The relevance to periodontal diseases. *Oral Dis* 1998;4:231-240.
18. Kunitatsu K, Mataka S, Tanaka H, et al. A cross-sectional study on osteocalcin levels in gingival crevicular fluid from periodontal patients. *J Periodontol* 1993;64:865-869.
19. Nakashima K, Roehrich N, Cimasoni G. Osteocalcin,

- prostaglandin E<sub>2</sub> and alkaline phosphatase in gingival crevicular fluid: Their relations to periodontal status. *J Clin Periodontol* 1994;21:327-333.
20. Nakashima K, Giannopoulou C, Andersen E, et al. A longitudinal study of various crevicular fluid components as markers of periodontal disease activity. *J Clin Periodontol* 1996;23:832-838.
  21. Wilson AN, Schmid MJ, Marx DB, Reinhardt RA. Bone turnover markers in serum and periodontal microenvironments. *J Periodontol Res* 2003;38:355-361.
  22. O'Leary TJ, Drake RB, Naylor JE. The plaque control record. *J Periodontol* 1972;43:38.
  23. Van der Velden U. Probing force and relationship of the probe tip to the periodontal tissues. *J Clin Periodontol* 1979;6:106-114.
  24. Machtei EE, Christersson LA, Grossi SG, Dunford R, Zambon JJ, Genco RJ. Clinical criteria for the definition of established periodontitis. *J Periodontol* 1992;63:207-215.
  25. Nomura T, Ishii A, Shimizu H, et al. Tissue inhibitor of metalloproteinases-1, matrix metalloproteinases-1 and -8, and collagenase activity levels in peri-implant crevicular fluid after implantation. *Clin Oral Implants Res* 2000;11:430-440.
  26. Gonzalez C, Guerrero JM, Elorza FL, Molinero P, Goberna R. Evaluation of maternal serum alpha-fetoprotein assay using dry blood spot samples. *J Clin Chem Clin Biochem* 1988;26:79-84.
  27. Chamoles NA, Blanco MB, Gaggioli C. Hurler-like phenotype: Enzymatic diagnosis in dried blood spots on filter paper. *Clin Chem* 2001;47:2098-2102.
  28. Lee HJ, Auken S, Kulkarni G, et al. Collagenase activity in recurrent periodontics: Relationship to disease progression and doxycycline therapy. *J Periodontol Res* 1991;26:479-485.
  29. World Health Organization. Assessment of fracture risk and its applications to screening for postmenopausal osteoporosis. *WHO Technical Report Series*. Geneva: WHO; 1994.
  30. Ducy P, Schinke T, Karsenty G. The osteoblast: A sophisticated fibroblast under central surveillance. *Science* 2000;289:1501-1504.
  31. Ivanovski S, Li H, Haase HR, Bartold PM. Expression of bone associated macromolecules by gingival and periodontal ligament fibroblasts. *J Periodontol Res* 2001;36:131-141.
  32. Ducy P, Desbois C, Boyce B, et al. Increased bone formation in osteocalcin-deficient mice. *Nature* 1996;382:448-452.
  33. Baumgrass R, Williamson MK, Price PA. Identification of peptide fragments generated by digestion of bovine and human osteocalcin with the lysosomal proteinases cathepsin B, D, L, H, and S. *J Bone Miner Res* 1997;12:447-455.
  34. Slovik DM, Gundberg CM, Neer RM, Lian JB. Clinical evaluation of bone turnover by serum osteocalcin measurements in a hospital setting. *J Clin Endocrinol Metab* 1984;59:228-230.
  35. Garnero P, Hausher E, Chapuy MC, et al. Markers of bone resorption predict hip fracture in elderly women: The EPIDOS prospective study. *Osteoporos Int* 1996;11:1531-1538.
  36. Bauer DC, Sklarin PM, Stone KL, Black DM. Biochemical markers of bone turnover and prediction of hip bone loss in older women: The study of osteoporotic fractures. *J Bone Miner Res* 1999;14:1404-1410.
  37. Looker AC, Bauer DC, Chesnut CH III, et al. Clinical use of biochemical markers of bone remodelling: Current status and future directions. *Osteoporos Int* 2000;11:467-480.
  38. Lian JB, Gundberg CM. Osteocalcin. Biochemical consideration and clinical applications. *Clin Orthop* 1988;226:267-291.

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