Correlation Between Salivary and Serum Markers of Bone Turnover in Osteopenic Rats

Gretel G. Pellegrini,* Chaves Macarena Gonzales,*† Julia C. Somoza,†‡ Silvia M. Friedman,* and Susana N. Zeni*†‡

Background: Previously, we measured bone alkaline phosphatase (b-ALP) and terminal C-telopeptide of collagen type I (CTX) in saliva. The present longitudinal experimental study sought to determine whether salivary concentrations of b-ALP and CTX have the same response as in serum samples under different conditions: normal, increased, and reduced bone remodeling.

Methods: Thirty rats were ovariectomized (OVX) to induce osteopenia 60 days after surgery, and 10 rats were sham operated. Then, the rats were divided into four groups and treated as follows for 45 days: group 1 (G1) = SHAM + vehicle; group 2 (G2) = OVX + 8 μg olpadronate (OPD)/100 g of body weight; group 3 (G3) = OVX + 4 μg OPD/100 g of body weight; and group 4 (G4) = OVX + vehicle. Saliva and serum CTX and b-ALP were determined at 60 days (baseline) and at 75 days (T_{75}). Lumbar spine and proximal tibia bone mineral density (BMD) was determined using dual-energy x-ray absorptiometry at baseline and at 105 days.

Results: SHAM baseline and T_{75} salivary b-ALP and CTX levels correlated with serum concentrations (P <0.01 and P <0.004, respectively). A correlation was observed between saliva and serum concentrations of b-ALP and CTX in OVX at baseline (P <0.0001 and P <0.004, respectively). Baseline salivary b-ALP and CTX levels were lower in SHAM animals compared to OVX groups (P <0.01). After treatment, T_{75} saliva and serum CTX remained higher in G4 compared to G1 (P <0.05), was lower in G2 than in G1 (P <0.01) and G3 (P <0.01), and was similar in G1 and G3. Changes in BMD were the result of variations in salivary CTX levels due to OPD treatment (P <0.05).

Conclusions: Saliva determinations may prove to be practical and reliable for the detection of systemic signs of increased bone remodeling, particularly in cases involving pediatric, obese, and elderly patients, and in screening large populations. Moreover, saliva CTX may be one of the best candidate markers to detect the activity and severity of periodontal disease. *J Periodontol 2008;79:158-165.*

KEY WORDS

Bisphosphonates; bone remodeling; osteopenia; periodontitis; rats; saliva/analysis.

* Oral and General Biochemistry Department, School of Dentistry, University of Buenos Aires, Buenos Aires, Argentina.
† National Council of Technical and Scientific Research, Buenos Aires, Argentina.
‡ Medical Bone Disease Service, "J. de San Martín" Clinical Hospital, School of Medicine, University of Buenos Aires.

Bone is a specialized connective tissue continuously undergoing remodeling as a result of the coordinated actions of osteoclasts and osteoblasts. Under normal conditions, bone turnover involves a cycle that, at the cellular level, takes place at discrete sites in cancellous and cortical bone. The team of cells carrying out this process of bone renewal is termed a basic multicellular unit (BMU).[1] At any particular period of time, >1 million BMUs work simultaneously throughout the healthy adult skeleton. The activity of all of these cellular packages can be measured biochemically by determining markers of bone turnover, which involves bone resorption and formation processes arising from osteoclasts and osteoblasts, respectively.[2]

Remodeling is not stable throughout life. In children, formation and resorption more or less parallel growth velocity, with a transient increase during puberty. Bone markers decrease in young adults and remain stable thereafter until menopause when they increase in women again as a consequence of estrogen withdrawal. It is well established that there is a dramatic increase in remodeling during the menopausal transition, which is characterized by a significant increase in bone resorption with a simultaneous but slower increase in bone formation.[3] This high bone turnover leads to a biphasic loss of mass[4] that increases the incidence of osteoporosis-related fractures.[3] In this

doi: 10.1902/jop.2008.070168

regard, during the first years postmenopause, the loss of trabecular and cortical bone is 20% to 30% and 5% to 10%, respectively. A second slow, continuous phase occurs throughout a woman's life; although the incidence of osteoporotic hip fractures is higher in women, the bone loss associated with aging is similar in both genders.[5]

Like postmenopausal women, ovariectomized (OVX) rats show an increase in bone formation and resorption markers and a loss of trabecular bone.[6,7] For this reason, the OVX rat is the most commonly used model to study bone loss associated with estrogen deficiency.[6,8]

The proper management of osteoporosis and other metabolic bone pathologies involves early diagnosis of the disease, which includes measurement of bone mineral density (BMD) using dual-energy x-ray absorptiometry (DXA). This technique involves the relative attenuation of two discrete energies, and it can measure bone mass with good accuracy and excellent precision. However, BMD is a static parameter that is critical to clinical evaluation, but it does not provide insight into the rate of bone remodeling. A dynamic assessment of bone turnover could enhance the predictive value of BMD as an indicator of fracture risk and could have diagnostic and therapeutic significance.[9] Although changes in bone architecture take time to manifest, changes in bone turnover can be ascertained relatively early by measuring bone markers in serum and/or urine, thereby helping clinicians to make therapeutic decisions.[3,10] In this regard, drugs used to treat individuals with a high fracture risk are designed to decrease bone resorption and/or increase bone formation.

Anticatabolic drugs, such as bisphosphonates, are used to treat bone loss because they induce a dose-dependent decrease in bone turnover.[11] The effect of these drugs on bone markers is rapid, resulting in decreases in resorption markers within 1 to 3 months and in bone formation markers within 3 to 6 months.[12] Consequently, bone turnover decreases within a few months after anticatabolic treatment, whereas ≥1 year is required to evaluate changes in BMD. For this reason, strong evidence now shows that changes in bone markers are predictive of the efficacy of treatment with regard to future BMD changes.[9,10]

Bone turnover is measured using serum or urinary biomarkers. However, saliva recently has gained significant recognition as a biologic sample for the detection of several oral and systemic illnesses. Because saliva is a biofluid that can be obtained readily via a non-invasive procedure, it could pose several advantages in identifying individuals with high bone turnover and in monitoring the progress of a specific treatment. Additional benefits of the use of saliva samples are related to certain clinical settings, such as examining normal pediatric or elderly patients, or performing screenings. In these cases, obtaining serum or urine samples to measure bone turnover markers may prove awkward or may seem invasive. Based on the above, using saliva samples to evaluate bone turnover seems to be a promising alternative in reaching the optimal goal, which is to reduce the morbidity and economic cost of bone disease and the mortality related to hip fractures.[3,13]

Saliva determinations also could be a useful diagnostic tool to provide information for differential diagnosis, disease severity, and treatment planning and to monitor the effectiveness of periodontal therapy.[14] Periodontitis is a chronic destructive category of periodontal disease that progresses to the resorption of alveolar bone, which, without proper treatment, leads to progressive bone destruction and subsequent tooth loss. As a consequence of resorption, breakdown products are released into the periodontal tissues, migrating toward the gingival sulcus and gathering from the surrounding site in whole saliva, where several of them have been identified.[3,15]

Previously, we found that bone alkaline phosphatase (b-ALP) and carboxyterminal telopeptide of collagen type I (CTX) can be measured in the saliva of rats and humans.[16] Based on the above, the aim of the present experimental study was to determine whether measuring salivary concentrations of bone markers is as effective as measuring serum concentrations in assessing changes in systemic bone turnover using osteopenic rats with no oral diseases.

MATERIALS AND METHODS

Drugs

Olpadronate§ (OPD), the aminobisphosphonate used in this study, was obtained as two saline solutions (3.2 or 6.4 mg/100 ml). The doses are in keeping with a previous experimental dose-response study.[17] Ketamine hydrochloride and acepromazine maleate (0.1 mg each/100 g body weight)‖ were used as light anesthesia. Saliva stimulation was induced with 0.2 μg pilocarpine hydrochloride¶/100 g body weight. The drugs were administered intraperitoneally.

Animals and Experimental Design

Forty female, virgin, adult Wistar rats (250 to 300 g) were housed at room temperature (21°C ± 1°C) with 55% ± 10% humidity under 12-hour light/dark cycles; they were fed standard rodent diet# and deionized water *ad libitum*. Body weight was recorded weekly. The rats were maintained in keeping with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

§ Gador Company, Buenos Aires, Argentina.
‖ Holliday-Scott, Buenos Aires, Argentina.
¶ Isoptocarpina 1%, Alcon Argentine Laboratories, Buenos Aires, Argentina.
# Granave, Buenos Aires, Argentina.

After 1 week of acclimatization, 10 animals were sham operated, and the remaining 30 rats were subjected to bilateral OVX.

Sixty days post-surgery, the animals were divided into four groups and treated as follows for 45 days: group 1 (G1) = SHAM + vehicle (saline solution); group 2 (G2) = OVX + 8 μg OPD/100 g body weight; group 3 (G3) = OVX + 4 μg OPD/100 g body weight; and group 4 (G4) = OVX + vehicle (saline solution).

The study was approved by the University of Buenos Aires Institutional Review Board.

Biochemical Determinations

Fasting blood and saliva samples were collected at the onset of treatment (baseline: day 60) and after 15 days (T_{75}: 75 days post-surgery). Blood was obtained from the tail under ethyl ether anesthesia; the serum was separated and kept frozen at –20°C until the analyses were performed. Salivation was stimulated by intraperitoneal injection of pilocarpine. Ten minutes post-stimulation, saliva was collected for 1 minute using a sterile syringe and centrifuged at 3,000 × g for 10 minutes to separate cells and large macromolecules. The supernatants were collected and frozen at –20°C until the studies were performed. All biochemical determinations were performed at the same time to avoid interassay variations.

Serum and salivary CTX (ng/ml) was measured using an enzyme-linked immunosorbent assay** with a 6% intra-assay variation coefficient (CV) and a detection limit of 2.5 ng/ml.[18] The b-ALP was measured using a colorimetric method†† after bone enzyme isoform precipitation with wheat germ lectin.[18]

DXA Measurements

Total skeleton BMD was scanned "in vivo" under light anesthesia at the beginning of the experiment (T_0), at treatment onset (baseline: day 60), and at the end of the experimental period (T_{105}: day 105) using a total body scanner with software designed specifically for small animals.‡‡ Following a previously described technique,[18,19] this scanner used a constant potential x-ray source at 76 kV (peak); the effective energies were 38 and 70 keV. The collimation (size of x-ray beam at the source) used was 0.84 mm. The size of each sample point during the scan was 0.6 × 1.2 mm. With the DXA scanner, a series of transverse scans was made from the top of the nose to the end of the tail at 1.2-mm intervals with a set scan area of 8 × 25 cm for each rat. Scans were performed at a transverse scan speed of 9.6 mm/second, giving a scan time of 50 minutes for the total body determination.

All rats were scanned under light anesthesia using an identical scan procedure. The precision of the software in determining total body BMD was assessed by measuring one rat five times after repositioning between scans, on the same day and on different days.[18,19] The CV was 0.9% for total skeleton BMD. The different subareas were analyzed on the image of the animal on the screen, using a region of interest for each segment. The BMD CV was 1.8% for the lumbar spine and 3.5% for the proximal tibia. All of the analyses were carried out by the same technician to eliminate interobserver variation.

Statistical Methods

Results are expressed as mean ± SE. Data were analyzed using one-way analysis of variance. The Bonferroni multiple comparisons test was performed when significant differences were encountered. The Pearson correlation test was used to analyze correlation between bone markers in saliva and serum samples. Statistical analyses were performed using a software package.§§ $P < 0.05$ was considered significant.

RESULTS

Correlation Between Bone Marker Determinations in Serum and Saliva Samples During Estrogen Repletion and Depletion

With regard to the SHAM group, and to increase the sample size, the levels of salivary bone formation and resorption markers of the 10 rats were plotted against their corresponding serum levels at baseline and at T_{75}.

A significant correlation was observed when baseline and T_{75} salivary b-ALP levels of SHAM rats were plotted against their corresponding serum b-ALP levels (N = 19; r = 0.57; $P < 0.01$) (Fig. 1A). A significant correlation also was found between saliva and serum levels of CTX (N = 18; r = 0.65; $P < 0.004$) (Fig. 1B).

In OVX rats, a significant correlation was observed when baseline salivary b-ALP levels were plotted against the corresponding serum b-ALP concentrations (N = 26; r = 0.80; $P < 0.0001$) (Fig. 2A). A significant correlation also was observed between baseline saliva and serum CTX in OVX animals (N = 14; r = 0.71; $P < 0.004$) (Fig. 2B).

As expected, mean baseline serum b-ALP (N = 26) and CTX (N = 14) levels in the OVX group were significantly higher than in the SHAM (N = 10) group (59.7 ± 19.2 IU/l versus 78.4 ± 31.7 IU/l [$P < 0.001$] and 21.8 ± 5.0 μg/ml versus 29.0 ± 10.7 μg/ml [$P < 0.02$], respectively). The same pattern was observed for baseline salivary b-ALP and CTX levels (63.1 ± 21.9 IU/l versus 77.2 ± 21.6 IU/l [$P < 0.005$] and 3.7 ± 1.0 μg/ml versus 6.0 ± 1.8 μg/ml [$P < 0.0001$], respectively).

** Rat Laps, Osteometer BioTech, Herlev, Denmark.
†† Boehringer Mannheim, Germany.
‡‡ DPX Alpha 8034, Small Animal Software, Lunar Radiation, Madison, WI.
§§ SPSS for Windows 11.0, SPSS, Chicago, IL.

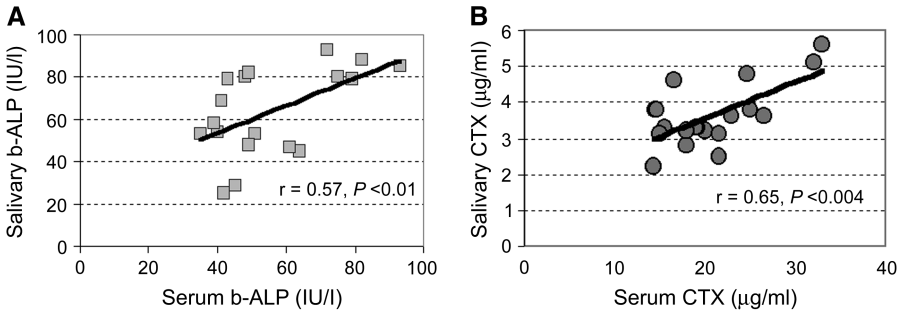

Figure 1.
Correlations between salivary and serum bone markers levels of samples obtained at baseline and after 15 days of treatment in SHAM rats. ***A)*** *Correlation between salivary and serum b-ALP (N = 19).* ***B)*** *Correlation between salivary and serum CTX (N = 18).*

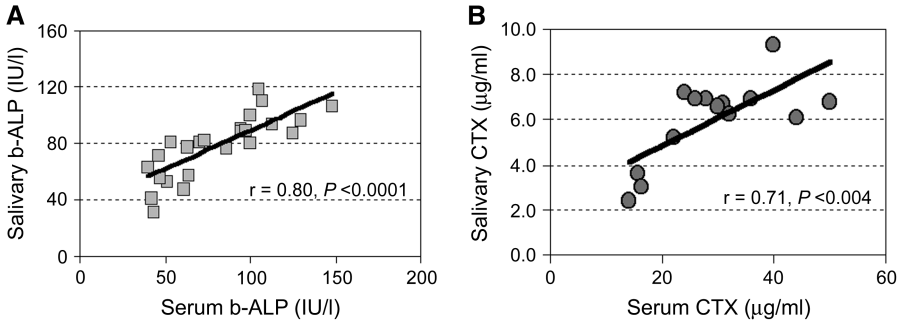

Figure 2.
Correlations between baseline salivary and serum bone markers levels in OVX rats. ***A)*** *Correlation between salivary and serum b-ALP (N = 26).* ***B)*** *Correlation between salivary and serum CTX (N = 14).*

Changes in Bone Markers After Anticatabolic Treatment

Figures 3A and 4A show the baseline b-ALP and CTX levels of the four studied groups, respectively. As expected, serum b-ALP and CTX levels were significantly lower in the SHAM group compared to the OVX groups ($P < 0.01$), and no differences were observed among the OVX groups. Similar results were obtained in baseline saliva samples ($P < 0.01$).

Figures 3B and 4B show b-ALP and CTX levels, respectively, of the four studied groups obtained at T_{75}. Serum and salivary bone marker levels were higher in the untreated OVX group (G4) than in the SHAM group (G1) ($P < 0.05$), whereas a dose-response pattern was observed in the OVX-OPD groups (G2 and G3). Regarding bone formation, no significant differences in serum or salivary b-ALP levels were observed between G2 (higher dose) and G1; G3 (lower dose) and G4 exhibited significantly higher levels than G1 ($P < 0.05$). Moreover, G2 had lower levels than G3 and G4 ($P < 0.05$), and no differences were observed between the latter two groups (Fig. 3B). Regarding bone resorption, G2 exhibited significantly lower CTX levels in saliva and serum samples than G1 ($P < 0.01$), G3 ($P < 0.01$), and G4 ($P < 0.01$). G3 showed significantly lower serum and salivary CTX levels than G4 and G1, but the difference only reached statistical significance compared to G1 ($P < 0.05$) (Fig. 4B).

Changes in BMD From Treatment Onset to the End of the Experiment

Figure 5 shows the changes in lumbar spine and proximal tibia BMD between days 60 and 105. As expected, untreated OVX rats exhibited a significant loss in BMD in both areas compared to the SHAM group ($P < 0.01$). OPD treatment prevented bone loss; however, the response to treatment was dose dependent. An increase in BMD was observed in both studied areas in G2 and in the proximal tibia in G3 compared to G1 ($P < 0.01$ and $P < 0.05$ for G2 and G3, respectively). The increase in proximal tibia BMD was significantly higher in G2 than in G3 ($P < 0.05$). Changes in both studied areas were statistically significant when comparing G2 and G3 to the OVX untreated group ($P < 0.01$ and $P < 0.05$ for G2 and G3, respectively).

The difference between CTX levels (Fig. 4) would account for the changes observed in BMD. Fifteen days after treatment onset, serum and salivary CTX levels remained unchanged in G1, were higher in G4, and showed a marked dose-dependent decrease after OPD treatment. In addition, the decrease in serum and saliva CTX levels were significantly higher in G2 than in G3 ($P < 0.05$).

DISCUSSION

The present study confirmed our previous findings regarding the possibility of measuring b-ALP and CTX in rat saliva samples.[16] To our knowledge, this is the first study in which the correlation between salivary and serum bone markers was investigated under different conditions that affect bone remodeling. The results showed that salivary samples responded like serum

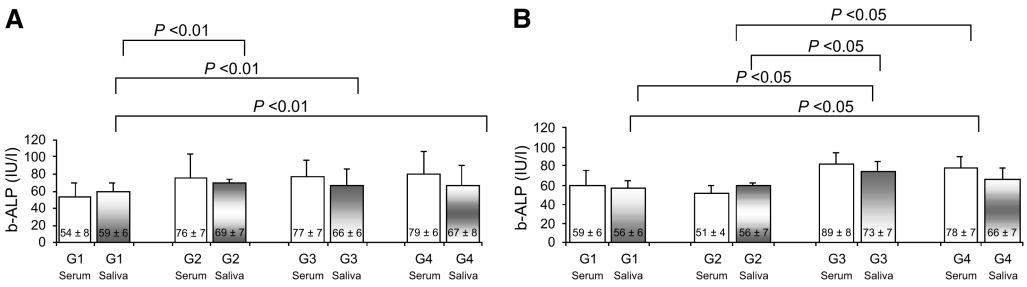

Figure 3.
Salivary and serum b-ALP levels for each group, respectively. ***A)*** *Baseline salivary and serum b-ALP levels in SHAM and OVX rats.* ***B)*** *T_{75} salivary and serum b-ALP levels in SHAM and OVX rats. (Numbers in bar graphs correspond to mean ± SD; N = 10.)*

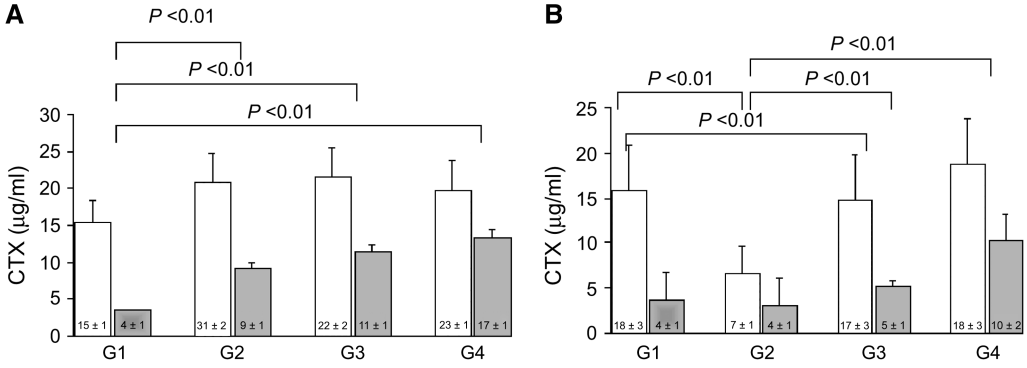

Figure 4.
Salivary and serum CTX levels for each group, respectively. ***A)*** *Baseline salivary and serum CTX levels in SHAM and OVX rats.* ***B)*** *T_{75} salivary and serum CTX levels in SHAM and OVX rats. (Numbers in bar graphs correspond to mean ± SD; N = 10.)*

samples in intact healthy rats and in conditions of increased or decreased bone turnover due to estrogen deficiency or anticatabolic treatment, respectively.

Bone remodeling is an essential part of bone health because bone sustains microfractures and fatigue damage daily, which must be repaired to maintain bone strength.[2] Several hormones regulate bone turnover. Among them, estrogen supports the balance between the function of osteoclasts and osteoblasts.[20] This balance is lost in estrogen deficiency, leading to an increase in the bone turnover rate.[21] Because bone resorption is faster than bone formation (12 days versus 3 months), any increase in remodeling results in bone loss and in a negative bone balance at the BMU level. For this reason, postmenopausal women are one of the risk groups for osteoporosis.[22]

Bone turnover can be evaluated by specific bone biochemical markers. These markers are complementary tests and are very useful to identify people with a high rate of bone remodeling who are at risk for osteoporosis or other metabolic bone disease, thus allowing treatment onset and monitoring of the response to therapy. Biochemical markers of bone turnover are divided broadly into markers of bone resorption and formation, which reflect osteoclast and osteoblast activity, respectively. As a result of osteoclast-mediated degradation of type I collagen during the process of bone resorption, amino and carboxy-terminal telopeptides (the pyridinoline cross-linked carboxyterminal telopeptide of type I collagen and CTX) are released into the bloodstream. CTX is considered one of the most specific and sensitive markers of bone resorption and is determined routinely in the assessment and monitoring of bone metabolic disorders.[2] b-ALP is one of the isoforms of alkaline phosphatase. It arises from osteoblast function and is essential for mineralization. However, its precise role in this process remains unclear. The serum concentration of b-ALP is considered a valid marker of bone formation in terms of distinguishing normal from disease states.[23]

Physiologic and pathologic conditions can cause significant changes in systemic bone turnover and, thus, can be reflected in bone marker concentrations.[24] In certain clinical settings, such as examining healthy children or elderly people, or performing screenings, obtaining serum or urine samples to measure bone turnover markers may prove awkward or may seem invasive. Saliva is an oral fluid, and interest in it as a diagnostic medium has advanced exponentially in the last 10 years.[14] Whole saliva–based diagnostic tests are being used in a broad range of applications, such as autoimmune disorders, cardiovascular disease, infectious diseases, and in monitoring drugs of abuse.[5]

In the present study, two bone markers were evaluated in the same serum and saliva samples. The

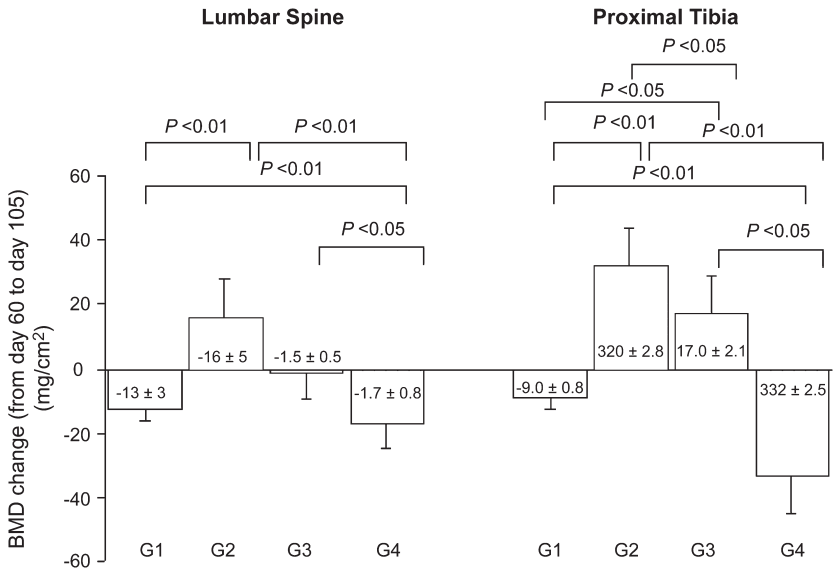

Figure 5.
Changes in lumbar spine and proximal tibia BMD. The changes were calculated as BMD values at the end of the experience (T_{105}) minus BMD values at the beginning of treatment (T_{60}). (Numbers in bar graphs correspond to mean ± SD; N = 10).

levels of b-ALP in serum and saliva were similar. However, salivary CTX levels were ~10% of those found in serum. Although the absolute concentration of CTX in saliva was slightly lower, the relative change within each subgroup could be assessed. The results showed significant correlation between salivary and serum concentrations of both of the studied biochemical markers under normal conditions and in estrogen deficiency. These results are in agreement with a previous study[25] in sheep reporting a lower salivary concentration and a correlation between salivary and serum deoxypyridinoline (D-Pyr) and osteocalcin. In our study, unlike CTX, salivary b-ALP was on the same order of magnitude as serum b-ALP. The high levels of b-ALP in saliva may be due, in part, to an increase in serum transudation and/or to local fibroblast production of b-ALP within the oral cavity.[26] In this regard, the fibroblast is the predominant cell type in the periodontal ligament, and it is implicated in tissue regeneration and repair. Osteoblasts and fibroblasts arise from the same progenitor mesenchymal stem cell that differentiates into a colony forming unit of fibroblasts that expresses alkaline phosphatase (ALP).[27]

Low bone mass and risk for bone fractures usually are determined using DXA. However, bone markers have proven to be good predictors of fracture risk, irrespective of bone mass measurement.[28] The results presented here demonstrate significant differences in the concentration of these two biomarkers among the studied groups, which presented low and high systemic bone turnover states. In this regard, a comparison between the OVX groups and the SHAM group showed that, similar to serum levels, salivary levels of b-ALP and CTX were higher in OVX animals on day 60, reflecting the presence of osseous metabolic activity and, consequently, the presence of bone loss. These results suggest that whole saliva may be an alternative biofluid to assess different levels of bone remodeling.

Anticatabolic treatment is known to reduce bone resorption with little effect on bone formation.[11] Changes in bone markers following bisphosphonate treatment were similar in saliva and serum determinations. The decrease in BMD observed in the untreated OVX group in sites of trabecular bone where metabolic activity is high, such as the lumbar spine and proximal tibia, was the result of persistent high levels of the bone resorption marker observed in serum and saliva. Conversely, the two OPD-treated groups presented a diminution in bone resorption reflected by a very important decrease in serum and salivary CTX levels. However, as expected, the decrease was dose dependent. In this regard, the lower dosages of bisphosphonate were associated with a lower reduction in bone resorption, as shown by the magnitude of bone loss in the spine and proximal tibia. These results demonstrate that, as occurs in serum, short-term changes in salivary levels of bone turnover markers could be valid predictors of long-term changes in BMD and could be used to monitor therapy with anticatabolic drugs. In agreement with these results, a previous report[15] using D-Pyr and osteocalcin showed that the salivary concentration of these two bone markers correlated with calcaneal T-scores in humans. These findings indicate that saliva is convenient to sample to monitor therapy in several situations, including in individuals with compromised venous access, hemophiliacs, children, obese people, and the elderly and infirm.[29]

The use of saliva as a diagnostic fluid to determine a systemic increase in bone turnover may have certain limitations in view of potential blood contamination or the presence of bone turnover markers from gingival crevicular fluid (GCF). In the present study, neither of the above occurred because animals were free of oral diseases, and blood contamination was avoided. Nevertheless, it is important to take into account that procedures that avoid potential blood contamination always should be followed when collecting saliva samples.[3] Moreover, three events occur during periodontitis: inflammation, collagen degradation, and bone turnover.

In this regard, Miller et al.[30] found that salivary levels of proinflammatory mediators (interleukin-1-beta) and tissue-destructive enzymes (matrix metalloproteinase-8) were significantly higher in patients affected by periodontitis. Conversely, Bullon et al.[22] demonstrated the lack of correlation in bone turnover marker levels between GCF and saliva, regardless of periodontitis status. Additionally, Miller et al.[30] found that saliva levels of osteoprotegerin (OPG), a glycoprotein that inhibits osteoclast differentiation and activity, are not well defined in health or existing periodontal disease. Furthermore, a previous investigation[14] correlated periodontal disease and total ALP, but not b-ALP, in saliva. This study demonstrated a higher enzyme activity in individuals with periodontal disease compared to nondiseased individuals, as well as a relationship between periodontal destruction and higher levels of salivary ALP. However, it failed to confirm salivary ALP as a predictive indicator for future periodontal breakdown.

Besides the usefulness of saliva samples to monitor systemic conditions that alter bone remodeling, their usefulness to accurately assess the severity of periodontal disease needs to be clarified. In this regard, further research in this field is required to identify the best candidate markers for the prediction, diagnosis, and monitoring of oral diseases. The aforementioned studies[14,25,30] showing the lack of a relationship between saliva bone markers and activity of periodontal disease could be due to the bone markers used in those studies (osteocalcin, ALP, and OPG); they may not be the most appropriate markers to assess active bone resorption. However, according to the present study, CTX may be considered one of the most specific and sensitive bone resorption markers and, therefore, could be evaluated further in saliva. According to the advantages offered by the analysis of salivary biomarkers that were suggested by Miller et al.,[30] it could be hypothesized that during the active resorption of bone as a result of advanced disease, CTX is released into the periodontal tissues, gathers in GCF, and is transferred to whole saliva, where it could be evaluated to assess disease activity and severity and to monitor periodontal therapy.

CONCLUSIONS

Under different conditions of systemic bone turnover, the results of the present longitudinal study provided evidence that bone marker concentrations in saliva samples follow a similar pattern to those in serum samples. Although further studies should be conducted to corroborate these findings, the results obtained in the present study suggest that saliva samples may be useful to evaluate systemic bone turnover status. In addition, further data obtained using saliva samples would be a promising way to find a better potential bone breakdown marker to establish the diagnosis and prognosis of severe periodontal disease as well as to monitor periodontal treatment outcome.

ACKNOWLEDGMENTS

This article is part of G.G. Pellegrini's doctoral thesis, which is to be submitted to the School of Dentistry, University of Buenos Aires, in partial fulfillment of the Ph.D. degree requirements. This study was performed with the support of the University of Buenos Aires (grant UBACyT M099). The authors thank Mr. Orzuza Ricardo, animal housing technician, Oral and General Biochemistry Department, School of Dentistry, University of Buenos Aires, for his technical assistance and Gardor Company, Buenos Aires, Argentina, for supplying the olpadronate. The authors report no conflicts of interest related to this study.

REFERENCES

1. Dempster DW. Bone remodeling. In: Core FL, Favus ME, eds. *Disorders of Bone Mineral Metabolism*, 2nd ed. Baltimore: Lippincott Williams and Wilkins; 2000: 315-343.
2. Camacho P, Kleerekoper M. Biochemical markers of bone turnover. In: Favus MJ, ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*, 6th ed. Washington, DC: ASBMR Press; 2006: 127-130.
3. Riggs BL, Melton IJ III. Involutional osteoporosis. *N Engl J Med* 1986;314:1676-1686.
4. Heaney RP, Recker RR, Saville PD. Menopausal changes in bone remodeling. *J Lab Clin Med* 1978;92:964-970.
5. Koka S, Forde MD, Khosla S. Systemic assessments utilizing saliva: Part 2. Osteoporosis and use of saliva to measure bone turnover. *Int J Prosthodont* 2006;19: 53-60.
6. Wronski TJ, Dann LM, Horner SL. Time course of vertebral osteopenia in ovariectomized rats. *Bone* 1989; 10:295-301.
7. Kalu DN, Liu CC, Salerno E, Hollis B, Echon R, Ray M. Skeletal response of ovariectomised rats to low and high doses of 17β-estradiol. *Bone Miner* 1991;14:175-187.
8. Turner RT, Vandersteenhove JJ, Bell NH. The effect of ovariectomy and 17β-estradiol on bone histomorphometry in growing rats. *J Bone Miner Res* 1987;2:115-122.
9. Souberbielle JC, Cormier C, Kindermans C. Bone markers in clinical practice. *Curr Opin Rheumatol* 1999; 11:312-317.
10. Srivastava AK, Vliet EL, Lewiecki EM, et al. Clinical use of serum and urine bone markers in the management of osteoporosis. *Curr Med Res Opin* 2005;21: 1015-1026.
11. Fleisch H. *Bisphosphonates in Bone Disease*, 2nd ed. New York: The Parthenon Publishing Group; 1997: 39-57.
12. Hammett-Stabler CA. The use of biochemical markers in osteoporosis. *Clin Lab Med* 2004;24:175-179.
13. Streckfus CF, Bigler LR. Saliva as a diagnostic fluid. *Oral Dis* 2002;8:69-76.
14. Kinney JS, Ramseier CA, Giannobile WV. Oral fluid-based biomarkers of alveolar bone loss in periodontitis. *Ann N Y Acad Sci* 2007;1098:230-251.

15. McGehee JW, Johnson RB. Biomarkers of bone turnover can be assayed from human saliva. *J Gerontol A Biol Sci Med Sci* 2004;59:196-200.
16. Pellegrini G, Gonzalez Chaves M, Somoza J, Friedman S, Zeni SN. Correlation between collagen type I carboxyterminal telopeptide (CTX) and bone alkaline phosphatase (b-AP) in serum and saliva of rats (in Spanish). *Medicina (B Aires)* 2006;66:245-248.
17. Roldan EJ, Mondelo N, Piccini E, Peluffo V, Montuori E. Olpadronic acid sodium salts. *Drugs on the Future* 1995;20:1123-1127.
18. Mastaglia S, Pellegrini G, Mandalunis P, Gonzales-Chaves M, Friedman S, Zeni SN. Vitamin D insufficiency reduces the protective effect of bisphosphonate on ovariectomy-induced bone loss in rats. *Bone* 2006; 39:837-844.
19. Zeni SN, Di Gregorio S, Gomez Acotto C, Mautalen C. Olpadronate prevents the bone loss induced by cyclosporine in the rat. *Calcif Tissue Int* 2002;70:48-53.
20. Manolagas SC, Bellido T, Jilka RL. Sex steroids, cytokines and the bone marrow: New concepts on the pathogenesis of osteoporosis. *Ciba Found Symp* 1995; 191:187-196.
21. Sypniewska G, Chodakowska-Akolinska G. Bone turnover markers and estradiol levels in postmenopausal women. *Clin Chem Lab Med* 2000;38:1115-1119.
22. Bullon P, Goberna B, Guerrero JM, Segura JJ, Perez-Cano R, Martinez-Sahuquillo A. Serum, saliva, and gingival crevicular fluid osteocalcin: Their relation to periodontal status and bone mineral density in postmenopausal women. *J Periodontol* 2005;76: 513-519.
23. Yang L, Grey V. Pediatric reference intervals for bone markers. *Clin Biochem* 2006;39:561-568.
24. Robins SP. Biochemical markers for assessing skeletal growth. *Eur J Clin Nutr* 1994;48(Suppl. 1):199S-209S.
25. Johnson RB, Gilbert JA, Cooper RC, et al. Effect of estrogen deficiency on skeletal and alveolar bone density in sheep. *J Periodontol* 2002;73:383-391.
26. Aubin JE, Lian JB, Stein GS. Bone formation: Maturation and functional activities of osteoblast lineage cells. In: Favus MJ, ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*, 6th ed. Washington, DC: ASBMR Press; 2006:20-29.
27. Chapple ILC, Glenwright HD, Mathews JB, Thorpe GHG, Lumley PJ. Site-specific alkaline phosphatase levels in gingival crevicular fluid in health and gingivitis: Cross-sectional studies. *J Clin Periodontol* 1994;21:409-414.
28. Garnero P, Sornay-Rendu E, Claustrat B, Delmas PD. Biochemical markers of bone turnover, endogenous hormones and the risk of fractures in postmenopausal women: The OFELY study. *J Bone Miner Res* 2000;15: 1526-1536.
29. Forde MD, Koka S, Eckert SE, Carr AB, Wong DT. Systemic assessments utilizing saliva: Part 1 general considerations and current assessments. *Int J Prosthodont* 2006;19:43-52.
30. Miller CS, King CP Jr., Langub MC, Kryscio RJ. Salivary biomarkers of existing periodontal disease: A cross-sectional study. *J Am Dent Assoc* 2006;137:322-329.

Correspondence: Dr. Susana N. Zeni, Medical Bone Disease Service, Clinical Hospital - University of Buenos Aires, Córdoba 2351-8vo, Piso, 1120 Capital Federal, Buenos Aires, Argentina. E-mail: osteologia@hospitaldeclinicas.uba.ar.

Submitted March 28, 2007; accepted for publication July 31, 2007.