Osteoporosis in hemodialysis patients revisited by bone histomorphometry: A new insight into an old problem

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Osteoporosis in hemodialysis patients is associated with high morbidity and mortality and, although extensively studied by noninvasive methods, has never been assessed through bone biopsy. The aim of this study was to use histomorphometry to evaluate osteoporosis and identify factors related to its development in hemodialysis patients. We conducted a cross-sectional study involving 98 patients (35 women and 63 men; mean age: 48.4 ± 13 years) on hemodialysis for 36.9 ± 24.7 months. Patients were submitted to transiliac bone biopsy with double tetracycline labeling. The bone metabolism factors ionized calcium, phosphorus, bone alkaline phosphatase, deoxypyridinoline, intact parathyroid hormone, and 25(OH) vitamin D were evaluated, as were the bone remodeling cytokines osteoprotegerin (OPG), soluble receptor-activator of NF-κβ ligand (sRANKL) and tumor necrosis factor-α (TNF)α. Osteoporosis was defined as trabecular bone volume (BV/TV) greater than 1 s.d. below normal (men <17.4%; women <14.7%). Forty-five patients (46%) presented osteoporosis, which was correlated with white race. We found BV/TV to correlate with age, OPG/sRANKL ratio, TNFα levels, and length of amenorrhea. In multiple regression analysis adjusted for sex and age, length of amenorrhea, white race, and OPG/sRANKL ratio were independent determinants of BV/TV. Histomorphometric analysis demonstrated that osteoporotic patients presented normal eroded surface and low bone formation rate (BFR/BS). Osteoporosis is prevalent in hemodialysis patients. Low BFR/BS could be involved in its development, even when bone resorption is normal. Cytokines may also play a role as may traditional risk factors such as advanced age, hypogonadism, and white race.

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Osteoporosis is a common bone disorder among the elderly. It occurs owing to disruption of the bone remodeling cycle, which leads to bone loss. In addition to its known association with fractures,[1] osteoporosis has recently been associated with vascular disease.[2] As uremia induces a derangement in bone and mineral homeostasis known as renal osteodystrophy (ROD), it is logical to assume that patients with chronic kidney disease (CKD) are at risk for developing osteoporosis and its comorbidities. In fact, such patients are more prone to fractures than are individuals in the general population,[3–5] and it has also been suggested that there is a relationship between ROD and vascular calcification.[6,7] The increasing surveillance of hemodialysis patients, together with the growing numbers of older individuals entering into this type of treatment, increases the importance of studying bone loss in hemodialysis patients.

The diagnosis of osteopenia/osteoporosis, as proposed by the World Health Organization (WHO),[1] is based on bone mineral density, measured by dual-energy X-ray absorptiometry. However, this method has proven to be reliable only in white women, and its accuracy in evaluating men, children, and individuals belonging to other ethnic groups remains uncertain, as does its applicability to all types of secondary osteoporosis, including ROD. Therefore, caution should be taken when extrapolating this diagnostic approach to these other situations.[8]

It has been suggested that bone biopsy, the gold standard method for the diagnosis of ROD, could be applied to characterize osteopenia/osteoporosis in CKD patients.[9] Bone biopsy followed by histomorphometric analysis is the first quantitative method that can be used to elucidate how bone remodeling abnormalities can cause bone loss.[10] This technique also provides qualitative information regarding microarchitectural changes in bone tissue. However, this approach has been underused in the evaluation of osteoporosis in CKD patients.

The aim of this study was to employ bone biopsy followed by histomorphometric analysis in order to assess the prevalence of osteoporosis in a population of patients on hemodialysis, as well as to identify factors that might contribute to its development.

RESULTS

According to our diagnostic criteria, patients were divided into osteoporotic (OP) and non-osteoporotic (NOP) groups that encompassed, respectively, 45 (46%) and 53 (54%) of the patients. Clinical and biochemical characteristics of both groups are shown in Table 1. There were no differences between the two groups regarding age, etiology of CKD, time on hemodialysis, or body mass index. Regarding gender, we observed a higher frequency of men in OP group, although statistically nonsignificant ($P = 0.08$). There was a strong correlation between osteoporosis and being of the white race (odds ratio = 3.58; 95% confidence interval = 1.40–9.27; $P = 0.005$), whereas calcitriol therapy tended to be a protective factor against osteoporosis (odds ratio = 0.40; 95% confidence interval = 0.15–1.05; $P = 0.06$). Osteoporosis was also associated with duration of amenorrhea longer than 48 months (odds ratio = 10; 95% confidence interval = 1.02–127.99; $P = 0.02$). We found that osteoporosis did not correlate with diabetes mellitus, previous corticotherapy, or smoking. No differences regarding biochemical parameters were noted, except for a significantly higher osteoprotegerin (OPG)/soluble receptor-activator of NF-$\kappa\beta$ ligand (sRANKL) ratio and significantly lower tumor necrosis factor-α (TNFα) levels in the OP group. Interestingly, mean levels of intact parathyroid hormone (iPTH) in both groups were near the target range for hemodialysis patients.[9]

Table 2 shows the result of the bone histomorphometry. Regarding static parameters, trabecular separation was significantly higher in the OP group than in the NOP group, whereas trabecular thickness, trabecular number, and trabecular bone volume (BV/TV), the last being the parameter used to diagnose osteoporosis, were significantly lower. No statistically significant difference was observed between the other static parameters. Analysis of the dynamic parameters indicated that bone formation rate (BFR/BS) was significantly lower and mineralization lag time was significantly higher in the OP group than in the NOP group.

Based on histomorphometry, adynamic bone disease ($n = 55$; 56.1%) was the most common type of ROD, followed by mixed uremic osteodystrophy ($n = 21$; 21.4%), predominant hyperparathyroid bone disease ($n = 12$; 12.2%), and osteomalacia ($n = 1$; 1.1%). Aluminum-related bone disease was detected in 24.5% of the patients. Normal bone

Table 1 | Clinical and biochemical characteristics of OP and NOP patients

Variable	OP (n=45)	NOP (n=53)
Age (years)	51 ± 14.1	46.3 ± 11.7
Gender		
Male	33 (73%)	30 (57%)
Female	12 (27%)	23 (43%)
Race		
White	33 (73%)†	23 (43%)
Non-white	12 (27%)	30 (57%)
Etiology of CKD		
Unknown	12 (27%)	15 (33%)
Hypertension	14 (31%)	13 (25%)
CGN	4 (9%)	13 (25%)
DM	3 (6%)	6 (11%)
Other	12 (27%)	6 (11%)
Time on HD (months)	33.9 ± 23.8	39.5 ± 25.5
BMI (kg/m^2)	24.1 ± 3.8	25.4 ± 4.6
History of DM	6 (13%)	10 (22%)
Calcitriol therapy	14 (41%)	33 (62%)
Corticotherapy	11 (24%)	12 (22%)
Smoking	11 (24%)	14 (26%)
Amenorrhea >48 months	5/12 (41.6%)†	2/23 (8.7%)
Ionized calcium (mmol/l)	1.23 ± 0.08	1.24 ± 0.09
Phosphorus (mg/dl)	7.0 ± 1.2	7.3 ± 2.1
BAP (U/l)*	22.5 ± 11.7	35.6 ± 40.9
Deoxypyridinoline (nmol/l)*	76.1 ± 66.4	142.2 ± 192.0
iPTH (pg/ml)	296.4 ± 245.7	388.3 ± 354.5
25(OH) vitamin D (ng/dl)*	34.8 ± 17.2	31.0 ± 12.0
OPG (pg/ml)*	187.7 ± 65.4	164.4 ± 76.0
sRANKL (pg/ml)*	4.6 ± 7.7	6.2 ± 9.5
OPG/sRANKL ratio*	96.4 ± 58.3†	74.2 ± 63.6
TNFα (pg/Ml)*	3.7 ± 4.0†	6.0 ± 8.0
Estradiol (ng/dl)**	3.0 ± 1.3	6.5 ± 6.6
Free testosterone (pg/ml)***	369.6 ± 153.5	384.8 ± 208.0

BAP, bone alkaline phosphatase; BMI, body mass index; CGN, chronic glomerulonephritis; CKD, chronic kidney disease; DM, diabetes mellitus; HD, hemodialysis; iPTH, intact parathyroid hormone; NOP, non-osteoporotic; OP, osteoporotic; OPG, osteoprotegerin; sRANKL, soluble receptor-activator of NF-κB ligand; TNFα, tumor necrosis factor-α.
Values shown as n (%) or as mean ± s.d.
*n=86; **n=28; ***n=50.
†$P < 0.05$.

Table 2 | Comparison of histomorphometric parameters between OP and NOP patients

Variable	OP (n=45)	NOP (n=53)	Reference range
BV/TV (%)	12.2 ± 2.6*	22.3 ± 5.1	25.5 ± 8.1(M); 22.0 ± 7.3(F)
OV/BV (%)	3.2 ± 3.2	4.0 ± 4.0	3.2 ± 3.0
Ob.S/BS (%)	4.4 ± 5.7	6.7 ± 7.9	2.13 ± 3.8
ES/BS (%)	4.2 ± 3.2	6.9 ± 6.1	3.15 ± 4.81
Oc.S/BS (%)	0.71 ± 0.69	1.1 ± 1.1	0.07 ± 0.22
Tb.Th (μm)	103.4 ± 15.7††	122.1 ± 18.4	126.5 ± 31.4
Tb.Sp (μm)	781.2 ± 231.15††	439.7 ± 93.1	402.7 ± 156.8
Tb.N/mm	1.32 ± 0.95††	1.8 ± 0.37	2.05 ± 0.72
Fb.V/TV (%)	0.11 ± 0.26	0.52 ± 1.78	0
Al.S/BS (%)	30.6 ± 32.9	18.9 ± 23.3	0
BFR/BS (μ^3/μ^2/day)	0.02 ± 0.04†	0.05 ± 0.07	0.13 ± 0.07
Mlt (days)	95.5 ± 104.7†	55.6 ± 55.7	21.3 ± 2.3

Al.S/BS, aluminum bone surface; BFR/BS, bone formation rate; BV/TV, trabecular bone volume; ES/BS, eroded surface; Fb.V/TV, fibrosis volume; Mlt, mineralization lag-time; Ob.S/BS, osteoblast surface; Oc.S/BS, osteoclast surface; OV/BV, osteoid volume; Tb.Sp, trabecular separation; Tb.N, trabecular number; Tb.Th, trabecular thickness.
Normal or more than 1 s.d. above the normal range, mineralization lag time more than 1 s.d. above the normal range.
Values expressed as mean ± s.d.
†$P < 0.05$; ††$P < 0.000001$.
*Not applicable.

histology was identified in four (4.1%) and isolated osteoporosis in three (3.1%) patients. Although osteoporosis was present in all forms of ROD (Figure 1), no association was found between osteoporosis and any type of ROD.

As shown in Table 3, BV/TV correlated positively with TNFα levels and negatively with age, length of amenorrhea, and OPG/sRANKL ratio. A borderline correlation was observed between BV/TV and body mass index. We found that BV/TV was not correlated with estradiol, free testosterone, iPTH, or 25(OH) vitamin D levels. All variables reaching the level of statistical significance in the univariate analysis were included in the regression model. Multiple stepwise regression analysis adjusted for sex and age (Table 4) revealed that being of the white race, length of amenorrhea and OPG/sRANKL ratio were independent determinants of BV/TV.

Other histomorphometric parameters also correlated with cytokine levels. We found a negative correlation between BFR/BS and OPG/sRANKL ratio ($P = 0.05$) and a positive correlation between BFR/BS and TNFα levels ($P = 0.01$). Eroded surface correlated weakly with OPG/sRANKL ratio ($P = 0.06$), but not at all with TNFα levels. Neither osteoclast surface nor osteoblast surface correlated with cytokine levels.

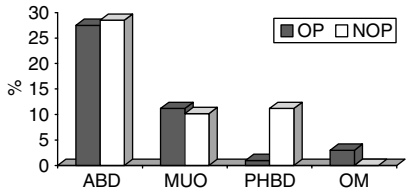

Figure 1 | Osteoporosis distribution among renal osteodystrophy types. ABD: adynamic bone disease; MUO: mixed uremic osteodystrophy; PHBD: predominant hyperparathyroid bone disease; and OM: osteomalacia.

Table 3 | Correlation coefficients between BV/TV and the various clinical and biochemical parameters

Variable	*r*	*P*-value
TNFα	0.32	0.002
OPG/sRANKL ratio	−0.29	0.005
Duration of amenorrhea	−0.51	0.005
Age	−0.27	0.006
OPG	−0.24	0.02
BMI	0.18	0.07
sRANKL	0.19	0.08

BMI, body mass index; OPG, osteoprotegerin; sRANKL, soluble receptor-activator of NF-κB ligand; TNFα, tumor necrosis factor.

Table 4 | Multiple stepwise linear regression analyses using log BV/TV as a dependent variable

Variable	β-Coefficient	*P*-value	95% CI
Duration of amenorrhea	−0.315	0.001	−0.0013 to −0.0003
White race	−0.255	0.008	−0.148 to −0.231
OPG/sRANKL	−0.215	0.026	−0.001 to −0.0001

95% CI, 95% confidence interval; OPG, osteoprotegerin; sRANKL, soluble receptor-activator of NF-κβ ligand.

DISCUSSION

In this bone histomorphometry-based study, we detected a high prevalence (46%) of osteoporosis in a relatively young population of hemodialysis patients. In addition, low BV/TV was observed in patients as young as 22 years old. These findings indicate that bone loss likely begins much earlier in hemodialysis patients than in the general population, in which such bone loss is not typically in patients less than 40 years of age.[11]

Many bone disorders, such as osteoporosis and ROD, develop as a result of an imbalance between bone formation and bone resorption. Bone biopsy followed by histomorphometric analysis is still the gold standard for evaluating such disorders.[12,13] In our study, osteoporosis was characterized by normal eroded surface and slightly increased osteoclast surface (both bone resorption parameters), followed by low BFR/BS. Bone microarchitecture was highly altered, as demonstrated by reduced trabecular thickness and trabecular number, as well as by increased trabecular separation. These changes would lead to both increased bone fragility and increased fracture risk.[14] In contrast to these bone biopsy results, the determination of serum levels of iPTH, as well as of serum markers of bone formation (bone alkaline phosphatase) and resorption (deoxypyridinoline), showed no difference between OP and NOP patients.

Another interesting finding of this study was the distribution of osteoporosis among the different forms of ROD. Osteoporosis was present in both low bone turnover states (adynamic bone disease and osteomalacia) and high bone turnover states (predominant hyperparathyroid bone disease and mixed uremic osteodystrophy). The well-known hallmark of ROD is an uncoupled bone remodeling cycle, which can lead to bone loss if bone resorption becomes proportionally greater than bone formation. Therefore, the high occurrence of osteoporosis (49%) in patients with adynamic bone disease could be owing to the low bone formation in the presence of normal to slightly increased bone resorption. The coexistence of bone loss with adynamic bone disease could, in part, explain its association with a high rate of fractures.[3,5] In predominant hyperparathyroid bone disease patients, the low prevalence of osteoporosis (Figure 1) could be related to the way PTH affects bone. Excess PTH generally has catabolic effects on cortical bone and anabolic effects on trabecular bone,[15–18] the latter being the type of tissue that was the focus of this study. Therefore, secondary hyperparathyroidism would be to some degree protective against trabecular bone loss. In contrast, as an iPTH level >1000 pg/ml was an exclusion criteria, we were unable to determine whether exposure to excessively elevated PTH levels, as seen in severe hyperparathyroidism, would have a deleterious effect on trabecular bone. The risk of osteoporosis should not be underestimated in patients with secondary hyperparathyroidism.

It is possible that cytokines involved in the regulation of bone remodeling contributed to the bone loss observed in our study. Recent studies using transgenic mice have

demonstrated that the OPG/RANK/RANKL pathway plays a role in regulating osteoclastogenesis.[19–21] However, initial studies involving uremic patients and evaluating the influence of increased circulating levels of OPG[22] in ROD have produced conflicting results.[23,24] In addition, it has been suggested that determining the OPG/sRANKL ratio is a more rational means of evaluating osteoclastogenesis than simply determining OPG levels.[25] In our study, OPG levels were above the reference range in both the OP and the NOP group. In the OP group, the OPG/sRANKL ratio was higher ($P = 0.04$) and there was a trend toward higher OPG levels ($P = 0.06$). Moreover, OPG levels and OPG/sRANKL ratio correlated negatively with BV/TV. These conflicting results could represent a homeostatic mechanism that limits the rate of bone loss by decreasing bone resorption. Supporting this hypothesis, a trend toward an inhibitory influence of OPG/sRANKL ratio in bone resorption was noted, as evidenced by the negative correlation between OPG/sRANKL ratio and the eroded surface ($P = 0.06$). Yano *et al.*[26] recently observed similar correlations in postmenopausal OP women.

Interestingly, the results for TNFα were just the opposite of those observed for OPG. In OP patients, TNFα levels were lower ($P = 0.006$) and were positively correlated with BV/TV. Previous studies have also suggested that TNFα acts like a skeletal catabolic agent by stimulating bone resorption.[27,28] Santos *et al.*[29] found decreased expression of TNFα in bone biopsy after parathyroidectomy in dialysis patients, suggesting that this cytokine is involved in the regulation of bone remodeling. Therefore, it seems reasonable to assume that TNFα could also be involved in the homeostatic mechanism of bone loss prevention. It is valid to question whether this proposed mechanism represents an innocuous response. As bone resorption precedes bone formation in the bone remodeling cycle, increased OPG and decreased TNFα levels would also lead to a delayed decrease in bone turnover. In agreement with this hypothesis, we noted that BFR/BS correlated negatively with OPG/sRANKL ratio ($P = 0.05$) and positively with TNFα levels ($P = 0.01$). Studies providing a better understanding of the bone microenvironment are needed in order to elucidate this seeming paradox.

Regarding sex hormones, we were unable to demonstrate any correlation of BV/TV with levels of estradiol or free testosterone. In the case of our female patients, this could be attributed to the small number of women included in this study and to the fact that most of them were amenorrheic (60%). However, three other findings suggested an effect of female hypogonadism on trabecular bone volume in our hemodialysis patients. First, amenorrheic women presented significantly lower BV/TV than did menstruating women ($15.9 \pm 5.5\%$ vs $22.9 \pm 9.6\%$; $P < 0.05$; data not shown). Second, longer duration of amenorrhea was negatively correlated with BV/TV. Finally, patients who were amenorrheic for more than 48 months were found to be at a 10-fold greater risk for osteoporosis. Recent reports have addressed the importance of sexual hormone status in the maintenance of bone mass in hemodialyzed women.[30,31] According to the hormonal evaluation, only four of our male patients presented hypogonadism. Owing to this small number of hypogonadic men, the statistical analysis could not be performed for this variable. In addition, there is clinical evidence that the role of estrogens in the maintenance of bone mass in males is more relevant than that of testosterone.[32]

Finally, factors that were predictive of osteoporosis in our study were similar to those found in dual-energy X-ray absorptiometry evaluations of CKD patients and of the general population.[8,33–40] Such factors included advanced age, being of the white race and, probably, low body mass index. Although dual-energy X-ray absorptiometry is an important diagnostic tool, it has limitations in the evaluation of osteoporosis in CKD patients. Primarily, there is as yet no agreement regarding which parameter (*T*-score or *Z*-score) should be used to classify CKD patients as osteopenic or OP.[41] This fact may cause a misleading interpretation of the data available. In addition, spinal osteophytes and aortic calcification (both common findings in CKD patients) may interfere with the accuracy of dual-energy X-ray absorptiometry in measuring bone mineral density in the lumbar spine.[42,43] Furthermore, bone mineral density correlates poorly with bone remodeling and, consequently, with ROD type.[14,41] Nevertheless, other aspects must be considered when evaluating osteoporosis through bone histomorphometry. First, as suggested by Meunier *et al.*[44], intersample variation should be taken in account to assess the minimal difference required in order to affirm that changes noted in a repeat biopsy are significant. Second, although supposition has yet to be proven, it has been suggested that another histomorphometric parameter might be more sensitive or specific than BV/TV in detecting bone loss.[45] Therefore, there is as yet no clear definition of osteoporosis based on bone histomorphometry, especially in CKD patients. In an elegant study involving OP patients with at least one vertebral compression, Meunier *et al.*[46] observed that BV/TV was always less than 16%. The same author also defined the 'vertebral fracture threshold' as a BV/TV of approximately 11%.[47] Kelepouris *et al.*[48] observed that men aged 20 to 44 years with primary osteoporosis had a mean BV/TV of $18.6 \pm 5.4\%$, whereas OP men aged 45 to 60 years had a mean BV/TV of $15.4 \pm 4.2\%$. In our study, patients had no history of fracture. In addition, choosing a cutoff point below the mean minus 1.0 s.d., it is supposed that only 16% of a given population would fall below the normal range. This being so, we can consider our cutoff point judicious for use in the diagnosis of osteoporosis. However, more studies using bone biopsy are required in order to clarify these aspects and to investigate the links between bone mineral density, bone histomorphometry, and clinical end points such as fracture and vascular calcification.

In conclusion, to the best of our knowledge, this is the first study that employed bone histomorphometry in order to characterize osteoporosis in CKD patients. By considering bone biopsy as the gold standard, osteoporosis was shown to

be a truly prevalent bone disorder in hemodialysis patients. In addition, we showed that even in the presence of normal bone resorption, low bone formation could be involved in the development of OP bone loss. Cytokines may also play a role in this bone derangement, as may traditional risk factors such as advanced age, hypogonadism, and white race.

MATERIALS AND METHODS

Patients

In this cross-sectional study, we evaluated 98 CKD patients on standard hemodialysis treatment for 36.9 ± 24.7 months (range, 4–91 months). The sample consisted of 63 males and 35 females, and the mean age was 48.4 ± 13.0 years. Of the 98 patients studied, 56 were white, eight were black, six were Asian, and 28 were of mixed ethnicity. Body mass index was calculated for all patients. None of the patients had a history of fracture. Data regarding smoking, diabetes mellitus, and amenorrhea were recorded.

The present study makes reference to baseline data from an ongoing randomized clinical trial comparing two phosphate binders, sevelamer and calcium acetate, in hemodialysis patients.[6] Therefore, based on the exclusion criteria for this study, the patients presented no HIV infection, chronic inflammatory disease, current use of corticosteroids, intact PTH levels >1000 pg/ml, or continuous use of antiseizure drugs, nor were there any pregnant or breastfeeding patients included.

The causes of renal failure were hypertension (27 patients), chronic glomerulonephritis (17 patients), diabetic nephropathy (nine patients), and other causes (18 patients). The etiology of the renal failure was unknown in 27 patients. Of the 98 patients studied, 95 were currently taking calcium-containing phosphate binders (either calcium acetate or calcium carbonate), and 52 had previously taken calcitriol. Calcium concentration in the dialysate was 1.25 mmol/l for 18 patients and 1.75 mmol/l for 80 patients. Of the 35 female patients, 21 were amenorrheic. None of the patients had previously undergone parathyroidectomy or were undergoing treatment with bisphosphonate or hormone replacement therapy.

The study was reviewed and approved by the ethics committee of the local institution, and all patients gave written informed consent.

Biochemical parameters

Blood samples for the determination of biochemical parameters were obtained before the first weekly hemodialysis session (after patients had fasted overnight). In menstruating women, blood sampling for measurement of sex hormones was performed during the first week of the menstrual cycle (follicular phase).

Laboratory evaluation included ionized calcium (reference range: 1.11–1.40 mmol/l) and serum phosphorus (reference range: 2.3–4.5 mg/dl), determined by automated methods. Bone alkaline phosphatase (reference range: 11.6–42.7 U/l) was measured by enzyme immunoassay (Metra Biosystem Inc., Mountain View, CA, USA). Serum deoxypyridinoline (reference range: 3.25 ± 0.66 nmol/l) was determined by enzyme immunoassay (Quidel Corporation, San Diego, CA, USA). Serum iPTH (reference range: 10–65 pg/ml) was measured by chemiluminescence assay (DPC; Medlab, San Antonio, TX, USA). Serum 25(OH) vitamin D (reference range: 18–62 ng/dl) was determined by radioimmunoassay (DiaSorin, Stillwater, MN, USA). Serum OPG (reference range: 30.45 ± 12.1 pg/ml) and sRANKL (detection limit: 1.5 pg/ml) were determined using a sandwich enzyme immunoassay (Immundiagnostik Laboratory, Bensheim, Germany). Serum TNFα (detection limit: 2 pg/ml) was measured by enzyme immunoassay (Pharmingen, San Diego, CA, USA). Serum estradiol (reference range: 1–30 ng/dl; menopause <3 ng/dl) and serum follicle-stimulating hormone (reference: <12 U/l; menopause >30 U/l; and male subjects <10 U/l) were measured by immunofluorometric assay. Free testosterone (reference range: 9–55 pg/ml) was measured by radioimmunoassay.

Bone biopsy

All patients were submitted to a transiliac bone biopsy using a Bordier trephine, following a course of double-labeling tetracycline (20 mg/kg/day) for 3 days, with a 10-day interval. The biopsy was performed 3–5 days after the last dose of tetracycline and no longer than 1 month after the blood sampling. Undecalcified bone fragments were submitted to standard processing for histological studies.[49] Sections were stained with toluidine blue (pH = 6.4). Acid solochrome azurine[50] and Perls' staining were employed to detect deposits of aluminum and iron, respectively, in bone tissue. Unstained sections were used for tetracycline fluorescence analysis.

Bone histomorphometry was conducted in a double-blind protocol, using the semiautomatic method provided in the Osteomeasure software (Osteometrics Inc., Atlanta, GA, USA). The static and dynamic parameters were analyzed following the standards established by the American Society of Bone and Mineral Research.[51] The reference ranges used for static parameters were obtained from our normal laboratory controls, whereas the dynamic followed those described elsewhere.[52]

On the basis of the histomorphometry, the patients were divided into the following groups: (1) predominant hyperparathyroid bone disease, defined as BFR/BS, as well as either osteoblast surface or osteoclast surface, more than 1 s.d. above normal range, osteoid volume within or above the normal range and marrow fibrosis >0.5%; (2) adynamic bone disease, defined as BFR/BS and osteoid volume more than 1 s.d. below the normal range and marrow fibrosis <0.5%; (3) osteomalacia, defined as BFR/BS more than 1 s.d. below the normal range and osteoid volume more than 1 s.d. above the normal range; and (4) mixed uremic osteodystrophy, defined as BFR/BS more than 1 s.d. below the normal range, osteoid volume and osteoblast surface more than 1 s.d. above the normal range and marrow fibrosis ⩾0.5%. Aluminum-related bone disease was defined as an aluminum bone surface greater than 25%.

In this study, osteoporosis was defined as BV/TV greater than 1 s.d. below normal range (M = 25.5 ± 8.1%; F = 22.0 ± 7.3%). Therefore, osteoporosis was defined as a BV/TV lower than 14.7% for female patients and lower than 17.4% for male patients.

Statistical analysis

Results are expressed as mean ± s.d. or percentage. Either Student's *t*-test for unpaired data or non-parametric Mann–Whitney *U*-test was used to elucidate differences between groups. Spearman's coefficient was used to describe correlations between BV/TV and other variables. A multiple linear regression analysis adjusted for sex and age was conducted in order to determine the relationship between BV/TV and demographic, clinical, and biochemical variable. All variables that reached statistical significance in the univariate analysis were included in the model of the multiple regression analysis. As BV/TV is not a normally distributed variable, we used log BV/TV as the dependent variable in the regression model. A *P*-value <0.05 was considered statistically significant. All statistical analyses were carried out using the true Epistat program (Epistat Services, Richardson, TX, USA).

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REFERENCES

1. Kanis JA. Diagnosis of osteoporosis and assessment of fracture risk. *Lancet* 2002; **359**: 1929–1936.
2. Tanko LB, Bagger YZ, Christiansen C. Low bone mineral density in the hip as a marker of advanced atherosclerosis in elderly women. *Calcif Tissue Int* 2003; **73**: 15–20.
3. Atsumi K, Kushida K, Yamazaki K *et al.* Risk factors for vertebral fractures in renal osteodystrophy. *Am J Kidney Dis* 1999; **33**: 287–293.
4. Stehman-Breen CO, Sherrard DJ, Alem AM *et al.* Risk factors for hip fracture among patients with end-stage renal disease. *Kidney Int* 2000; **58**: 2200–2205.
5. Coco M, Rush H. Increased incidence of hip fractures in dialysis patients with low serum parathyroid hormone. *Am J Kidney Dis* 2000; **36**: 1115–1121.
6. Barreto DV, Barreto FC, Carvalho AB *et al.* Coronary calcification in hemodialysis patients: the contribution of traditional and uremia related risk factors. *Kidney Int* 2005; **67**: 1576–1582.
7. London GM, Marty C, Marchais SJ *et al.* Arterial calcifications and bone histomorphometry in end-stage renal disease. *J Am Soc Nephrol* 2004; **15**: 1943–1951.
8. Moe SM, Yu BO, Sprague SM. Maintenance of bone mass in patients receiving dialytic therapy. *Am J Kidney Dis* 1993; **22**: 300–307.
9. Eknoyan G, Levin A, Levin NW, National Kidney Foundation. Bone metabolism and disease in chronic kidney disease. *Am J Kidney Dis* 2003; **42**(Suppl 3): 1–201.
10. Meunier P, Courpron P, Giroux JM *et al.* Bone histomorphometry as applied to research on osteoporosis and to the diagnosis of 'hyperosteoidosis states'. *Calcif Tissue Res* 1976; **21**: 354–360.
11. Manolagas SC, Jilka RL. Bone marrow, cytokines, and bone remodeling. Emerging insights into the pathophysiology of osteoporosis. *N Engl J Med* 1995; **332**: 305–311.
12. Malluche H, Faugere MC. Renal bone disease 1990: an unmet challenge for the nephrologist. *Kidney Int* 1990; **38**: 193–211.
13. Hruska KA, Teitelbaum SL. Renal osteodystrophy. *N Engl J Med* 1995; **333**: 166–174.
14. Stehman-Breen C. Osteoporosis and chronic kidney disease. *Semin Nephrol* 2004; **24**: 78–81.
15. Parfitt AM. A structural approach to renal bone disease. *J Bone Miner Res* 1998; **13**: 1213–1220.
16. Eriksen EF. Primary hyperparathyroidism: lessons from bone histomorphometry. *J Bone Miner Res* 2002; **17**(Suppl 2): 95–97.
17. Khosla S, Melton III J. Fracture risk in primary hyperparathyroidism. *J Bone Miner Res* 2002; **7**(Suppl2): 103–107.
18. Khan A, Bilezikian J. Primary hyperparathyroidism: pathophysiology and impact on bone. *Can Med Assoc J* 2000; **25**: 184–187.
19. Simonet WS, Lacey DL, Dunstan CR *et al.* Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. *Cell* 1997; **89**: 309–319.
20. Bucay N, Sarosi I, Dunstan CR *et al.* Osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification. *Genes Dev* 1998; **12**: 1260–1268.
21. Kong YY, Yoshida H, Sarosi I *et al.* OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. *Nature* 1999; **397**: 315–323.
22. Avbersek-Luznik I, Malesic I, Rus I, Marc J. Increased levels of osteoprotegerin in hemodialysis patients. *Clin Chem Lab Med* 2002; **40**: 1019–1023.
23. Coen G, Ballanti P, Balducci A *et al.* Serum osteoprotegerin and renal osteodystrophy. *Nephrol Dial Transplant* 2002; **17**: 233–238.
24. Haas M, Leko-Mohr Z, Roschger P *et al.* Osteoprotegerin and parathyroid hormone as markers of high-turnover osteodystrophy and decreased bone mineralization in hemodialysis patients. *Am J Kidney Dis* 2002; **39**: 580–586.
25. Hruska KA, Saab G, Chaudhary LR *et al.* Kidney–bone, bone–kidney, and cell–cell communications in renal osteodystrophy. *Semin Nephrol* 2004; **24**: 25–38.
26. Yano K, Tsuda E, Washida N *et al.* Immunological characterization of circulating osteoprotegerin/osteoclastogenesis inhibitory factor: increased serum concentrations in postmenopausal women with osteoporosis. *J Bone Miner Res* 1999; **14**: 518–527.
27. Nanes MS. Tumor necrosis factor-alpha: molecular and cellular mechanisms in skeletal pathology. *Gene* 2003; **321**: 1–15.
28. Lee WY, Baek KH, Rhee EJ *et al.* Impact of circulating bone-resorbing cytokines on the subsequent bone loss following bone marrow transplantation. *Bone Marrow Transplant* 2004; **34**: 89–94.
29. Santos FR, Moyses RM, Montenegro FL *et al.* IL, IL-1beta, TNF-alpha, TGF-beta, and bFGF expression in bone biopsies before and after parathyroidectomy. *Kidney Int* 2003; **63**: 899–907.
30. Taal MW, Masud T, Green D, Cassidy MJ. Risk factors for reduced bone density in haemodialysis patients. *Nephrol Dial Transplant* 1999; **14**: 1922–1928.
31. Weisinger JR, Bellorini-Font E. Postmenopausal osteoporosis in the dialysis patients. *Curr Opin Nephrol Hypertens* 2003; **12**: 381–386.
32. Slemenda CW, Longcope C, Zhou L *et al.* Sex steroids and bone mass in older men. Positive associations with serum estrogens and negative associations with androgens. *J Clin Invest* 1997; **100**: 1755–1759.
33. Lobão R, Carvalho AB, Cuppari L *et al.* High prevalence of low bone mineral density in pre-dialysis chronic kidney disease patients: bone histomorphometric analysis. *Clin Nephrol* 2004; **62**: 432–439.
34. Spasovski GB, Bervoets AR, Behets GJ *et al.* Spectrum of renal bone disease in end-stage renal failure patients not yet on dialysis. *Nephrol Dial Transplant* 2003; **18**: 1159–1166.
35. Asaka M, Iida H, Entani C *et al.* Total and regional bone mineral density by dual photon absorptiometry in patients on maintenance hemodialysis. *Clin Nephrol* 1992; **38**: 149–153.
36. Gabay C, Ruedin P, Slosman D *et al.* Bone mineral density in patients with end-stage renal failure. *Am J Nephrol* 1993; **13**: 115–123.
37. George A, Tracy JK, Meyer WA *et al.* Racial differences in bone mineral density in older men. *J Bone Miner Res* 2003; **18**: 2238–2244.
38. Stein MS, Packham DK, Ebeling PR *et al.* Prevalence and risk factors for osteopenia in dialysis patients. *Am J Kidney Dis* 1996; **28**: 515–522.
39. Stehman-Breen CO, Sherrard D, Walker A *et al.* Racial differences in bone mineral density and bone loss among end-stage renal disease patients. *Am J Kidney Dis* 1999; **33**: 941–946.
40. Harris S, Dallal GE, Dawson-Hughes B. Influence of body weight on rates of change in bone density of the spine, hip, and radius in postmenopausal women. *Calcif Tissue Int* 1992; **50**: 19–23.
41. Cunningham J, Sprague SM, Cannata-Andia J *et al.* Osteoporosis Work Group. Osteoporosis in chronic kidney disease. *Am J Kidney Dis* 2004; **43**: 566–571.
42. Johnson DW, McIntyre HD, Brown A *et al.* The role of DEXA bone densitometry in evaluating renal osteodystrophy in continuous ambulatory peritoneal dialysis patients. *Periton Dial Int* 1996; **16**: 34–40.
43. Masud T, Langley S, Wiltshire P *et al.* Effect of spinal osteophytosis on bone mineral density measurements in vertebral osteoporosis. *BMJ* 1993; **307**: 172–173.
44. Chavassieux PM, Arlot ME, Meunier PJ. Intersample variation in bone histomorphometry: comparison between parameter values measured on two contiguous transiliac bone biopsies. *Calcif Tissue Int* 1985; **37**: 345–350.
45. Birkenhäger-Frenkel DH, Courpron P, Hüpscher EA *et al.* Age-related changes in cancellous bone structure – a two-dimensional study in the transiliac and iliac crest biopsy sites. *Bone Miner* 1988; **4**: 197–216.
46. Meunier PJ, Chavassieux P. Histomorphometry. Method for evaluating the bone mass. *Rev Rheum Mal Osteoartic* 1985; **52**: 669–673.
47. Meunier P, Courpron P, Edouard C *et al.* Physiological senile involution and pathological rarefaction of bone. Quantitative and comparative histological data. *Clin Endocrinol Metab* 1973; **2**: 238–256.
48. Kelepouris N, Harper KD, Gannon F *et al.* Severe osteoporosis in men. *Ann Intern Med* 1995; **123**: 452–460.
49. Malluche HH, Faugere MC (eds) *Methodology of Mineralized Bone Histology, in ATLAS of Mineralized Bone Histology*. Karger: Basel, Switzerland, 1986, pp 17–24.
50. Dos Reis LM, Martins CT, Jorgetti V. Histochemical staining of bone aluminum: comparison of aluminon and acid solochrome azurine and their correlation with bone aluminum content. *Rev Hosp Clin Fac Med S Paulo* 1997; **52**: 171–174.
51. Parfitt AM, Drezner MK, Glorieux FH *et al.* Bone histomorphometry: standardization of nomenclature, symbols and units. *J Bone Miner Res* 1987; **2**: 595–610.
52. Sherrard DJ, Baylink DJ, Wergedal JE, Maloney NA. Quantitative histological studies on the pathogenesis of uremic bone disease. *J Clin Endocrinol Metab* 1974; **39**: 119–135.