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Konstantinos K. Doumouchtsis · Alkis I. Kostakis
Stergios K. Doumouchtsis · Marios P. Tziamalis
Charalambos P. Stathakis · Evanthia Diamanti-Kandarakis
Dimitrios Dimitroulis · Despoina N. Perrea

Associations between osteoprotegerin and femoral neck BMD in hemodialysis patients

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Abstract Numerous humoral factors are involved in the development of renal osteodystrophy, causing perturbations in bone mineral density (BMD) in patients with end-stage renal disease (ESRD). The RANKL/OPG cytokine system appears to mediate the effects of many of these factors on bone turnover, contributing to the pathogenesis of renal bone disease. The aim of this study was to evaluate the clinical and biochemical correlations of BMD measurements in patients on chronic hemodialysis. Fifty-four hemodialysis patients underwent measurement of BMD at the proximal femur and the lumbar spine (L2–L4). Intact parathyroid hormone (PTH), osteoprotegerin (OPG), sRANKL, and main bone biochemical markers were also measured in serum samples of all patients. BMD of the femoral neck was negatively correlated with OPG levels ($r = 0.333$, $P = 0.014$). OPG levels were significantly different among normal, osteopenic, and osteoporotic tertiles defined according to BMD of the femoral neck. The highest OPG levels were measured in the lowest T-score (osteoporotic) tertile and were higher than in the osteopenic and normal tertiles ($P < 0.05$). A threshold level for OPG at 21.5 pmol/l enabled the detection of osteoporotic patients with 76.5% sensitivity and 62.2% specificity. BMD values of trabecular bone-rich sites of the skeleton such as lumbar spine (L2–L4), trochanter, and Ward' s triangle were inversely correlated with total ALP levels ($P < 0.05$). Hemodialysis patients with low BMD of the femoral neck demonstrated higher OPG levels than patients with normal BMD. Those with lumbar spine (L2–L4), trochanteric, and Ward's triangle BMDs below the normal range presented higher total ALP levels. These results suggest that OPG and total ALP may be clinically useful markers in the detection of significant femoral neck and trabecular bone mineral deficit in hemodialysis patients, warranting further investigations.

Key words osteoprotegerin · total alkaline phosphatase · hemodialysis · renal osteodystrophy · bone mineral density

K.K. Doumouchtsis (✉) · S.K. Doumouchtsis · D. Dimitroulis · D.N. Perrea
Laboratory for Experimental Surgery and Surgical Research, Athens University, 15B Saint Thomas Street, Athens 11527, Greece
Tel. +03-02-10643-4691; Mobile +03-06976-793870;
Fax +03-02107-462539
e-mail: konsdoum@yahoo.gr

A.I. Kostakis
2nd Department of Propedeutic Surgery, Athens University, Athens, Greece

M.P. Tziamalis
Department of Nephrology and Dialysis, Kastoria General Hospital, Kastoria, Greece

C.P. Stathakis
Department of Nephrology and Dialysis, Laikon General Hospital, Athens, Greece

E. Diamanti-Kandarakis
1st Department of Medicine, Laiko General Hospital, Athens University, Athens, Greece

Introduction

In chronic renal failure, several types of metabolic bone disease occur as a result of perturbations related to the parathyroid and vitamin D system. The term renal osteodystrophy encompasses all types of complex disorders of the skeleton from which patients with chronic renal disease suffer. As glomerular filtration rate (GFR) declines, phosphate retention, hyperphosphatemia, and impaired production of calcitriol occur, leading to reciprocal decrease of serum ionized calcium concentration. Low serum calcium levels are caused either by the formation of complexes of calcium and phosphorus that precipitate into skin and soft tissues or by inadequate production of calcitriol [1]. Decreased concentrations of ionized calcium and calcitriol as well as hyperphosphatemia persistently stimulate parathyroid hormone (PTH) secretion. Continuous stimulation of PTH secretion induces hyperplasia of the parathyroid glands, causing secondary hyperparathyroidism (sHPT) [2]. Excess PTH can produce a condition of high turnover bone disease. A common abnormality associated with renal osteodystrophy is increased skeletal resistance to PTH, rec-

ognized as a blunted calcemic action of PTH [3]. This perturbation determines higher PTH concentrations are needed in patients with end-stage renal disease (ESRD) to maintain normal homeostasis in bone metabolism. These concentrations range from three- to fourfold greater than the upper limit of normal for the intact PTH assay [4,5]. Moreover, new therapeutic modalities and interventions designed to control excess PTH, such as calcitriol pulse therapy, have made it possible to suppress serum PTH to normal or lower levels in uremic patients [6]. Consequently, skeletal resistance to PTH and relative hypoparathyroidism are recognized as major factors in the development of low turnover or adynamic bone diseases [7].

RANKL and osteoprotegerin (OPG) is a newly identified cytokine system that is involved in the development of the various types of renal bone disease [8]. PTH appears to stimulate RANKL and inhibit OPG secretion, affecting their serum levels in renal failure [9]. In this way the RANKL/OPG cytokine system mediates the effects of PTH and other humoral factors on bone metabolism, thus reflecting their impact on bone composition in several types of renal osteodystrophy.

Quantitative imaging methods of bone densitometry are noninvasive procedures used to assess bone mineral density (BMD) at various sites in patients with ESRD. BMD is thought to be an effective parameter for the diagnosis and surveillance of osteoporosis; however, its application to studying the effect of chronic renal disease on the skeleton has revealed controversial results and inconsistent conclusions [10,11]. Although it has not been possible to discriminate the different types of renal osteodystrophy by BMD measurements, this technique is a useful method to quantify bone changes among patients on hemodialysis (HD). In combination with biochemical markers of bone turnover, it is a useful approach in identifying patients with ESRD with marked bone mineral deficit. BMD is reduced in patients with chronic renal failure and especially in HD patients with sHPT, but the identified differences are related to various factors such as sex, bone site, duration of HD, clinical factors, and humoral markers [12].

This study aimed to assess BMD using dual X-ray absorptiometry (DXA) in HD patients as a whole and in male and female subgroups. It also investigated the possible correlations of BMD with clinical parameters as well as serum levels of sRANKL, OPG, PTH, and other humoral factors.

Materials and methods

Patients

Fifty-four (54) patients, 27 men and 27 women aged from 30 to 78 years (mean, 58.11 ± 12.04 years) on maintenance hemodialysis therapy, were recruited from three dialysis units. The patients were fully counseled, and informed consent was obtained. The duration of dialysis ranged from 4 to 298 months (mean, 73.27 ± 68.19 months). All female patients were amenorrheic for longer than 1 year. Exclusion criteria were presence of clinical or biochemical evidence of malignancy, hyperthyroidism, and active infection as well as administration of medications affecting bone metabolism such as steroids, hormone replacement regimens, bisphosphonates, and calcitonin during the past year preceding the study. Most of the patients had undergone treatment with vitamin D or its active metabolites at some stage of their disease in accordance with the last K/DOQI Clinical Practice Guidelines for renal bone disease (based on serum levels of intact PTH, calcium, phosphorus, and Ca-P product). The main phosphate chelating agents used were calcium salts and sevelamer.

Bone mineral density (BMD) measurements

BMD of the proximal femur and the lumbar spine (L2–L4) was measured by dual-energy X-ray absorptiometry (DEXA) using a Lunar DPX-L bone densitometer (Lunar Corporation, Madison, WI, USA) in each hospital's local nuclear medicine laboratory. BMD was expressed in absolute values (g/cm^2), as well as Z-scores and T-scores (deviation from the peak BMD). Z-scores were defined as numbers of standard deviations from the mean BMD of age-, weight-, and ethnic-matched normals. T-scores were defined as the number of standard deviations from the mean BMD from sex-matched young controls. Patients were grouped into normal, osteopenic, and osteoporotic tertiles according to World Health Organization (WHO) criteria (deviation from the peak BMD). Their femoral neck T-scores were normal if the T-score was higher than –1, osteopenic if the T-score ranged between –1 and –2.5, and osteoporotic if the T-score was lower than –2.5 compared to the control values.

Biochemical analyses

Serum samples were obtained from all patients just before dialysis procedure and stored at –80°C until assayed. The following biochemical markers were measured: intact parathyroid hormone (iPTH), sRANKL, osteoprotegerin (OPG), osteocalcin (OC), total alkaline phosphatase (total ALP), and tartrate-resistant acid phosphatase (TRAP-5b).

Intact PTH (iPTH) was measured by enzyme-linked immunosorbent assay (ELISA) (Biometrika, Santa Monica, CA, USA). The reported intraassay and interassay coefficients of variation (CV) of the assay were <3% and <8%, respectively; the sensitivity was 0.9 pg/ml.

Total sRANKL was quantitatively determined by ELISA (Immundiagnostik, Bensheim, Germany; Apotech, Epalinges, Switzerland). Intra- and interassay coefficients of variation (CV) of the assay were <4% and <10%, respectively, and the sensitivity was 1.56 pg/ml.

Serum concentrations of OPG were quantitated using an ELISA kit (Biovendor Laboratory Medicine, Czech Republic) that detects both monomer (5%) and dimer (95%) forms. The intraassay CV was <7%, the interassay CV was <7.5%, and the sensitivity was 0.4 pmol/l.

Osteocalcin (OC) was determined with a commercially available ELISA kit (Nordic Bioscience Diagnostics A/S,

Denmark). The intra- and inter-assay CVs were <3%, and <5% respectively, and the detection limit was 0.5 ng/ml.

TRAP-5b of serum was assayed by solid-phase immunofixed enzyme activity assay (SBA-Sciences, Suomen Bioanalytiica Oy, Finland). Intra- and interassay CVs of the assay were <14% and <10%, respectively, and the limit of quantitation was <1 U/l.

Total alkaline phosphatase (total ALP) was determined using standard colorimetric methods.

Statistical analysis

The results were expressed as mean ± SD. Statistical evaluation was performed using Statistical Package for the Social Sciences software (SPSS 13, Chicago, IL, USA) on a PC. Evaluations were based on Spearman's nonparametric correlation coefficient and the Kruskal–Wallis test, whereas a receiver-operating characteristic (ROC) curve was used to assess the predictive accuracy of OPG. Statistical significance was set at the 0.05 level.

Results

BMD values: comparison between male and female patients

In the present study, the average absolute BMD of the femoral neck of the patients was 0.75 ± 0.13 g/cm^2, whereas the average BMD Z-score and BMD T-score were –1.02 ± 0.97 and –2.12 ± 1.08, respectively. The mean absolute BMD of the lumbar spine (L2–L4) was 1.095 ± 0.16 g/cm^2, the mean Z-score was –0.13 ± 1.3, and the mean T-score was –1.017 ± 1.29. Statistically significant higher values of femoral neck, trochanter, total hip, and lumbar spine (L2–L4) BMD were identified in males (0.79 ± 0.15 g/cm^2, 0.69 ± 0.11 g/cm^2, 0.92 ± 0.16 g/cm^2, and 1.16 ± 0.18 g/cm^2, respectively) than in females (0.71± 0.11 g/cm^2, 0.57 ± 0.11 g/cm^2, 0.8 ± 0.12 g/cm^2, and 1.028 ± 0.112 g/cm^2, respectively) (Table 1).

Femoral neck BMD correlation

Femoral neck BMDs were negatively correlated with the age of the patients (r = –0.290, P = 0.034), duration of dialysis (r = –0.326, P = 0.016), and OPG levels (r = –0.333, P = 0.014) (Fig. 1). A weak positive correlation was found between BMD of the femoral neck and RANKL/OPG ratio (r = –0.275, P = 0.044), whereas no other relationship was found with intact PTH, RANKL, and other indices of bone turnover such as TRAP-5b, total ALP, and OC (Table 2).

Femoral neck BMD T-scores decreased as the patient's age increased (r = –0.325, P = 0.016), whereas no significant association was revealed between T-scores and the duration of HD (r = –0.247, P = 0.072). Significant negative correlation between femoral neck BMD T-scores and OPG levels (r = –0.333, P = 0.014) was identified. BMD T-scores showed a weak positive correlation with RANKL/OPG ratio (r = –0.272, P = 0.047), but they were not associated with other humoral factors and indices of bone turnover. Furthermore, the patients were stratified into tertiles according to the WHO criteria. The first tertile included 7 patients with

Table 1. Comparison of clinical characteristics and bone mineral density (BMD) between male and female patients

	Total	Male patients	Female patients
n	54	27	27
Age (years)	58.11 ± 12.04	57.81 ± 12.64	58.4 ± 11.63
HD time (months)	73.27 ± 68.19	60.74 ± 60.26	85.81 ± 74.30
Neck			
g/cm^2	0.75 ± 0.13	0.79 ± 0.15	0.71 ± 0.11*
T-score	–2.12 ± 1.08	–2.01 ± 1.22	–2.22 ± 0.92
Z-score	–1.02 ± 0.97	–0.91 ± 1.09	–1.12 ± 0.84
Trochanter			
g/cm^2	0.63 ± 0.12	0.69 ± 0.11	0.57 ± 0.11*
T-score	–1.84 ± 0.96	–1.75 ± 0.96	–1.92 ± 0.99
Z-score	–1.22 ± 0.92	–1.26 ± 0.86	–1.99 ± 0.99
Ward's triangle			
g/cm^2	0.59 ± 0.144	0.62 ± 0.15	0.57 ± 0.13
T-score	–2.35 ± 1.09	–2.16 ± 1.14	–2.53 ± 1.04
Z-score	–0.87 ± 0.96	–0.83 ± 0.96	–0.91 ± 0.99
Total hip			
g/cm^2	0.86 ± 0.15	0.92 ± 0.16	0.8 ± 0.12*
T-score	–1.42 ± 1.11	–1.27 ± 1.27	–1.56 ± 0.94
Z-score	–0.59 ± 1.07	–0.42 ± 1.24	–0.76 ± 0.88
L2–L4			
g/cm^2	1.095 ± 0.16	1.16 ± 0.18	1.02 ± 0.11*
T-score	–1.01 ± 1.29	–0.56 ± 1.51	–1.43 ± 0.93
Z-score	–0.13 ± 1.3	–0.06 ± 1.74	–0.2 ± 0.77

HD, hemodialysis
*Compared with male patients, P < 0.05

Table 2. Correlation coefficient of BMD (g/cm^2) with clinical characteristics and biochemical bone markers

	Proximal femur				Lumbar spine L2–L4 vertebrae
	Neck	Trochanter	Ward's triangle	Total hip	
Age (years)	–0.290*	–0.046	–0.057	–0.173	0.023
Hemodialysis (HD) time (months)	–0.326*	–0.563**	–0.550**	–0.179	–0.368
iPTH (pg/ml)	–0.161	–0.354	–0.237	–0.139	–0.320
sRANKL (pmol/l)	0.058	–0.106	–0.031	0.162	0.122
OPG (pmol/l)	–0.333*	–0.126	–0.152	–0.160	–0.090
sRANKL/OPG	0.275*	0.082	0.170	0.272	0.140
OC (ng/ml)	–0.094	–0.152	0.039	–0.194	–0.277
Total ALP (U/l)	–0.217	–0.396*	–0.441*	–0.261	–0.516*
TRAP-5b (U/l)	0.209	0.2	0.398*	–0.4	–0.196
Ca (mg/dl)	0.362	0.308	0.318	0.356	0.126
PO_4 (mg/dl)	–0.049	0.007	0.028	–0.135	0.013
$Ca \times PO_4$	0.015	0.08	0.073	–0.054	0.042

* $P < 0.05$; ** $P < 0.01$

Table 3. Comparison of clinical characteristics and biochemical bone markers among patients with normal, osteopenic, and osteoporotic BMD T-scores at the femoral neck

T-scores	Normal	Osteopenia	Osteoporosis	*P* value
N	7	30	17	
Sex (male/female)	3/4	15/15	8/7	0.981
Age (years)	48.5 ± 13.08	57.93 ± 13	61.88 ± 8.36	0.108
HD time (months)	88.66 ± 97	53.3 ± 51.93	101.64 ± 76.44	0.22
iPTH (pg/ml)	132.71 ± 113.26	185.71 ± 297.81	215.76 ± 321.11	0.957
sRANKL (pmol/l)	0.396 ± 0.172	0.629 ± 0.659	0.757 ± 0.76	0.314
OPG (pmol/l)	18.05 ± 6.57	21.58 ± 11.45	28.02 ± 13.03	0.035*
sRANKL/OPG	0.024 ± 0.01	0.038 ± 0.042	0.031 ± 0.032	0.83
OC (ng/ml)	80 ± 35.23	78.25 ± 32.96	76.69 ± 33.04	0.969
Total ALP (U/l)	69.32 ± 44.37	145.32 ± 151.57	106.17 ± 80.78	0.244
TRAP-5b (U/l)	3.46 ± 1.37	3.03 ± 1.92	2.52 ± 1.58	0.348
Ca (mg/dl)	10.35 ± 0.37	9.96 ± 0.77	9.93 ± 0.54	0.537
PO_4 (mg/dl)	4.85 ± 1.08	5.24 ± 1.79	4.56 ± 1.32	0.635

* $P < 0.05$

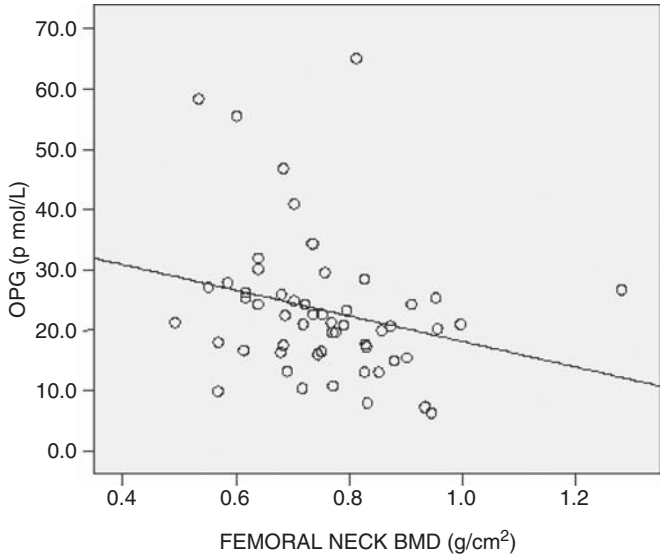

Fig. 1. Relationship between osteoprotegerin (*OPG*) and femoral neck bone mineral density (*BMD*) in the hemodialysis patients. Femoral neck BMDs were negatively correlated with OPG levels ($r = –0.333$, $P = 0.014$)

femoral neck BMD T scores from 0 to –1 (considered as normal), the second tertile included 30 patients with T scores from –1.0 to –2.5 (considered as osteopenic), and the third tertile included 17 patients with T score lower than –2.5 (considered as osteoporotic). There was no significant difference in sex and age distribution, HD time in months, and serum levels of Ca and PO_4 between the three tertiles (Table 3). However, OPG levels differed significantly among these three patient groups. The highest OPG levels were noted in the lowest T-score (third) tertile (28.02 ± 13.03) and were significantly higher than in the second and first tertiles (21.58 ± 11.45 and 18.05 ± 6.57, respectively; $P = 0.035$) (Fig. 2). No statistically significant difference was found in the levels of PTH, RANKL, total ALP, OC, TRAP-5b, and RANKL/OPG ratio among the three tertiles.

Using these results, we constructed a receiver-operating characteristic (ROC) curve of OPG to detect osteoporosis (T-score below –2.5) at the femoral neck (Fig. 3). The optimum threshold level for OPG was 21.5 pmol/l, which enabled the detection of osteoporotic patients with 76.5% sensitivity and 62.2% specificity [area under the curve (AUC), 0.71; $P < 0.05$].

Femoral neck BMD Z-scores of our HD patients were inversely correlated with duration of hemodialysis ($r =$

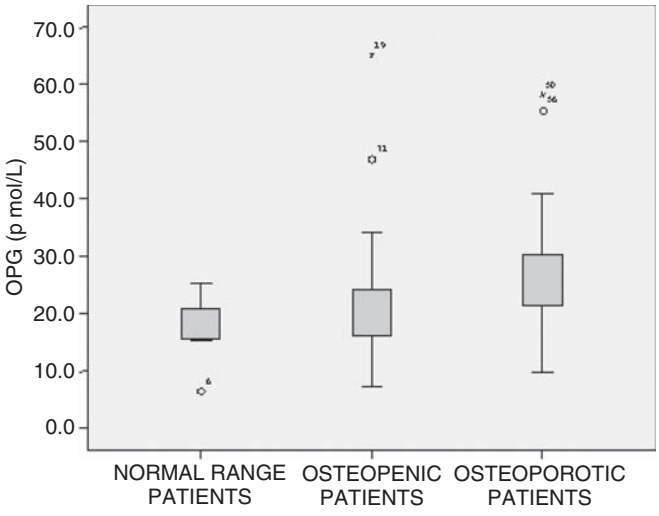

Fig. 2. Comparison of serum osteoprotegerin (OPG) levels among patients with normal, osteopenic, and osteoporotic BMD T-scores at the femoral neck. Serum OPG levels were significantly higher in the osteoporotic tertile in comparison with osteopenic and normal tertiles ($P = 0.035$)

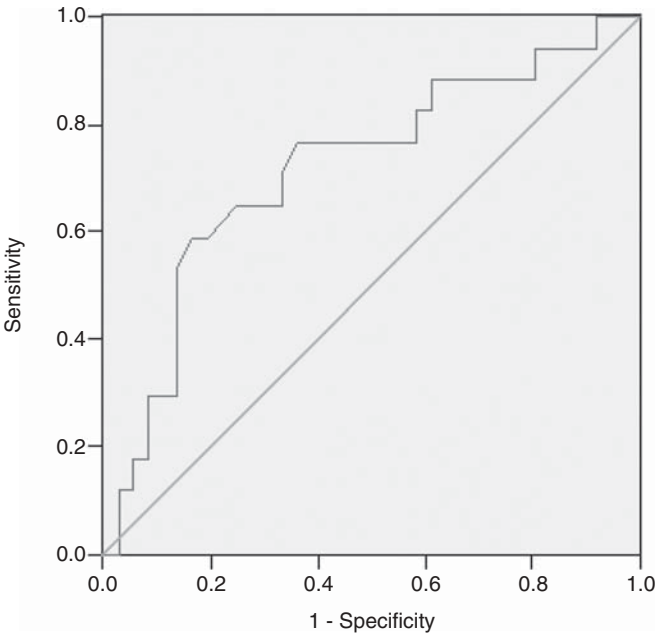

Fig. 3. Receiver operating characteristic (ROC) curve of OPG for prediction of osteoporosis (T-score below –2.5) at the femoral neck. A threshold level for OPG at 21.5 pmol/l enabled the detection of osteoporotic patients with 76.5% sensitivity and 62.2% specificity [area under the curve (AUC), 0.71, $P < 0.05$]

–0.354, $P = 0.009$). No significant association between Z-scores and iPTH, RANKL, TRAP-5b, total ALP, OC, and RANKL/OPG ratio was established.

Trochanteric and Ward's triangle BMD correlation

Trochanteric and Ward' s triangle BMD values were inversely correlated with total ALP levels ($r = –0.396$, $P = –0.027$ and $r = –0.441$, $P = 0.013$, respectively). Their absolute values, T-scores, and Z-scores demonstrated strong negative correlations with the duration of dialysis (see Table 2).

Lumbar spine (L2–L4) BMD correlation

Lumbar spine (L2–L4) BMDs showed a trend to negatively correlate with the duration of dialysis ($r = –0.368$, $P = 0.084$) and were significantly inversely associated with serum levels of total ALP ($r = –0.516$, $P = 0.017$). No other significant correlation with RANKL, OPG, PTH, and biochemical markers was identified (see Table 2). Similar correlations were found between lumbar spine (L2–L4) T-scores, duration of dialysis ($r = –0.366$, $P = 0.086$), and serum levels of total ALP ($r = –0.456$, $P = 0.038$). Z-scores were significantly correlated positively with age ($r = –0.475$, $P = 0.022$) and negatively with the duration of dialysis ($r = –0.497$, $P = 0.016$).

No significant correlations were found between BMDs at the skeletal sites studied and serum levels of calcium, phosphorus, and $Ca \times PO_4$ product.

Discussion

The involvement of RANKL/OPG cytokine system in the regulation of bone turnover has been established in previous studies. However, its role in the pathogenesis of renal osteodystrophy has not yet been fully elucidated. BMD measurements have been used to study the effect of ESRD and its treatment on several skeletal sites and the possible correlation of BMD with clinical and biochemical markers and bone histology [10,13].

This study showed that BMD as assessed by the average BMD Z-score was significantly decreased at the proximal femur and lumbar spine (L2–L4), which is in accordance with data from most studies that used the same technique (DEXA) for evaluation of bone mass [11,14,15]. The mean absolute BMD, T-score, and Z-score were lower in the femoral neck, where cortical bone is predominant, than in the lumbar spine, a trabecular bone-rich component [16]. This finding is in agreement with the statement that bone loss in renal bone disease is site specific and that in primary and secondary hyperparathyroidism there is greater cortical than trabecular bone loss, whereas in postmenopausal osteoporosis trabecular bone loss is more prominent [17]. Female patients presented more pronounced bone loss in both cortical and trabecular bone than male patients, as higher values of BMD have been found in males rather than females at several skeletal sites [16]; this probably reflects an additional potential for bone resorption in women as a consequence of postmenopausal changes in female hormones.

In our study the OPG levels were significantly higher in patients with marked bone loss at the femoral neck than those without. These findings are consistent with previous studies, which demonstrated a negative correlation between serum OPG levels and BMD in healthy individuals as well as higher OPG concentrations in women with postmeno-

pausal osteoporosis than in aged-matched healthy controls [18–20]. Setting a threshold of OPG of 21.5 pmol/l gave a sensitivity of 76.5% and a specificity of 62.2% for prediction of osteoporosis. Our data suggest that OPG could be used as a screening tool for the detection of HD patients with suspected low femoral neck BMD who qualify for BMD assessment. Higher OPG levels could be attributed not only to reduced renal clearance and lack of its elimination through the polysulfon hemodialysis membrane [21] but may also reflect a homeostatic mechanism for bone protection. Research on OPG and BMD in HD patients supports the concept that high OPG levels may also exert a protective action to limit bone resorption [22,23].

Lumbar spine (L2–L4), trochanteric, and Ward's triangle BMD values were inversely correlated with total ALP levels, which accords with the pattern of bone loss in some HD patients, with rapid bone loss associated with high levels of total ALP [24,25]. Some authors have used total and bone ALP in predicting BMD in HD patients [10,26]. The discrepancies in the correlations with humoral factors in different sites of the skeleton suggest that renal osteodystrophy has a marked variability in the model of bone mineral deficit at several skeletal sites [11,27].

BMD declines with aging and longer duration of HD, so the aging process implies perturbations in bone turnover, especially in HD patients. Our results are in agreement with previous studies showing that patients on dialysis for a longer period had BMDs below the normal range [28,29]. These studies highlighted the deleterious effects of uremia even before the onset of dialysis and in correlation with the severity of renal failure [16,30,31].

Although trochanter BMD showed a tendency to negatively correlate with iPTH, we failed to demonstrate any other correlation between BMDs and PTH, which is the major regulator of bone metabolism. This finding was expected, given that the skeleton is exposed to fluctuating levels of PTH during the course of hemodialysis. This finding is also in keeping with the absence of any significant relationship between BMD and calcium, phosphorus, and $Ca \times PO_4$ product, probably because calcium and phosphorus levels are not stable in the serum of HD patients. Moreover, skeletal resistance to PTH in renal failure suggests that other humoral factors which may accumulate in uremic serum, such as OPG, could exert more potential effect on bone turnover in patients with renal failure [32]. Nevertheless, some previous studies have shown that iPTH levels could influence BMD, and the least reduced levels of BMD were identified in patients with normal bone turnover [11,33].

A number of factors should be assessed in concert with our findings. Bone disease as associated with chronic renal impairment is complex and multifactorial. Consequently, BMD in HD patients is influenced by several factors such as previous medical history, comorbidities, treatment modalities, and humoral factors of the uremic serum. The small number of patients reduces our study's statistical power and probably limits the applicability of our observations in the overall HD patient population. Further studies are needed to establish the significance of OPG to identify HD patients with several types of renal osteodystrophy, possibly including histomorphometric analysis.

However, it may be concluded that HD patients with low femoral neck BMD demonstrate higher OPG levels than patients with normal BMD and that those with lumbar spine (L2–L4), trochanteric, and Ward's triangle BMDs below the normal range present higher total ALP levels. These results suggest that OPG and total ALP could be used in conjunction with other parameters as a screening tool for the detection of HD patients with marked femoral neck and trabecular bone loss, respectively, who probably need to undergo further investigations.

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