25-hydroxyvitamin D levels and bone histomorphometry in hemodialysis renal osteodystrophy

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Background. The importance of 25-hydroxyvitamin D (25-OHD) serum levels in hemodialysis chronic renal failure has not been so far histologically evaluated. Information still lacking relate to the effect of 25-OHD deficiency on serum parathyroid hormone (PTH) levels and on bone and its relationship with calcitriol levels.

Methods. This retrospective study has been performed on a cohort of 104 patients on hemodialysis from more than 12 months, subjected to transiliac bone biopsy for histologic, histomorphometric, and histodynamic evaluation. The patients, 61 males and 43 females, mean age 52.9 ± 11.7 years, hemodialysis length 97.4 ± 61.4 months, were treated with standard hemodialysis and did not receive any vitamin D supplementation. Treatment with calcitriol was not underway at the time of the biopsy. Transiliac bone biopsies were performed after double tetracycline labels. In addition, serum intact PTH (iPTH), alkaline phosphatase, and 25-OHD were measured. Calcitriol serum levels was also measured in a subset of patients ($N = 53$). The patients were divided according to serum 25-OHD levels in three groups: (*1*) 0 to 15 (15 patients), (*2*) 15 to 30 (38 patients), and (*3*) >30 ng/mL (51 patients).

Results. There was no significant difference in average age, hemodialysis age, serum PTH [490 ± 494, 670 ± 627, and 489 ± 436 pg/mL, respectively (mean $\pm$ SD)], alkaline phosphatase, and calcitriol between the three groups. The parameters double-labeled surface, trabecular mineralizing surface, and bone formation rate were significantly lower in group 1 than in the other groups ($P < 0.03$, < 0.03, and < 0.02, respectively). Osteoblast surface and adjusted apposition rate were borderline significantly lower in group 1 ($P < 0.06$ and < 0.10). There was no statistical difference in the biochemical and bone parameters between groups 2 and 3. A positive significant correlation was found between several bone static and dynamic parameters and 25-OHD levels in the range 0 to 30 ng/mL, showing a vitamin D dependence of bone turnover at these serum levels. However,

actual evidence of an effect on bone of 25-OHD deficiency was found at serum levels below 20 ng/mL. With increasing 25-OHD levels beyond 40 ng/mL, a downslope of parameters of bone turnover was also observed.

Conclusion. Since PTH serum levels are equally elevated in low and high 25-OHD patients, while calcitriol levels are constantly low, an effect of 25-OHD deficiency (group 1) on bone, consisting of a mineralization and bone formation defect, can be hypothesized. The effect of vitamin D deficiency or bone turnover is found below 20 ng/mL. The optimal level of 25-OHD appears to be in the order of 20 to 40 ng/mL. Levels of the D metabolite higher than 40 ng/mL are accompanied by a reduction of bone turnover.

Serum levels of 25-hydroxyvitamin D (25-OHD) provide an evaluation of body stores of the vitamin, since hepatic 25-hydroxylation of cholecalciferol is poorly regulated and depends on the availability of the substrate. Low levels of serum 25-OHD are a frequent finding in the general population [1–3], with special regard to elderly or disabled or chronically ill people, unsufficiently exposed to sun and ultraviolet light, or affected by malnutrition. Serum 25-OHD levels are decreased to insufficiency mainly during winter and early spring periods [4, 5]. It is also known that patients with chronic renal failure have levels of 25-OHD lower than normal with a progressive decrease in the serum with decreasing renal function [6, 7]. The deficiency of 25-OHD is associated to osteomalacia in subjects with normal renal function, and is a cofactor of impaired mineralization and osteomalacia in renal patients [8, 9]. However, the state of 25-OHD deficiency and insufficiency, with special regard to chronic renal failure, has not been clearly defined. While in nonrenal patients vitamin D insufficiency is considered to be present at the serum levels associated with curtailed calcitriol synthesis, in chronic renal failure, calcitriol production is hampered even at relatively elevated 25-OHD levels. The role and extent of extrarenal calcitriol synthesis in uremic patients and its contribution to vitamin D effect on target organs is not known. In addition, we do not know whether this metabolite of vitamin

D plays a direct and specific role on bone metabolism and on parathyroid secretion, independent of calcitriol serum levels, notoriously low in chronic renal failure. In facts, we are accustomed to believe that all the vitamin D activity is exhibited by calcitriol, the vitamin D metabolite which we commonly utilize when it is necessary to administer vitamin D in chronic renal failure. An independent biologic activity of 25-OHD is still a matter of doubt. It is not known which are the appropriate and desirable 25-OHD serum levels, in chronic renal failure, which would avoid adverse consequences on bone and on parathyroid hormone (PTH) secretion. Most of our knowledge derives from the study of subjects with normal renal function and, in the definition of optimal serum 25-OHD levels, reference is usually made to the vitamin D serum levels which are unable to induce secondary hyperparathyroidism [3, 5]. However, in chronic renal failure, there are many factors of secondary hyperparathyroidism, so that an increased secretion of the hormone due to vitamin D deficiency or insufficiency cannot be clearly demonstrated. In addition, while it is known that elderly subjects with normal renal function and low 25-OHD levels have more frequent hip fractures [10, 11], increased incidence of fractures are known to be present in patients on hemodialysis [12, 13] independently of age and vitamin D status of the patients. Deficiency of 25-OHD in chronic renal failure appears to be only one and probably not very decisive factor of bone fractures.

Therefore, a better understanding of the specific role of 25-OHD deficiency in chronic renal failure should be obtained from a large study based on bone biopsies with histologic, histomorphometric, and histodynamic evaluation. Data of this kind are not yet available in the medical literature. This study compares cross-sectionally patients with different levels of serum 25-OHD from biochemical and histologic point of view providing new data on the effect of low levels of the vitamin D metabolite on bone static and dynamic parameters.

METHODS

This study has been performed retrospectively on a cohort of 104 patients on hemodialysis for more than 12 months, with a wide range of PTH serum levels, who volunteered to undergo a bone biopsy for histologic, histomorphometric, and histodynamic evaluation. The patients, 61 males and 43 females, had a mean age of 52.9 ± 11.7 years, and a mean dialytic length of 97.4 ± 61.4 months. The causes of terminal renal failure were the following: chronic glomerulonephritis in 36 patients, tubulointerstitial nephropathy in 24 patients, hypertensive/ischemic nephropathy in 16 patients, polycystic kidney disease in 12 patients, diabetic nephropathy in four patients, renal stone disease and obstructive nephropathy in two patients, and unknown in ten patients. They did not receive any cholecalciferol or 25-OHD supplementation. Most of the patients had been treated previously with relatively limited doses of calcitriol administered orally or intravenously (1.5 to 3.5 µg/week), but had discontinued this treatment at least 3 months before bone biopsy in two patients and more than 5 months in the other patients.

The patients did not receive corticosteroids, nonsteroidal anti-inflammatory drugs (NSAIDs), anticoagulants, antiepileptics, estrogens, and androgens, at least since they were on hemodialysis. Phosphate binders were mainly calcium salts and sevelamer. In the majority of the patients, regular erythropoietin intravenous treatment was underway. All the patients were treated with standard hemodialysis, 12 hours a week, divided in three sessions.

The patients were subjected to transiliac bone biopsy with a Bordier trocar, following a double-labeling course with tetracycline per mouth, with a 12-day interval period. The biopsy was performed 3 to 5 days after the end of tetracycline administration. At the same time, a blood sample was drawn for the following assays: calcium, phosphate, 25-hydroxycholecalciferol, intact PTH (iPTH), osteocalcin, total alkaline phosphatase, serum C-terminal cross-linked telopeptide of collagen type I (ICTP), and 1,25-dihydroxycholecalciferol.

Serum 25-hydroxycholecalciferol was measured with a radioimmunoassay provided by IDS (Boldon, UK). Following an extraction procedure with acetonitrile, the assay is carried out with anti-25-OHD ovine antibody, and phase separation is performed with anti-ovine IgG antiserum. The assay measures $25\text{-}OHD_3$ and $25\text{-}OHD_2$. Intra-assay and interassay variability are 5% and 8%, respectively. The normal range in our population is 10.0–60.0 ng/mL.

Serum PTH was measured by means of a commercial (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA) immunoradiometric assay, directed to the "intact" molecule, based on a double-antibody technique. Both 1-84 and "7-84" PTH species are measured together. Normal range of values is 10 to 65 pg/mL.

Serum osteocalcin, or BGP, was measured by an immunoradiometric assay (IRMA) (Nichols Institute Diagnostics). The intra- and interassay variation coefficients were less than 5.2% and 7.1%, respectively. The normal range is 6.8 to 32.2 ng/mL.

1,25-dihydroxycholecalciferol was measured (in a subset of 53 patients) with a radioimmunoassay provided by Nichols Institute Diagnostics. Normal range of values is 19.9 to 67 pg/mL.

ICTP was measured by a radioimmunoassay from Orion Diagnostica (Oulu, Finland). Normal values in the general population are within 1.8 and 5 ng/mL serum.

Serum total calcium was determined by a spectrophotometric assay using cresolftaleine as substrate. Serum phosphate and alkaline phosphatase measurements were

Table 1. Histomorphometric variables

Bone volume (BV/TV)	Percent of whole trabecular bone volume occupied by calcified and uncalcified bone tissue
Osteoid volume (OV/BV)	Percent of bone volume consisting of osteoid
Osteoid thickness (O.Th.) μm	Thickness of osteoid seams
Osteoid surface (OS/BS)	Percent of trabecular surface covered by osteoid tissue
Osteoblast surface (Ob.S/BS)	Percent of trabecular surface covered by osteoid lined by active osteoblasts
Eroded Surface (ES/BS)	Percent of trabecular surface with Howship's lacunae
Osteoclast surface (Oc.S/BS)	Percent of trabecular surface covered by osteoclasts
Osteoclast number (Oc.N/BS)	Number of osteoclasts per mm^2 of trabecular surface
Single-labeled surface (sLS/BS)	The extent of tetracycline single-labeled surface in percentage of trabecular surface
Double-labeled surface (sLS/BS)	The extent of tetracycline double-labeled surface in percentage of trabecular surface
Mineralizing surface (MS/BS)	The extent of double-labeled plus half the extent of single-labeled surface in percentage of trabecular surface
Mineralizing surface (MS/OS)	The extent of double-labeled plus half the extent of single-labeled surface in percentage of osteoid surface
Mineral apposition rate (MAR) $\mu m/day$	The distance between the midpoints of two consecutive labels, divided by the time interval between the midpoints of the two labelling periods
Bone formation rate (BFR/BS) $\mu m^3/\mu m^2/day$	The volumetric amount of new mineralized bone per unit of trabecular bone surface per day
Adjusted apposition rate (Aj.AR) $\mu m/day$	The mineral apposition rate corrected for osteoid surface
Mineralization lag time (Mlt)	The mean time interval between deposition of osteoid matrix and its mineralization
Osteoid mineralization rate (OMR)	The reciprocal value of mineralization lag time

performed spectrophotometrically (DU-65 Beckman; Beckman Instruments, Fullerton, CA, USA), using molybdate and p-nitrophenyl-phosphate as respective substrates. The normal range for the adult population is 2.5 to 4.5 mg/dL and 60 to 170 U/L, respectively.

Bone specimens were fixed using 4% paraformaldehyde in 0.1 mol/L phosphate buffer (pH 7.2). They were then longitudinally halved, dehydrated in acetone, and embedded without decalcification, in glycol-metacrylate, as previously described [14]. Sections were cut with a Reichert-Jung Autocut microtome equipped with a tungsten carbide knife. Three to four micrometer thick sections of the specimens, together with positive controls, were stained by the aluminum histochemical staining technique [15]. Alternate sections, 1 to 2 μm thick, were stained with azure II-methylene blue for histomorphometric measurement of structural and static variables. Alternate sections, about 5 μm thick, were also prepared unstained to be examined under ultraviolet light for histodynamic evaluation of tetracycline fluorescent labels. Histomorphometric and histodynamic measurements were obtained using an interactive color video-based image analysis system (IAS 2000; Delta Sistemi, Rome, Italy) with a personalized software developed for bone histomorphometry. The measured variables [16] are reported in Table 1.

Bone volume/tissue volume (BV/TV) was evaluated at objective magnification of 2.5× and static and dynamic variables were evaluated at objective magnification of 10× [17]. Normal values (Table 2) for the histomorphometric [17] and histodynamic [14] parameters were obtained in our own histomorphometric laboratory.

Bone pathology was classified as predominant hyperparathyroidism, osteomalacia, mixed osteodystrophy, and adynamic bone disease. The classification was made on the basis of morphologic criteria, as already reported

[14, 18]. The designation of predominant hyperparathyroidism implied a general increase in bone turnover rate and predominant osteomalacia was characterized by a decrease in bone turnover rate associated with an increase of both osteoid surface and thickness. Mixed osteodystrophy included all the intermediate features between pure hyperparathyroidism and osteomalacia [19] and adynamic bone disease was characterized by reduced bone turnover associated with thin osteoid seams, bone cell paucity, and a decrease in tetracycline uptake.

Statistical evaluation was carried out with a statistical package (BMDP statistical software, Cork, Ireland) on a personal computer. Correlation analysis was made with the Spearman rank order correlation test and stepwise multiple regression analysis, while comparison of groups was evaluated by parametric [analysis of variance (ANOVA) and Tukey test] and nonparametric (Kruskal-Wallis and Mann-Whitney) tests. Probability < 0.05 was considered significant. Values are expressed as mean ± SD.

RESULTS

Clinical data and the results of laboratory and bone histologic, histomorphometric, and histodynamic evaluation of all patients, together with normal reference values, are reported in Table 2. The monthly distribution of blood sampling for 25-OHD assay and their values are reported in Figure 1. No circannual pattern of 25-OHD levels was found. The patients were divided in three groups, according to the serum levels of 25-OHD. The subdivision in groups was made following the indication of population studies [20, 21]. Therefore the patients were arbitrarily divided in a first group, with 25-OHD levels between 0 and 15 ng/mL, while patients with 25-OHD levels between 15 and 30 ng/mL and above 30 ng/mL were in a

Table 2. Clinical, biochemical, and bone parameters of all patients, with normal values

	All	Normal values	Normal range
Number	104	–	
Male/female	61/43	–	
Age *years*	53.4 ± 11.9	–	
Hemodialysis length *months*	96.8 ± 63.4	–	
Intact parathyroid hormone *pg/mL*	555.4 ± 524.2	15–55	
25-OHD *ng/mL*	32.0 ± 18.1	10–40	
BGP *ng/mL*	170.2 ± 151.2	1.4–12.2	
Alkaline phosphatase *U/L*	398.9 ± 432.2	70–170	
ICTP *ng/mL*	148.4 ± 143.5	1.8–5.00	
Serum calcium *mg/dL*	10.47 ± 1.1	8.4–9.5	
Serum phosphate *mg/day*	5.48 ± 1.60	3.5–5.5	
Bone volume/tissue volume (BV/TV) %	24.14 ± 7.03	19.01 ± 4.5	9.12–29.13
Osteoid volume/bone volume (OV/BV) %	9.56 ± 8.8	1.39 ± 1.08	0.03–3.25
Osteoid thickness (O.Th,) μm	16.22 ± 6.4	9.55 ± 3.32	5.19–18.68
Osteoid surface/bone surface (OS/BS) %	40.88 ± 21.1	9.58 ± 6.88	0.59–28.03
Osteoblast surface/bone surface (Ob.S/BS) %	17.36 ± 14.1	0.20 ± 0.49	0.00–3.01
Eroded surface/bone surface (ES/BS) %	10.05 ± 5.6	1.52 ± 1.28	0.00–4.97
Osteoclast number/bone surface (Oc.N/BS) *no./mm²*	1.18 ± 0.9	0.05 ± 0.05	0.00–0.27
Osteoclast surface/bone surface (Oc.S/BS) %	3.49 ± 2.3	0.18 ± 0.19	0.00–0.83
Single-labled surface/bone surface (sLS/BS) %	12.45 ± 10.0	7.16 ± 2.88	4.16–11.38
Double-labeled surface/bone surface (dLS/BS) %	18.59 ± 14.5	6.74 ± 4.26	3.74–14.67
Mineralizing surface/bone surface (MS/BS) %	24.82 ± 16.3	10.35 ± 4.91	6.40–18.11
Mineralizing surface/osteoid surface (MS/OS) %	61.57 ± 28.1	70.83 ± 19.10	62.23–99.15
Mineral apposition rate (MAR) μm/day	1.04 ± 0.5	0.64 ± 0.13	0.45–0.87
Bone formation rate/bone surface (BFR/BS) μm³/μm² *day*	0.319 ± 0.3	0.066 ± 0.037	0.029–0.136
Adjusted apposition rate (Aj.AR) μm/day	0.718 ± 0.5	0.441 ± 0.123	0.360–0.648
Mineralization lag time (Mlt) *day*	119.17 ± 439.4	33.80 ± 10.18	20–46
Osteoid mineralization (OMR) %/day	4.620 ± 2.9	3.23 ± 1.15	2.16–5.01

Abbreviations are: 25-OHD, 25-hydroxyvitamin D; BGP, osteocalcin; ICTP, serum C-terminal cross-linked telopeptide of collagen type 1.
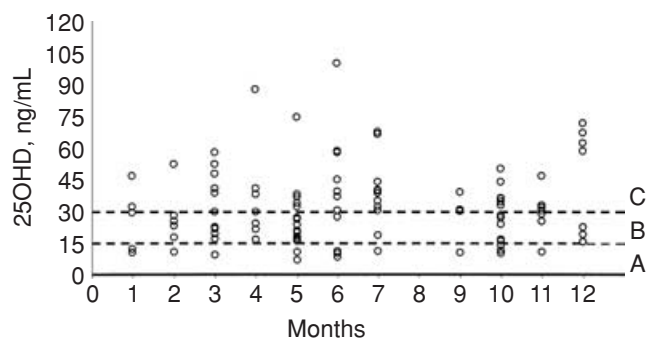

Fig. 1. Monthly distribution of blood sampling for 25-hydroxyvitamin D (25-OHD) assay. The lines set the limits of 25-OHD groups.

second group and third group, respectively. Means and standard deviations of the clinical, biochemical, and histologic, histomorphometric, and histodynamic parameters in the three 25-OHD groups are reported in Table 3, together with significance levels for parametric and non-parametric statistical tests.

There was no significant difference between the 25-OHD groups for what concerns average age, hemodialysis age, serum iPTH, serum calcium, phosphate, alkaline phosphatase, and ICTP. The values of serum 1,25(OH)$_2$D levels (Table 3), pertaining to approximately one half of the entire case series, did not

show significant difference between the three 25-OHD groups. Similarly to iPTH, the average value of calcitriol did not change with increasing 25-OHD serum levels. The scatter of calcitriol vs. 25-OHD values is represented in Figure 2.

The aluminum stain was negative in all patients. The histomorphometric and histodynamic parameters revealed some interesting and significant differences between the three groups. In all groups there were cases of hyperparathyoidism, mixed osteodystrophy, and low turnover osteodystrophy. The mean values for the parameters of group 1 were higher than normal for what concerns osteoid volume/bone volume (OV/BV), osteoid thickness (O.Th), osteoid surface/bone surface (OS/BS), osteoblast surface/bone surface(Ob.S/BS), eroded surface/bone surface (ES/BS), osteoclast surface/bone surface (Oc.S/BS), single-labeled surface/bone surface (sLS/BS), double-labeled surface/bone surface (dLS/BS), mineralizing surface/bone surface (MS/BS), and mineralization lag time (Mlt) (Tables 2 and 3). Bone formation rate/bone surface (BFR/BS) in group 1 was, as well, on average, elevated, with a mean value at the upper limit of normal (mean plus 2 SD). However, the group with low 25-OHD levels (group 1) showed significantly lower average values of dLS/BS, MS/BS, and BFR/BS compared to groups 2 and 3. In addition osteoblast surface/bone surface (Ob.S/BS) and adjusted apposition rate

Table 3. Clinical, biochemical, and bone parameters of the 25-hydroxyvitamin D (25-OHD) groups with significance of parametric and nonparametric tests

25-OHD ng/mL (range)	1 (0–15)	2 (16–30)	3 (>30)	ANOVA P value	Kruskal-Wallis test P value
Number	15	38	51	–	–
Male/female	5/10	23/15	33/18	–	–
Age *years*	57.27 ± 12.0	52.53 ± 11.5	52.92 ± 12.3	NS	NS
Hemodialysis length *months*	102.53 ± 76.6	95.16 ± 62.9	96.52 ± 60.9	NS	NS
Intact parathyroid hormone *pg/mL*	490.71 ± 494.8	670.0 ± 627.2	489.15 ± 436.7	NS	NS
25-OHD *ng/mL*	10.47 ± 1.59	22.03 ± 4.6	45.90 ± 15.7	–	–
$1,25(OH)_2D_3$ *pg/mL*	11.07 ± 5.8 (15)	7.86 ± 5.3 (25)	7.26 ± 4.3 (13)	NS	NS
BGP *ng/mL*	149.9 ± 181.0	212.48 ± 174.5	145.54 ± 115.9	NS	NS
Alkaline phosphatase *U/L*	348.2 ± 264.9	457.09 ± 532.8	350.47 ± 380.5	NS	NS
ICTP *ng/mL*	108.6 ± 59.18	180.2 ± 186.9	137.06 ± 122.0	NS	NS
Serum calcium *mg/dL*	9.83 ± 1.26	9.97 ± 1.04	10.00 ± 1.1	NS	NS
Serum phosphate *mg/dL*	5.85 ± 1.28	5.7 ± 1.73	5.21 ± 1.5	NS	NS
Bone volume/tissue volume (BV/TV) %	21.27 ± 8.00	24.56 ± 6.9	24.56 ± 6.8	NS	NS
Osteoid volume/bone volume (OV/BV) %	9.23 ± 13.3	10.41 ± 8.7	9.03 ± 7.3	NS	NS
Osteoid thickness (O.Th,) μm	17.60 ± 19.59	17.75 ± 6.8	16.11 ± 6.3	NS	NS
Osteoid surface/bone surface (OS/BS) %	38.39 ± 23.8	41.84 ± 21.2	40.87 ± 20.5	NS	NS
Osteoblast surface/bone surface (Ob.S/BS) %	11.18 ± 8.3	22.18 ± 16.4	15.57 ± 12.7	0.06	<0.10
Eroded surface/bone surface (ES/BS) %	8.43 ± 6.3	11.20 ± 4.9	9.65 ± 5.7	NS	NS
Osteoclast number/bone surface (Oc.N/BS) *no./mm²*	0.94 ± 0.8	1.41 ± 1.0	1.08 ± 0.8	NS	NS
Osteoclast surface/bone surface (Oc.S/BS) %	2.68 ± 2.1	3.90 ± 2.2	3.41 ± 2.3	NS	NS
Single-labled surface/bone surface (sLS/BS) %	12.14 ± 9.2	13.69 ± 13.1	11.66 ± 7.7	NS	NS
Double-labeled surface/bone surface (dLS/BS) %	9.097 ± 7.4	22.21 ± 13.6	18.67 ± 15.5	<0.03	<0.05[a,b]
Mineralizing surface/bone surface (MS/BS) %	15.17 ± 8.6	29.06 ± 15.2	24.51 ± 17.6	<0.03	<0.08[a,b]
Mineralizing surface/osteoid surface (MS/OS) %	42.44 ± 22.4	69.68 ± 26.6	61.17 ± 28.4	NS	NS
Mineral apposition rate (MAR) μm/day	0.766 ± 0.40	1.09 ± 0.6	1.08 ± 0.4	NS	NS
Bone formation rate/bone surface (BFR/BS) μm³/μm² *day*	0.143 ± 0.11	0.373 ± 0.34	0.32 ± 0.4	<0.02[a,b]	<0.09
Adjusted apposition rate (Aj.AR) μm/day	0.392 ± 0.28	0.823 ± 0.53	0.73 ± 0.5	<0.10	NS
Mineralization lag time (Mlt) *day*	162.31 ± 398.03	93.73 ± 253.93	126.02 ± 546.2	NS	NS
Osteoid mineralization (OMR) %/day	3.223 ± 2.41	4.990 ± 3.24	1.37 ± 2.6	NS	NS
Hyperparathyroidism/mixed osteodystrophy/low turnover osteodystrophy (adynamic bone disease)	7/4/4 (2)	27/8/3 (2)	26/17/7 (3)	–	–

Abbreviations are: ANOVA, analysis of variance; BGP, osteocalcin; ICTP, serum C-terminal cross-linked telopeptide of collagen type 1.
[a]Significance of difference group 1 vs. group 2; [b]Significance of difference group 1 vs. group 3.
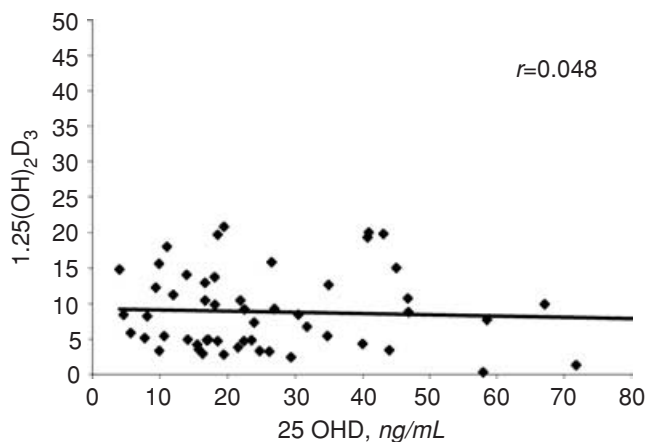
Fig. 2. Serum calcitriol versus 25-hydroxyvitamin D (25-OHD) in 53 patients.

(Aj.AR) were borderline significantly lower in group 1 compared to the other groups. No statistical difference between groups 2 and 3 for the biochemical and histologic parameters was observed.

A significant positive correlation was found in the combined patients of groups 1 and 2 versus serum 25-OHD for the following parameters: dLS/BS, MS/BS, and BFR/BS while no significant correlation was found between these parameters and 25-OHD in the patients of group 3. The scatters of data, together with graphic representation of regression lines, are reported in Figure 3 for the parameter BFR/BS. The results of a stepwise multiple regression analysis in the patients with 25-OHD levels 0 to 30 and in those with levels >30 are reported in Table 4. Serum 25-OHD was a significant independent variable. Correlations of serum 25-OHD levels vs histodynamic parameters were significantly positive for the interval 0 to 30 ng/mL [with the exception of mineralizing surface/osteoid surface (MS/OS)] and significantly negative in the group with 25-OHD levels >30 ng/mL. Age, hemodialysis age, and gender were variables excluded by the model.

From the scatter of data (Fig. 3), it was evident that values of the histodynamic parameter at 25-OHD levels in the order of 30 ng/mL were on average at the same level of those with higher 25-OHD serum values. In order to better evaluate the threshold of vitamin D sufficiency, the
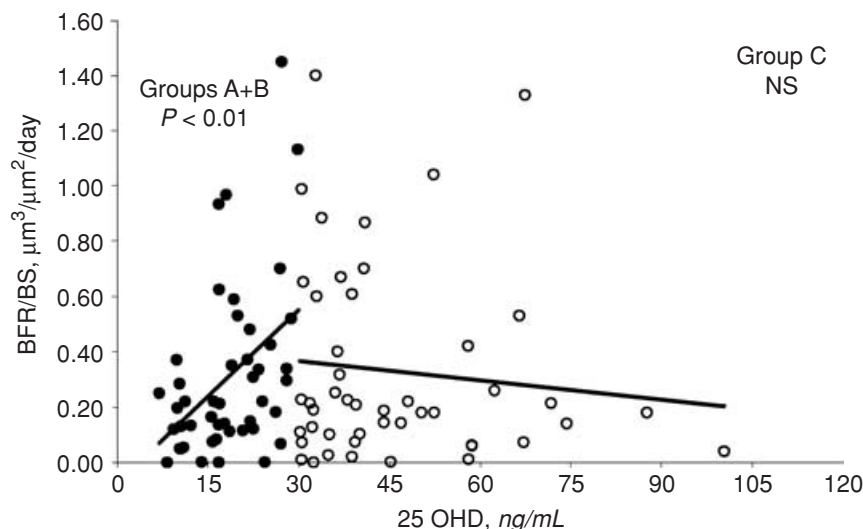
Fig. 3. Bone formation rate/bone surface (BFR/BS) versus 25-hydroxyvitamin D (25-OHD) serum levels in groups 1 + 2 and group 3. Regression lines are also reported.

Table 4. Multiple regression analysis, step-wise

	Independent variables			
	PTH		25-OHD	
25-OHD, 0 to 30 ng/mL	β	*P*	β	*P*
Dependent variables				
Bone formation rate/bone surface (BFR/BS)	0.000267	<0.001	0.0143	<0.025
Double-labeled surface/bone surface (dLS/BS)	0.0097	<0.003	0.842	<0.002
Adjusted apposition rate (Aj.AR)	0.00051	<0.0001	0.0215	<0.03
Mineralizing surface/bone surface (MS/BS)	0.0117	<0.002	0.782	<0.012
Mineralizing surface/osteoid surface (MS/OS)	0.0268	<0.0001	–	–
25-OHD, >30 ng/mL				
Bone formation rate/bone surface (BFR/BS)	0.00059	<0.0001	−0.0049	<0.045
Double-labeled surface/bone surface (dLS/BS)	0.0251	<0.0001	−0.232	<0.035
Adjusted apposition rate (Aj.AR)	0.00083	<0.0001	−0.0081	<0.04
Mineralizing surface/bone surface (MS/BS)	0.029	<0.0001	−0.295	<0.02
Mineralizing surface/osteoid surface (MS/OS)	0.0315	<0.001	−0.502	<0.035

patients were divided in narrower 25-OHD intervals, as shown in Figure 4. The histodynamic parameter BFR/BS was on average lower, with reduced bone turnover, up to the 25-OHD level of 20 ng/mL. There was a downslope of this histodynamic parameter at values of 25-OHD higher than 40 ng/mL.

In spite of the evidence of a relatively lower bone turnover due to vitamin D deficiency in the 0 to 20 ng/mL 25-OHD group, there was no increase in the parameters osteoid thickness and osteoid volume/bone volume. This unexpected finding could be due to the wide range of PTH serum levels of the patients. The relationship between serum PTH and the parameter osteoid thickness was investigated as shown in Figure 5. A positive significant correlation was found between the two parameters in the patients with 25-OHD values 0 to 20 ng mL, while the increase of this parameter was not significant in the patients with 25-OHD levels higher than 30 ng/mL.

DISCUSSION

The data presented in this study are in favor of a direct role of 25-OHD on bone metabolism, apparently not mediated through calcitriol, in patients with chronic renal failure on hemodialysis, affected by renal osteodystrophy, with a wide range of severity in terms of bone turnover. The patients with levels of 25-OHD currently defined as vitamin D deficiency, show on average PTH serum levels higher than the normal range, nonetheless not higher than the levels observed in the other 25-OHD groups. Several factors are at play in promoting hyperparathyroidism in chronic renal failure, probably masking the effect of vitamin D deficiency. In addition, the levels of circulating calcitriol do not seem to be influenced by the different levels of 25-OHD, at least in the range of serum values of this metabolite spontaneouly observed in our cohort, as already reported by Ishimura et al [22]. An increase in calcitriol serum levels has been reported only following administration of pharmacologic doses of 25-OHD
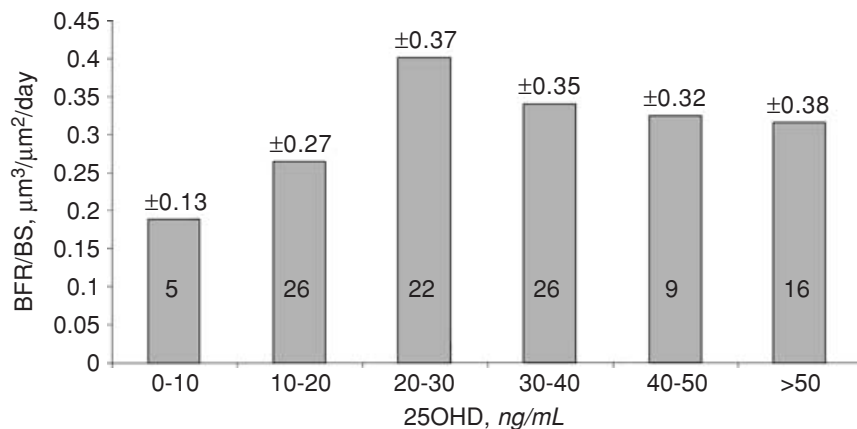
Fig. 4. Bone formation rate/bone surface (BFR/BS) in the patients with increasing 25-hydroxyvitamin D (25-OHD) levels. Number of patients in each group are recorded.
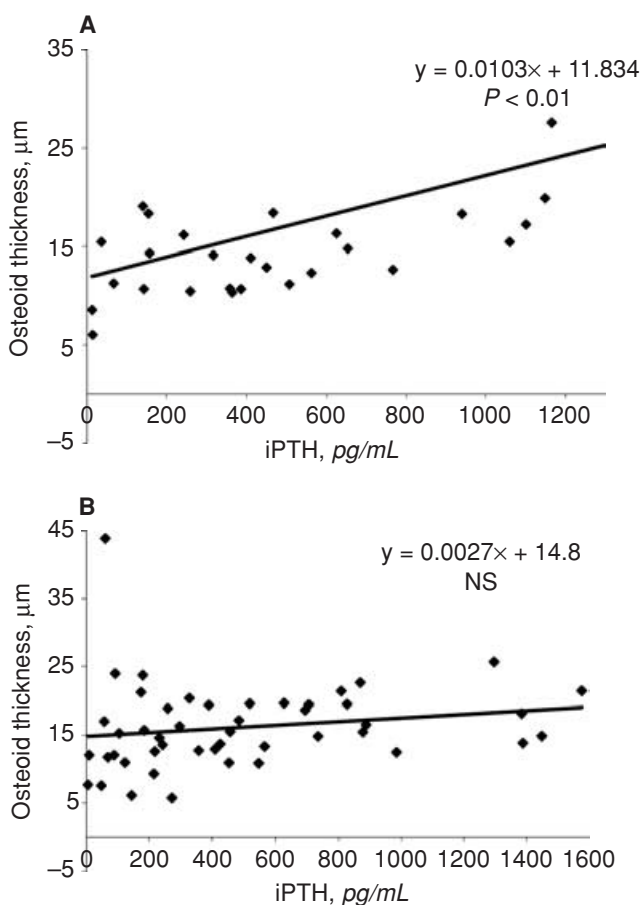

Fig. 5. Osteoid thickness versus intact parathyroid hormone (iPTH) serum levels in the patients with serum 25-OHD values 0–20 (*A*) and >30 ng/mL (*B*).

[23]. Therefore, our results favor the conclusion that, independently of serum PTH and calcitriol serum levels, 25-OHD holds a direct and autonomous influence on the process of bone formation and mineralization. Very low levels of 25-OHD in chronic renal failure seem to be compatible with increased bone turnover, presumably mainly PTH-dependent, with an increase of several parameters which are an expression of bone formation and resorption. However, serum values of 25-OHD lower than 20 ng/mL appear to moderate the extent of bone formation rate and the velocity of osteoid synthesis and mineralization, which would be allowed by higher levels of the vitamin D metabolite. As shown by other reports [9], one would expect, with low levels of 25-OHD, an increase in the evidence of osteomalacia, histologically characterized by an increase of the parameters osteoid volume/bone volume and osteoid thickness. However, our finding is the average result of patients of the experimental groups with a wide range of values of iPTH serum levels. With correlation analysis, a progressive increase in the parameter osteoid thickness was observed with increasing PTH serum levels in the low 25-OHD group, while this positive correlation was not observed at 25-OHD levels >30 ng/mL. This finding indicates that the increase in histologic evidence of a mineralization defect can be better detected with increased rate of osteoid synthesis induced by elevated PTH serum levels, ending up in the increase of the parameter osteoid thickness. The study of Ghazali et al [9] confirms that the finding of osteomalacia is found in those cases of low serum 25-OHD levels when combined with elevated PTH levels, while decreased 25-OHD associated to relatively low PTH serum levels was not associated to radiologic evidence of osteomalacia.

Despite the relatively reduced bone formation rate, deficiency of 25-OHD could not be recognized as a cause of adynamic bone disease, since cases of adynamic bone disease can be found in all three groups of 25-OHD levels.

Despite the absence of a significant difference in average histodynamic values between groups 2 and 3, some more information derives from the multiple regression analysis. Due to the relatively small number of cases in group 1, they were combined to cases of group 2. 25-OHD showed an independent significant positive correlation with several histomorphometric and histodynamic parmeters in the range 0 to 30 ng/mL. A negative

correlation was found for the same parameters as dependent variables in the patients of group 3. This finding is in favor of a dependence from 25-OHD of bone turnover in the 25-OHD serum level range, including vitamin D deficiency and insufficiency (0 to 15 and 15 to 30 ng/mL, respectively). However, from further subdivision of the patients, a real defect in 25-OHD can be identified in cases with a 25-OHD levels below 20 ng/mL. In addition, an optimal 25-OHD serum level can be identified at around 30 ng/mL, since with higher levels of the vitamin D metabolite the total vitamin effect seems to be excessive, since these levels were accompanied by a decrease in the parameters of bone turnover.

A direct effect of 25-OHD on bone cells should be envisaged as an action mediated by the vitamin D receptor, where calcitriol normally acts with an elevated affinity. Relatively recent studies in vitro, using transcriptional activation techniques, have shown that 25-OHD is only 500 times less active than calcitriol [22]. Therefore, when calcitriol serum levels are in the low range, concentration of serum 25-OHD >30 ng/mL should be sufficiently high to act on the vitamin D receptors in the place of calcitriol, at least for what concerns osteoblastic stimulation and maturation. Therefore, It is likely that in chronic renal failure with low levels of calcitriol, 25-OHD contributes a significant part of the total vitamin D effect. However, an extrarenal peripheral production of calcitriol not detected by increased serum levels cannot be entirely ruled out [25]. As for the decrease of bone histomorphometric parameters for 25-OHD levels above 40 ng/mL, a direct vitamin D effect on bone of 25-OHD or through an increase in the synthesis of $24,25(OH)_2D_3$ levels [26, 27] can be hypothesized.

On a practical ground, in chronic renal failure, with special regard to hemodialysis and peritoneal dialysis patients, evaluation of 25-OHD serum levels is necessary, and in case of values lower than 30 ng/mL, vitamin D should be administered as cholecalciferol or as 25-OHD [28], at doses which should avoid excessive increases of 25-OHD serum levels.

This study shows some limitations due to the fact of being a cross-sectional retrospective study. A prospective study with administration of vitamin D, correction of serum levels of 25-OHD and reassessment of bone histology would have been a more comprehensive protocol. Nevertheless, this is the only study with a relatively large histology based data relating 25-OHD levels to bone histomorphometry and histodynamic evaluation in hemodialysis chronic renal failure.

CONCLUSION

25-OHD levels below 20 ng/mL are accompanied by relatively lower bone turnover. The optimal level of 25-OHD appears to be 20 to 40 ng/mL, while levels of the vitamin D metabolite higher than 40 ng/mL are probably associated to excessive vitamin D effect. In addition the current findings support the recent recommendations by National Kidney Foundation Dialysis Outcomes Quality Initiative (KDOQI) [28] on the assessment of 25-OHD serum levels in patients with chronic kidney disease.

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