Original Article

25-Hydroxyvitamin D3, arterial calcifications and cardiovascular risk markers in haemodialysis patients

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Abstract

Background. Decreased vitamin D serum levels have been recently related to arterial stiffening and vascular calcifications in haemodialysis (HD) patients, but the pathophysiology of this association is not yet clear. The aim of this study was to evaluate the relationship between vascular calcifications, cardiovascular risk factors [including brain natriuretic peptide (BNP), pulse pressure (PP) and left ventricular mass index] and 25-hydroxyvitamin D3 (25(OH)D3) and 1,25-dihydroxyvitamin D3 [1,25(OH)2D3] serum levels.

Methods. We performed a cross-sectional study with 223 prevalent HD patients, 48% females, 27% diabetics, with the mean age of 62.7 ± 15.3 years and the mean HD time of 42.9 ± 39.3 months. Forty-seven percent of the patients were taking active forms of vitamin D.

Results. Serum levels of [25(OH)D3] were low (21.6 ± 12.2 ng/mL) and negatively correlated with age (r = −0.31, P < 0.001), diabetes mellitus (DM) (r = −0.20, P = 0.004), C-reactive protein (r = −0.25, P < 0.001), log10 BNP (r = −0.22, P = 0.002), PP > 65 mmHg (r = −0.21, P = 0.003) and vascular calcifications (r = −0.26, P < 0.001). Levels of [25(OH)D3] were positively correlated with [1,25(OH)2D3] (r = 0.25, P < 0.001) and albumin (r = 0.23, P = 0.001). On multivariate analysis, levels of [25(OH)D3] were independently associated with DM (P < 0.001), lower albumin levels (P = 0.003), higher BNP values (P = 0.005), PP > 65 mmHg (P = 0.006) and a higher vascular calcification score (≥ 3) (P = 0.002).

Conclusions. These results suggest that lower levels of [25(OH)D3] are a cardiovascular risk marker in HD patients, since they are strongly associated with higher BNP levels, increased PP and with the presence of vascular calcifications. The exact role of [25(OH)D3] deficiency on cardiovascular morbi-mortality needs to be clarified in large randomized controlled trials.

Keywords: haemodialysis; mortality; vascular calcifications; vitamin D

Introduction

Cardiovascular disease is the most common cause of death in dialysis patients [1]. Increasing evidence shows that abnormalities in mineral metabolism may play an important role in cardiovascular disease in patients with chronic kidney disease (CKD), as hyperphosphataemia, hypercalcaemia, high calcium–phosphorus product and secondary hyperparathyroidism have all been associated with increased mortality in dialysis patients [2,3].

Advanced CKD leads to divalent cation and metabolic derangements as well as decreased production of 1,25-dihydroxyvitamin D3 [1,25(OH)2D3] (calcitriol) all of which can cause parathyroid gland hyperplasia and development of bone disease [4,5]. One of the major actions of vitamin D is to maintain calcium and phosphate serum concentrations in the normal range and to allow for mineralization of newly synthesized bone [4]. Its main sites of action are the small intestine, bone and kidney [4]. Vitamin D has also been recognized to have numerous non-calcaemic functions, probably associated with the wide distribution of the vitamin D-receptor (VDR), namely in brain, heart, skeletal muscle, smooth muscle cells, pancreas, activated T and B lymphocytes and monocytes [5]. CKD also interferes with the interaction of the VDR with DNA, the nuclear uptake of the calcitriol-receptor complex and the synthesis and expression of the receptor [5].

Patients with renal failure frequently have low serum 25-hydroxyvitamin D [25(OH)D3] (the substrate of [1,25(OH)2D3]) [6–8]. There are several reasons for this [25(OH)D3] deficiency or insufficiency in these patients: they are inactive and have decreased exposure to sunlight, have reduced ingestion of foods that are natural sources of vitamin D, and the endogenous synthesis of vitamin D in the skin is also compromised in uraemic patients [6,7].

Studies on the general population have shown that [25(OH)D3] deficiency is associated with chronic heart
failure and hypertension [9–11]. Reduced vitamin D levels have also been recently associated with arterial stiffening in dialysis patients [12].

Vascular calcifications are highly prevalent in dialysis patients and have been associated with an increased risk of total mortality and cardiovascular mortality [13]. Recent studies have demonstrated that vascular calcification is an active cellular process, similar to bone formation [14–16]. Vascular smooth muscle cells (VSMCs) can differentiate into osteoblasts due to different stimuli, like hyperphosphatemia and hypercalcaemia [17]. Reduction of calcification inhibitors, such as fetuin-A or matrix-Gla protein, may be another factor associated with the development of calcification [18]. The presence of VDR in VSMCs has been recently described and may explain a possible mechanism of the action of vitamin D in vascular calcifications [5].

Some mechanisms linking vascular calcifications with cardiovascular risk, such as the association between vascular calcifications and arterial stiffness, have also been recognized [19]. The loss of arterial distensibility is associated with increased pulse pressure (PP) [20], left ventricular hypertrophy (LVH), decrease in coronary perfusion during diastole and cardiac failure [21].

The aim of this study was to evaluate the relationship between vitamin D status (accessed by [25(OH)D3] and [1,25(OH)2D3] serum levels), cardiovascular risk factors [including brain natriuretic peptide (BNP) plasma levels, PP and left ventricular mass index (LVMi)] and the presence of vascular calcifications.

**Subjects and methods**

**Study design**

This was an observational, cross-sectional, single-centre study of a cohort of prevalent haemodialysis (HD) patients.

**Population**

The study included 223 patients, 116 (52%) males and 107 (48%) females, with mean age of 62.7 ± 15.3 years. All the patients were dialyzed with high-flux membranes (helixone-Fresenius)® and ultrapure water (evaluated monthly by a cinetic chromogenic test). The mean HD time was 42.9 ± 39.3 months.

Sixty patients (27%) were diabetics and 71 (32%) hypertensives. Coronary artery disease was diagnosed if the patient had a typical history of angina pectoris or had suffered a myocardial infarction, had a positive stress test or had undergone a percutaneous coronary intervention or coronary bypass surgery. According to these criteria, coronary artery disease was diagnosed in 65 (29%) patients.

One hundred and four (47%) patients were taking active forms of vitamin D: 14% (n = 15) oral calcitriol, with a mean dose of 1.1 ± 0.5 (0.25–1.75) µg/week, and 86% (n = 89) iv paricalcitol, with a mean dose of 7.1 ± 4.3 (2.5–30) µg/week. None of the patients were receiving any other form of vitamin D, including ‘native’ or supplementation. One hundred and seventy-four patients (78%) were under therapy with phosphate binders: 14 (8%) were taking calcium carbonate with a mean dose of 1.7 ± 0.9 (1–4) g/day and 160 (92%) were taking sevelamer with a mean dose of 3.9 ± 1.9 (0.8–7.2) g/day.

PP was evaluated in the two mid-week HD sessions in which blood chemistry analysis was obtained, based on blood pressure (BP) measurement before HD. PP was calculated by the formula PP = SBP − DBP (SBP, systolic blood pressure; DBP, diastolic blood pressure).

**Biochemical analysis**

Serum [25(OH)D3] and [1,25(OH)2D3] were measured on two occasions (after summer, in November of 2006 and after winter, in June of 2007). Serum [25(OH)D3] and [1,25(OH)2D3] were measured with a radioimmunoassay provided by IDS (Boldon, UK). Following an extraction procedure, the assay is carried out with the anti-25-hydroxyvitamin D or the anti-1,25-dihydroxyvitamin D ovine antibody and phase separation is performed with anti-ovine IgG antiserum. The assay measures [25(OH)D3] and 25-hydroxyvitamin D2 or [1,25(OH)2D3] and 1,25-dihydroxyvitamin D2. Intra-assay and interassay variability are 5% and 8%, respectively. The normal range for [25(OH)D3] is 10–60 ng/mL and for [1,25(OH)2D3] is 20–46 pg/mL.

Definitions of deficiency (<15 ng/mL), insufficiency (<30 ng/mL) and normal values (>30 ng/mL) for [25(OH)D3] were based on K-DOQI guidelines for predialysis [22], whereas the definitions used for [1,25(OH)2D3] were based on biochemical normality of the assay (deficiency <20 pg/mL and normal values >20 pg/mL).

Serum calcium (Ca), serum phosphorus (P), Ca × P product, total intact parathyroid hormone (iPTH), bone alkaline phosphatase (bAP), haemoglobin, albumin and C-reactive protein (CRP) were measured simultaneously with [25(OH)D3] and [1,25(OH)2D3]. Total iPTH was evaluated by immunochemiluminescence using a second-generation assay, and the normal range of values is 10–65 pg/mL.

The levels of BNP were determined in June of 2007, in pre-HD-collected EDTA plasma samples, using the AxSYM BNP Assay (MEIA) on the AxSYM 2 Immunochemical Analyser (Abbott Laboratories, Chicago IL, USA). In a previous study [23], we found no significant difference in BNP-measured levels before or after dialysis.

**Ecocardiographic evaluation**

Between November of 2006 and June of 2007, each patient underwent an ecocardiographic examination (M mode and 2D), and LVMi was calculated using the Devereux formula [24] and indexed to body surface area. The presence of LVH was defined on the basis of a LVMi >125 g/m² for both men and women [25].

**Vascular calcification score**

To evaluate vascular calcifications, we used a simple vascular calcification score (SVCS) developed by Adragão et al. [21]. This vascular calcification score is based on the analysis of plain radiographic films of pelvis and hand.
Pelvis films were divided into four sections by two imaginary lines: a horizontal line over the upper limit of both femoral heads and a median vertical line over the vertebral column. Hand films were divided for each hand by a horizontal line over the upper limit of the metacarpal bones. Pelvis films evaluated iliac and femoral arteries (ilio-femoral score) and hand films evaluated radial and digital arteries (hand score). Any vascular calcification lining the vessel walls either in an irregular pattern or in a linear pattern was considered. The presence of vascular calcifications in each section was rated as 1 and its absence as 0. The final score was the sum of all sections and ranged from 0 to 8.

Adragão et al. [21] found that an SVCS > 3 was associated with an increase in cardiovascular events and mortality.

Statistical analysis

For statistical analysis, the arithmetic media of the two measurements (November 2006 and June 2007) was used. Variables were expressed as frequencies for categorical variables, mean values with SD for normally distributed variables. Comparison between groups was performed using Mann–Whitney U and chi-square tests. Spearman correlation was used for univariate analysis and linear regression for multivariate analysis (confidence interval of 95%). Variables entered in multivariate analysis were diabetes, albumin, BNP, PP and vascular calcification score.

Statistical analysis was performed with the SPSS system 14.0 (SPSS Inc., Chicago, IL, USA). For all comparisons, a P < 0.05 was considered statistically significant. For multiple correlations, Bonferroni adjustment was performed.

Results

The demographic, clinical, biochemical and vascular characteristics are reported in Table 1. Both [25(OH)D3] and [1,25(OH)2D3] serum levels were low and positively correlated (r = 0.25, P < 0.001). Serum [25(OH)D3] was below sufficiency values (<30 ng/mL) in almost 80% of our patients (Figure 1), and levels of [1,25(OH)2D3] were considered insufficient (<20 pg/mL) in more than 95% of the patients (Figure 2). Comparing serum levels obtained in June of 2007 with those of November of 2006, [25(OH)D3] serum values were higher in November (following summer), but not statistically significant (22.6 ± 16.0 versus 20.8 ± 12.3; P > 0.05). [1,25(OH)2D3] serum levels were similar on the two occasions (6.4 ± 7.6 versus 6.4 ± 7.7; P > 0.05). Patients taking paricalcitol showed significantly lower [1,25(OH)2D3] levels (4.7 ± 4.6; P < 0.001), but with the same [25(OH)D3] values. Patients under calcitriol showed similar levels compared with the studied population. A comparison of studied variables according to levels of [25(OH)D3] is shown in Table 2 and according to [1,25(OH)2D3] levels is shown in Table 3.

Plasma BNP values were high (574 ± 696 pg/mL), comparing with the general population with no cardiac insufficiency [26]. PP was elevated (68.8 ± 18.3 mmHg), and 127 (57%) patients had ecocardiographic criteria of left ventricular hypertrophy (LVMI >125 g/m²). Vascular

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients (n = 223)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>62.7 ± 15.3</td>
</tr>
<tr>
<td>Male gender</td>
<td>116 (52%)</td>
</tr>
<tr>
<td>HD duration (months)</td>
<td>429 ± 39.3</td>
</tr>
<tr>
<td>Diabetes</td>
<td>60 (27%)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>71 (32%)</td>
</tr>
<tr>
<td>Coronary disease</td>
<td>65 (29%)</td>
</tr>
<tr>
<td>Active vitamin D therapy</td>
<td>104 (47%)</td>
</tr>
<tr>
<td>Calcium carbonate therapy</td>
<td>14 (6%)</td>
</tr>
<tr>
<td>Sevelamer therapy</td>
<td>160 (72%)</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>8.5 ± 0.6 (6.9–10.5)</td>
</tr>
<tr>
<td>Phosphorus (mg/dL)</td>
<td>4.6 ± 1.5 (1.7–8.7)</td>
</tr>
<tr>
<td>Calcium×phosphorus (mg/dL)²</td>
<td>48.6 ± 15.5 (16.5–83.2)</td>
</tr>
<tr>
<td>IPTh (pg/mL)</td>
<td>277 ± 3332 (3–4461)</td>
</tr>
<tr>
<td>bAP (µg/L)</td>
<td>20.3 ± 14.3 (2.2–119.5)</td>
</tr>
<tr>
<td>Haemoglobin (g/dL)</td>
<td>12.3 ± 1.3 (8.2–15.4)</td>
</tr>
<tr>
<td>C-reactive protein (mg/dL)</td>
<td>0.9 ± 1.6 (0.1–17.4)</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>4.1 ± 0.4 (3.1–5.1)</td>
</tr>
<tr>
<td>[25(OH)D3] (ng/mL)</td>
<td>21.6 ± 12.2 (5.6–152.8)</td>
</tr>
<tr>
<td>[1,25(OH)2D3] (pg/mL)</td>
<td>5.9 ± 5.7 (0.1–49.1)</td>
</tr>
<tr>
<td>BNP (pg/mL)</td>
<td>596 ± 689 (11–3851)</td>
</tr>
<tr>
<td>PP (mmHg)</td>
<td>69 ± 18 (33–116)</td>
</tr>
<tr>
<td>LVMI, left ventricular mass index; SVCS, simple vascular calcification score.</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Serum 25-hydroxyvitamin D3 distribution.

Mean value: 21.6 ± 12.2 ng/mL.

Fig. 2. Serum 1,25-dihydroxyvitamin D3 distribution.

Mean value: 5.9 ± 5.7 pg/mL.
Table 2. Comparison of studied variables according to 25-hydroxyvitamin D3 serum levels

<table>
<thead>
<tr>
<th>Variable</th>
<th>&lt;30 ng/mL (n = 177)</th>
<th>&gt;30 ng/mL (n = 46)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>63.8 ± 15.4</td>
<td>58.5 ± 14.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male gender</td>
<td>85 (48%)</td>
<td>24 (52%)</td>
<td>NS</td>
</tr>
<tr>
<td>HD duration (months)</td>
<td>43.9 ± 41.3</td>
<td>39.2 ± 30.6</td>
<td>0.03</td>
</tr>
<tr>
<td>Diabetes</td>
<td>65 (37%)</td>
<td>10 (21%)</td>
<td>0.01</td>
</tr>
<tr>
<td>Hypertension</td>
<td>60 (34%)</td>
<td>14 (31%)</td>
<td>NS</td>
</tr>
<tr>
<td>Coronary disease</td>
<td>57 (32%)</td>
<td>13 (28%)</td>
<td>NS</td>
</tr>
<tr>
<td>Active vitamin D therapy</td>
<td>73 (41%)</td>
<td>24 (52%)</td>
<td>NS</td>
</tr>
<tr>
<td>Calcium carbonate therapy (g/day)</td>
<td>1.5 ± 0.6</td>
<td>1.7 ± 0.8</td>
<td>NS</td>
</tr>
<tr>
<td>Sevelamer therapy (g/day)</td>
<td>3.6 ± 1.8</td>
<td>3.9 ± 1.7</td>
<td>NS</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>8.6 ± 0.6</td>
<td>8.7 ± 0.7</td>
<td>NS</td>
</tr>
<tr>
<td>Phosphorus (mg/dL)</td>
<td>4.5 ± 1.5</td>
<td>4.7 ± 1.6</td>
<td>NS</td>
</tr>
<tr>
<td>Calcium × phosphorus (mg/dL)²</td>
<td>49.3 ± 13.8</td>
<td>51.9 ± 15.1</td>
<td>NS</td>
</tr>
<tr>
<td>iPTH (pg/mL)</td>
<td>312 ± 284</td>
<td>337 ± 271</td>
<td>NS</td>
</tr>
<tr>
<td>bAP (µg/L)</td>
<td>20.3 ± 18.1</td>
<td>20.2 ± 12.3</td>
<td>NS</td>
</tr>
<tr>
<td>Haemoglobin (g/dL)</td>
<td>12.3 ± 1.3</td>
<td>12.6 ± 1.5</td>
<td>NS</td>
</tr>
<tr>
<td>C-reactive protein (mg/dL)</td>
<td>1.0 ± 1.8</td>
<td>0.5 ± 0.7</td>
<td>0.002</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>4.1 ± 0.4</td>
<td>4.4 ± 0.2</td>
<td>0.004</td>
</tr>
<tr>
<td>[1,25(OH)₂D₃] (pg/mL)</td>
<td>4.5 ± 5.2</td>
<td>6.0 ± 5.7</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Comparison between means: Mann–Whitney U-test; comparison between frequencies: chi-square test.

calculations were identified in 130 (58%) patients. An SVCS > 3 was observed in 80 (36%) patients. There was no difference in serum calcium, phosphorus, Ca × P product and iPTH between patients with or without vascular calcifications. CRP and albumin were negatively correlated (r = −0.27, P < 0.001).

Serum levels of [25(OH)D3] were negatively correlated with age (r = −0.31, P < 0.001), DM (r = −0.20, P = 0.004), CRP (r = −0.25, P < 0.001), log10 BNP (r = −0.22, P = 0.002), PP > 65 mmHg (r = −0.21, P = 0.003) and SVCS (r = −0.26, P < 0.001) (Figure 3).

[25(OH)D3] levels were positively correlated with albumin (r = 0.23, P = 0.001) (Table 4). LVMI was not correlated with [25(OH)D3] levels.

Serum levels of [1,25(OH)₂D₃] were only negatively correlated with DM (r = −0.16, P = 0.02) and did not show any correlation with BNP, PP, LVMI or vascular calcifications. Therapy with active vitamin D was negatively correlated with the presence of vascular calcifications (r = −0.21, P = 0.004).

On multivariate analysis, serum levels of [25(OH)D3] were independently associated with diabetes (P < 0.001), lower albumin levels (P = 0.003), higher log10 BNP values (P = 0.005), PP > 65 mmHg (P = 0.006) and a higher SVCS (≥3) (P = 0.002) (Table 5). Active vitamin D therapy was associated with a lower SVCS (<3) (P = 0.03).

Discussion

According to our results, from a large prevalent HD population from a ‘sunny country’, [25(OH)D3] insufficiency seems much more frequent than suspected and may also contribute to the [1,25(OH)₂D₃] deficiency (observed in >95% of the patients). Levels of [25(OH)D3] as expected were higher, but not significantly, after summer because of increased sun exposure. [1,25(OH)₂D₃] levels did not show any seasonal variation.

In our study, as already described in other studies, older patients and diabetics showed lower levels of [25(OH)D3]. Elderly patients have reduced sun exposure, due to poor health and immobility, and have reduced ingestion of foods that are natural sources of vitamin D, and dermal synthesis of vitamin D is reduced with increasing age [27]. Diabetes may predispose to an even greater risk of vitamin D deficiency because of bowel motility disturbances, fat mal-absorption and an association with coeliac disease [28]. In this study, lower levels of [1,25(OH)₂D₃] were also present in diabetics, probably reflecting lower production of the substrate.

Dialysis patients have increased cardiovascular morbidity and mortality [1]. BNP plasma levels have been shown to be a good cardiovascular risk marker in the general population and in patients with renal failure [29,30]. Plasma BNP is synthesized mainly in cardiomyocytes in response to ventricular stretch and pressure overload [31]. Elevated BNP concentrations are seen in patients with cardiac insufficiency and renal failure [30]. Increased BNP levels in these patients are thought to be the result of water retention and ventricular volume increase [29–31], but they may also be a result of concomitant coronary artery disease or left ventricular dysfunction [32]. Recent studies have indicated that the degree of BNP elevation in patients with chronic renal failure may predict left ventricular function and future cardiac events [28,30–32]. We have also previously
found an increase in cardiovascular morbidity and mortality in HD patients with higher BNP plasma levels [23]. In this study, as reported in other studies, mean levels of BNP were high (comparing with the general population), because of renal failure and in some patients simultaneous cardiac disease.

CKD patients have stiffer vessels compared to the general population [13], contributing to reduced arterial compliance. In CKD, there is a similar magnitude of atherosclerotic plaque burden and intimal thickness, but there is a markedly increased medial calcification [33]. This phenomenon is a major determinant of left ventricular pressure overload and of abnormal coronary perfusion [34]. Left ventricular hypertrophy (LVH) is found early in the course of renal failure and progresses with further impairment of renal function [35]. LVMI is an independent predictor of survival in patients with CKD [36]. Our patients presented increased PP and more than 50% showed ecocardiographic criteria of LVH, both strong predictors of increased cardiovascular risk.

CKD patients have increased predisposition to develop vascular calcifications [17–19]. Calcification of the vessel walls may occur in two sites: the intima and the media. Intimal calcification mainly derives from the inflammation and calcification of the atherosclerotic plaques and they are localized in two functionally relevant arteries, such as the aorta and the coronaries. Medial calcification or Monckeberg’s arteriolosclerosis occurs in the elastica lamina of large and medium–small size arteries. Both types of calcification are present in patients with CKD, but the complications of these two types of vascular calcification are different. The former is mainly associated with occlusion of the vessels and the latter is associated with vascular stiffness. Both have an adverse impact on cardiovascular mortality in CKD patients [37,38]. The presence of hyperphosphataemia, hypercalcaemia and increased Ca × P product have been considered major pathogenic factors leading to vascular and soft tissue calcification in uraemic patients [17–19]. In this study, we found no correlation between serum calcium, serum phosphorus, Ca × P product and iPTH and vascular calcifications, probably because mean values were low compared with other studies.

The role of vitamin D in vascular calcifications is still controversial. In experimental models, very high doses of some vitamin D metabolites have been associated with an increase in vascular calcifications [39,40]. In contrast, epidemiological observational studies have shown that the currently used doses of different vitamin D metabolites and analogues have been associated with an improvement in vascular function and survival [3,12,41–45]. In our study, patients taking vitamin D (mainly paricalcitol) showed less vascular calcifications, probably because the used doses were not high and the serum levels of calcium, phosphorus, Ca × P product and iPTH were well controlled.

On the other hand, recent studies have shown that serum [25(OH)D3] levels were negatively correlated with vascular calcifications [37] and both [25(OH)D3] and [1,25(OH)2D3] serum levels were negatively correlated with aortic pulse wave velocity and positively correlated with brachial artery distensibility [12], suggesting a protective role for vitamin D in vascular function.

In other studies, vitamin D has shown to suppress transcription of renin [46]. Vitamin D therapy decreased circulating renin and angiotensin II levels in vitamin D-deficient
animals [46], and lower circulating vitamin D levels correlated with increased BP and LVMI in humans [47,48]. Furthermore, the 1α-hydroxylase enzyme that converts [25(OH)D3] to [1,25(OH)2D3] is expressed in a variety of tissues, including human endothelial cells and VSMCs [4,5]. These data suggest a paracrine effect of [25(OH)D3] that is independent of circulating [1,25(OH)2D3] levels, which challenges the traditional notion that biological activity of vitamin D is primarily dependent on conversion in renal proximal tubule [4].

An observational study by Wolf et al. [49] have found that, in incident HD patients, deficient values of vitamin D were associated with early mortality and that treatment with active vitamin D could probably reverse this situation.

Our results demonstrate that high [25(OH)D3] serum levels may have a protective role in cardiovascular disease, since deficient [25(OH)D3] levels were associated with higher BNP plasma values, increased PP (>65 mmHg) and higher vascular calcification score (≥3). These results were in accordance with the study published by London et al. [12]. A similar correlation could not be demonstrated for [1,25(OH)2D3], which can be explained by the fact that the vast majority of our patients showed very low levels of [1,25(OH)2D3] and were treated mainly with a vitamin D receptor activator (paricalcitol), which does not interfere/is not measured by the [1,25(OH)2D3] assay. Patients treated with paricalcitol also showed lower [1,25(OH)2D3] levels, which could be explained by a possible negative feedback mechanism. This effect was not observed in the 14 patients treated with calcitriol, probably due to the low doses that were used.

Several studies have shown that vitamin D has numerous nonclassic actions, including a protective role in innate immunity [5,9,50]. London et al. [12] showed that [25(OH)D3] levels significantly correlated with markers of arteriolosclerosis and endothelial dysfunction in dialysis patients. Low vitamin D levels were also associated with decreased pulmonary function test results, increased PP, congestive heart failure and increased carotid intima–media thickness [51,52]. Recent findings indicated that low [25(OH)D3] levels were associated with an increased risk of cancer and autoimmune diseases such as type 1 diabetes and rheumatoid arthritis [9].

In our study, the negative correlation between [25(OH)D3] and CRP and the positive correlation with albumin may only illustrate [25(OH)D3] as another marker of inflammation or represent the already described immunomodulator and anti-inflammatory actions of this hormone.

In this study, we used an SVCS based on plain radiographic films of pelvis and hand. This SVCS evaluated in plain X-ray was previously demonstrated to be associated with coronary artery disease and peripheral artery disease and to be a predictor of cardiovascular mortality and cardiovascular hospitalizations in dialysis patients [21]. This vascular calcification score is an inexpensive and valuable tool that can be used for screening for the presence of vascular calcifications in dialysis patients. Computed tomography scans are more accurate for the quantitative assessment of vascular calcifications but may be inadequate for an initial screening of vascular calcifications because of their greater price and limited availability in some areas [53]. The K/DOQI guidelines also recommend the utilization of plain X-ray for identification of vascular calcifications in dialysis patients [22].

This study shows some limitations due to the fact of being an observational and cross-sectional study. Although it shows a relevant association between [25(OH)D3] insufficiency and cardiovascular risk factors, a large randomized prospective study is needed to clarify the role of [25(OH)D3] on the increased cardiovascular morbidity and mortality observed in uremic patients. Nevertheless it seems relevant that serum levels of [25(OH)D3] should be evaluated in patients with CKD and insufficiency/deficiency treated. Recent studies show that [25(OH)D3] levels should also be evaluated in high-risk individuals in the general population [54,55].

### Conclusions

Deficiency of [25(OH)D3] and [1,25(OH)2D3] is very prevalent in dialysis patients. Our results suggest that lower levels of [25(OH)D3] are a cardiovascular risk marker in HD patients, since they are independently associated with higher BNP plasma levels, increased PP and with the presence of a higher vascular calcification score. Active vitamin D therapy seems to protect patients from developing vascular calcifications.

Conflict of interest statement. None declared.

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