Cholecalciferol Supplementation in Hemodialysis Patients: Effects on Mineral Metabolism, Inflammation, and Cardiac Dimension Parameters

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Background and objectives: Vitamin D deficiency is highly prevalent in chronic kidney disease. The aim of this study was to evaluate the effects of oral cholecalciferol supplementation on mineral metabolism, inflammation, and cardiac dimension parameters in long-term hemodialysis (HD) patients.

Design, setting, participants, & measurements: This 1-year prospective study included 158 HD patients. Serum levels of 25-hydroxyvitamin D [25(OH)D], 1,25-dihydroxyvitamin D [1,25(OH)2D], intact parathyroid hormone, and plasma brain natriuretic peptide as well as circulating bone metabolism and inflammation parameters were measured before and after supplementation. Baseline 25(OH)D and 1,25(OH)2D levels were measured twice (end of winter and of summer, respectively). Therapy with paricalcitol, sevelamer, and darbepoietin was evaluated.

Results: There was an increase in serum 25(OH)D and 1,25(OH)2D levels after supplementation. Conversely, serum calcium, phosphorus, and intact parathyroid hormone were decreased. There was a reduction in the dosage and in the number of patients who were treated with paricalcitol and sevelamer. Darbepoietin use was also reduced, with no modification of hemoglobin values. Serum albumin increased and C-reactive protein decreased during the study. Brain natriuretic peptide levels and left ventricular mass index were significantly reduced at the end of the supplementation.

Conclusions: Oral cholecalciferol supplementation in HD patients seems to be an easy and cost-effective therapeutic measure. It allows reduction of vitamin D deficiency, better control of mineral metabolism with less use of active vitamin D, attenuation of inflammation, reduced dosing of erythropoiesis-stimulating agents, and possibly improvement of cardiac dysfunction.


Patients with chronic kidney disease (CKD) frequently have low serum 25-hydroxyvitamin D [25(OH)D] or calcidiol levels, which is the substrate of 1,25-dihydroxyvitamin D [1,25(OH)2D] or calcitriol (1–3). With CKD progression, this tendency to vitamin D substrate insufficiency, coupled with the demonstrated loss of the renal 1α-hydroxylase activity, leads to progressive calcitriol deficiency (4,5).

Levels of 25(OH)D have been shown to be the best indicator of vitamin D status (6). In some studies (7,8), 25(OH)D showed inverse correlations with age, female gender, diabetes, and intact parathyroid hormone (iPTH) level. Calcidiol concentration also depends on the season, with higher serum levels after summer time (8).

The importance of measuring 25(OH)D levels is also supported by the newly emerging concept that an extrarenal 1α-hydroxylase is expressed in many sites outside the kidney. The extrarenal pool of 1α-hydroxylase (unlike its renal pool) seems to remain intact in kidney disease. This locally produced 1,25(OH)2D seems to have “autocrine or paracrine” effects and to promote additional roles for vitamin D, beyond its classical functions in mineral metabolism. Indeed, studies have demonstrated that 1,25(OH)2D acts as a cell-differentiating factor and antiproliferative agent on a variety of tissues (9–11). By influencing gene expression in multiple tissues, including the immune system, skin, muscle, pancreas, kidney, and brain, it is believed that vitamin D is involved in the pathogenesis of psoriasis, certain types of cancer, multiple sclerosis, diabetes, and BP regulation (9,12). Deficient levels of 25(OH)D have also been associated with cardiovascular risk factors in patients with and without CKD (12–15).

The evaluation of calcidiol serum levels in patients with stage 5D CKD is suggested by the Kidney Disease: Improving Global Outcomes (KDIGO) recommendations, and the proposed strategy for correction of 25(OH)D deficiency/insufficiency is similar to that used in the general population (16). The aim of this study was to evaluate the effects of oral cholecalciferol supple-
mentation in mineral metabolism, inflammation markers, and cardiac dysfunction in patients with stage 5D CKD.

Materials and Methods

Study Design

This was a 1-year follow-up, prospective study of a cohort of prevalent hemodialysis (HD) patients from a single center (two dialysis units sharing the medical team). Serum 25(OH)D and 1,25(OH)₂D were measured on two occasions with a 6-month interval (end of winter and of summer, respectively) and at the end of the study, after 6 months of cholecalciferol supplementation.

Population

All patients were included in the study, except for those who were taking cinacalcet, oral calcitriol, or calcium (Ca) carbonate and those who had undergone parathyroidectomy. Patients who were lost to follow-up were also excluded from the analysis (25 patients died, 20 were transferred and six received a transplant).

The study included 158 patients: 74 (47%) men and 84 (53%) women with mean age of 62.8 ± 14.8 years. All patients underwent dialysis with high-flux membranes (helixone-Frenesius) and ultrapure water (evaluated monthly by cinetic chromogenic test). The dialysate Ca concentration was 1.5 mmol/L for all patients during the study. Mean HD vintage was 44.3 ± 32.4 months.

Thirty-nine (25%) patients had diabetes, and 54 (34%) had hypertension. Coronary artery disease was diagnosed when the patient had a typical history of angina pectoris or had sustained 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 49 (31%) patients.

At baseline, 70 (44%) patients were taking paricalcitol (the only active vitamin D used during the study), at a mean dosage of 7.2 ± 4.5 μg/wk (range 2.5 to 30.0 μg/wk). A total of 145 (92%) patients were on darbepoietin therapy, with a mean dosage of 0.042 μg/kg per wk per g/dl. The target for ferritin was between 200 and 800 ng/ml for all patients, and, when needed, only intravenous iron saccarate was used.

Systolic (SBP) and diastolic BP (DBP) were measured in all HD sessions in the month before the beginning of supplementation and in the last month of cholecalciferol supplementation. Pulse pressure (PP) was also calculated (on the basis of BP measurements before HD) by the formula PP = SBP – DBP. Antihypertensive medication, including angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin II receptor blockers (ARBs), and therapy with statins were evaluated at the beginning and at the end of the study.

Oral cholecalciferol (Vigantol) was prescribed once or thrice weekly, after each HD session, ensuring 100% adhesion. Supplementation was started for all patients at the same time and was maintained for 6 months. It was based on baseline calcidiol serum levels: 50,000 IU (2.5 ml, or 75 drops) once a week for patients with 25(OH)D levels between 16 and 30 ng/ml, and 2700 IU (0.5 ml, or 15 drops) three times per week when levels where >30 ng/ml.

Biochemical Analysis

Serum 25(OH)D and 1,25(OH)₂D levels were measured with a RIA provided by IDS (Boldon, UK). After an extraction procedure, the assay is carried out with anti-25(OH)D or anti-1,25(OH)₂D ovine antibody, and phase separation is performed with anti-ovine IgG antiserum. The assay measures 25(OH)D₂ and 25(OH)D₃ or 1,25(OH)₂D₂ and 1,25(OH)₂D₃. Intra-assay and interassay variability are 5 and 8%, respectively. The normal range for 25(OH)D is 10 to 60 ng/ml and for 1,25(OH)₂D is 20 to 46 pg/ml, as indicated by the manufacturer. The inferior detection limit is 1.6 ng/ml and 2.5 pg/ml, respectively.

Serum Ca, serum phosphorus (P), Ca-P product, total intact parathyroid hormone (iPTH), bone alkaline phosphatase, hemoglobin, ferritin, albumin, and C-reactive protein (CRP) were measured before the beginning of supplementation and at the end of the study. Ca was corrected for hypoalbuminemia by addition of 0.8 mg/dl to the Ca concentration for each 1-g/dl decrease in albumin concentration from 4.0 g/dl (17). Total iPTH was evaluated by immunochemiluminescence using a second-generation assay (Immulite 2000; Siemens Medical Solutions Diagnostics, Los Angeles, CA) and normal range of values is 10 to 65 pg/ml. Albumin was measured using a colorimetric assay and CRP was evaluated by an immunoturbidimetric assay.

The levels of brain natriuretic peptide (BNP) were determined before the beginning of supplementation and at the end of the study in EDTA plasma samples that were collected before HD, using the AxSYM BNP Assay (MEIA) on the AxSYM 2 Immunochemical Analyser (Abbott Laboratories, Chicago, IL). In a previous study (18), we found no significant difference on BNP measured levels before or after dialysis. All blood samples were collected before dialysis in midweek sessions.

Echocardiography Evaluation

At the beginning and at the end of the study, each patient underwent an echocardiography examination, and left ventricular mass index (LVM) was calculated using the Devereux formula (19) and indexed to body surface area. This examination was performed on a midweek nondialysis day and in the same cardiologic center.

Statistical Analysis

For statistical analysis, the arithmetic mean of the two measurements of 25(OH)D and 1,25(OH)₂D at baseline (end of winter and of summer, respectively) was used. Demographic and laboratory data were compared at baseline and after 6 months of cholecalciferol supplementation. Variables were expressed as frequencies for categorical variables, mean values with SD for normally distributed variables, and median (interquartile ranges) values for non-normally distributed variables. Comparison between two groups was performed using paired Wilcoxon test. Spearman correlation was used for univariate analysis. Statistical analysis was performed with SPSS 15.0 (SPSS Inc., Chicago, IL). P < 0.05 was considered statistically significant.

Results

At baseline, both 25(OH)D and 1,25(OH)₂D serum levels were low and positively correlated (r = 0.25, P < 0.001). Serum 25(OH)D was bellow sufficiency values (<30 ng/ml) in almost 80% of our patients, and levels of 1,25(OH)₂D were considered insufficient (<20 pg/ml) in >95% of patients. Serum levels of 25(OH)D were higher after summer compared with after winter but were not statistically significant (22.6 ± 16.0 versus 20.8 ± 12.3 ng/ml; P > 0.05). Calcitriol serum levels were similar on both occasions (4.7 versus 4.6 pg/ml; P > 0.05). Older patients showed lower levels of 25(OH)D (r = −0.31, P < 0.001). Patients with diabetes presented lower concentrations of 25(OH)D (24.2 ± 12.5 versus 18.5 ± 9.7 ng/ml; P < 0.001) and 1,25(OH)₂D (4.4 versus 4.9 pg/ml; P = 0.02) than patients without diabetes.

Postwinter and postsummer baseline measurements were compared with the values that were obtained after 6 months of oral cholecalciferol supplementation. There was a significant increase in 25(OH)D (22.3 ± 12.0 versus 42.0 ± 12.1 ng/ml; P <
0.001) and 1,25(OH)₂D (4.6 versus 5.9 pg/ml; P = 0.001) levels (Figure 1). In 136 (86%) patients, 25(OH)D levels became normal (>30 ng/ml) at the end of the study, in comparison with only 32 (20%) at baseline (Figure 2). Most of the patients whose 25(OH)D levels did not increase had diabetes (P < 0.001). None of the patients achieved a toxic level of 25(OH)D: >100 ng/ml (20).

Clinical and biochemical parameters at the beginning and at the end of supplementation are reported in Table 1. Serum Ca (P = 0.014) and P (P = 0.011) showed a significant reduction with supplementation. This was independent of paricalcitol therapy, because patients who were not administered active vitamin D during the studied period also presented this reduction. Levels of iPTH (P = 0.01) showed a significant decrease with supplementation. There were no episodes of hypercalcemia >10.5 mg/dl during the study. Only 11 (7%) patients of those who had iPTH levels between 150 and 300 pg/ml at the beginning of the study showed values <150 pg/ml after supplementation.

In association with the cholecalciferol supplementation, a significant reduction in the number of treated patients as well as in active vitamin D dosage was observed (P < 0.001). Patients who were taking paricalcitol during the study showed significantly lower 1,25(OH)₂D levels (3.9 versus 6.8 pg/ml; P < 0.001) despite a same increase in 25(OH)D values (Figure 3). There was also a significant decrease in patients who were treated with sevelamer after supplementation (P < 0.001). Darbepoietin use also decreased (P = 0.013), with no modification of hemoglobin and ferritin values. Serum albumin increased (P < 0.001) and CRP decreased (P = 0.004) with supplementation.

Mean SBP, DBP, PP, and interdialytic weight gain in the last month before supplementation and in the last month of supplementation were similar. The percentage of patients who were on antihypertensive therapy, namely ACEIs and ARBs, and treated with statins was not different at the beginning and at the end of the study.

Although BNP plasma levels were high compared with the general population with no cardiac disease, they showed a significant reduction during the study (P = 0.008). LVMI also had a significant decrease after supplementation (P = 0.01). There was a significant negative correlation between the percentage of change in 25(OH)D and the percentage of change in BNP levels (r = -0.21, P = 0.009) and LVMI (r = -0.24, P = 0.003; Figure 4). The percentage of change in BNP was also positively correlated with the percentage of change in LVMI (r = 0.19, P = 0.02).

Discussion

Our study confirms the high incidence of vitamin D deficiency or insufficiency in HD patients and the apparently safety and efficacy of oral supplementation with native vitamin D. Patients with stage 5 CKD lack the capacity of the kidney to perform 1α-hydroxylation, thereby making it impossible to produce the necessary amount of 1,25(OH)₂D. Studies have also described the presence of a phosphaturic bone protein fibroblast growth factor 23 that is elevated in patients with CKD and may amplify the inhibition of renal 1α-hydroxylase (11,21); however, extrarenal production of 1,25(OH)₂D in anephric patients has been reported (22). Also, it was recently described that calcitriol levels in HD patients increased after 6 months of supplementation with 25(OH)D (23).

The expression of 1α-hydroxylase was also reported in other organs, such as the parathyroids, pancreas, adrenal medulla, endothelium, smooth vascular cells, skin, and cerebellum (11,24). This may indicate an autocrine/paracrine function of this enzyme in the control of cell proliferation and differentiation (25).

In our study, as already described in other studies, older patients and patients with diabetes showed lower levels of 25(OH)D. Elderly patients have reduced sun exposure as a result of poor health and immobility and have reduced ingestion of natural sources of vitamin D, and dermal synthesis of vitamin D is reduced with increasing age (26,27). In this study, lower levels of 1,25(OH)₂D were also present in patients with diabetes, probably reflecting lower production of the substrate. It is interesting that, as we found previously (15), patients who were receiving paricalcitol therapy showed lower levels of 1,25(OH)₂D. The exact reason for this is not completely known, but vitamin D₂ activates the catabolism of 1,25(OH)₂D through...
the activation of 24-hydroxylase (28). Although this enzyme acts in 25(OH)D and 1,25(OH)₂D, it has a 10-fold higher affinity for the latter molecule (29). Another hypothesis is that because paricalcitol acts in vivo as an active vitamin D, the target cell might downregulate the production of 1,25(OH)₂D.

An in vitro study also reported that 25(OH)D may directly activate the vitamin D receptor in the parathyroid gland, independent of 1,25(OH)₂D, contributing to the control of secondary hyperparathyroidism (30). Our study shows a decrease in Ca and P and an improvement in hyperparathyroidism control after cholecalciferol supplementation. This reduction in serum Ca and P was more significant than the reduction seen in iPTH and was independent of paricalcitol therapy, because patients who never used this medication also showed this reduction. The observed increase in serum albumin may also have contributed to the decrease in Ca levels. The active vitamin D therapeutic dosage was also significantly reduced and might contribute to a less calcemic and phosphoremic action seen at the end of the study. Conversely, lower Ca and P are potent stimulus of 1α-hydroxylase (11,24,25), and that could explain some of the increase in 1,25(OH)₂D levels.

The role of vitamin D metabolism has also been involved in the improvement of erythropoiesis by a direct effect on erythroid precursor proliferation (31) and/or, marginally, by controlling secondary hyperparathyroidism (32). In our study, the dosage of the erythroid-stimulating agent was reduced, with no modification of hemoglobin values.

### Table 1. Clinical, biochemical, and echocardiographic parameters before and after supplementation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before Supplementation</th>
<th>After Supplementation</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>25(OH)D (ng/ml; mean ± SD)</td>
<td>22.3 ± 12.0</td>
<td>42.0 ± 12.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>1,25(OH)₂D (pg/ml)</td>
<td>4.6</td>
<td>5.9</td>
<td>0.001</td>
</tr>
<tr>
<td>Ca (mg/dl; mean ± SD)</td>
<td>8.6 ± 0.8</td>
<td>8.4 ± 0.7</td>
<td>0.014</td>
</tr>
<tr>
<td>P (mg/dl; mean ± SD)</td>
<td>4.7 ± 1.3</td>
<td>4.5 ± 1.3</td>
<td>0.011</td>
</tr>
<tr>
<td>iPTH (pg/ml)</td>
<td>233</td>
<td>208</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>bAP (µg/L)</td>
<td>18.6</td>
<td>18.0</td>
<td>NS</td>
</tr>
<tr>
<td>Hemoglobin (g/dl; mean ± SD)</td>
<td>12.1 ± 1.2</td>
<td>11.9 ± 1.4</td>
<td>NS</td>
</tr>
<tr>
<td>Ferritin (mg/dl; mean ± SD)</td>
<td>438 ± 182</td>
<td>431 ± 196</td>
<td>NS</td>
</tr>
<tr>
<td>Albumin (g/dl; mean ± SD)</td>
<td>3.9 ± 0.5</td>
<td>4.2 ± 0.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CRP (mg/dl)</td>
<td>0.4</td>
<td>0.2</td>
<td>0.004</td>
</tr>
<tr>
<td>BNP (pg/ml)</td>
<td>338</td>
<td>296</td>
<td>0.008</td>
</tr>
<tr>
<td>eKt/V (mean ± SD)</td>
<td>1.4 ± 0.2</td>
<td>1.4 ± 0.3</td>
<td>NS</td>
</tr>
<tr>
<td>nPCR (g/kg per d; mean ± SD)</td>
<td>1.24 ± 0.50</td>
<td>1.23 ± 0.60</td>
<td>NS</td>
</tr>
<tr>
<td>Paricalcitol (%)</td>
<td>44</td>
<td>33</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Paricalcitol dosage (µg/wk; mean ± SD)</td>
<td>7.2 ± 4.5</td>
<td>6.0 ± 4.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sevelamer (%)</td>
<td>66</td>
<td>48</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sevelamer dosage (mg/d; mean ± SD)</td>
<td>3738 ± 1761</td>
<td>3284 ± 1391</td>
<td></td>
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<tr>
<td>Darbepoietin (%)</td>
<td>91</td>
<td>89</td>
<td>NS</td>
</tr>
<tr>
<td>Darbepoietin dosage (µg/kg per wk per g/dl)</td>
<td>0.042</td>
<td>0.033</td>
<td>0.013</td>
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<td>Antihypertensive therapy (%)</td>
<td>71</td>
<td>69</td>
<td>NS</td>
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<tr>
<td>ACEIs (%)</td>
<td>43</td>
<td>44</td>
<td>NS</td>
</tr>
<tr>
<td>ARBs (%)</td>
<td>9</td>
<td>7</td>
<td>NS</td>
</tr>
<tr>
<td>Statins (%)</td>
<td>18</td>
<td>19</td>
<td>NS</td>
</tr>
<tr>
<td>SBP (mmHg; mean ± SD)</td>
<td>132 ± 21</td>
<td>129 ± 23</td>
<td>NS</td>
</tr>
<tr>
<td>DBP (mmHg; mean ± SD)</td>
<td>64 ± 16</td>
<td>63 ± 15</td>
<td>NS</td>
</tr>
<tr>
<td>PP (mmHg; mean ± SD)</td>
<td>69 ± 15</td>
<td>68 ± 17</td>
<td>NS</td>
</tr>
<tr>
<td>Interdialytic weight gain (ml; mean ± SD)</td>
<td>1647 ± 968</td>
<td>1662 ± 897</td>
<td>NS</td>
</tr>
<tr>
<td>Dry weight (kg; mean ± SD)</td>
<td>66.3 ± 13.1</td>
<td>65.7 ± 12.8</td>
<td>NS</td>
</tr>
<tr>
<td>LVMI (g/m²; mean ± SD)</td>
<td>134 ± 31</td>
<td>121 ± 32</td>
<td>0.01</td>
</tr>
</tbody>
</table>

bAP, bone alkaline phosphatase; nPCR, normalized protein catabolic rate.

**Figure 3.** 25(OH)D and 1,25(OH)₂D serum levels before and after supplementation in patients without and with paricalcitol therapy.
Several studies have shown that vitamin D has numerous important nonclassic actions, including a protective role in innate immunity (33), antiatherosclerotic effects, and normalization of inflammatory reactions (10,11,14). Low vitamin D levels were also associated with decreased pulmonary function test results, increased PP, congestive heart failure, and increased carotid intima-media thickness (25,34). Our study, like others (35,36), indicates a reduction in the inflammatory parameters, with an increase in albumin and a reduction in CRP. This could reflect 25(OH)D as another marker of inflammation or represent the already described immunomodulator and anti-inflammatory actions of this hormone.

Dialysis patients have increased cardiovascular morbidity and mortality (37). BNP plasma levels have been shown to be a good cardiovascular risk marker in the general population as well as in patients with CKD (38,39). Plasma BNP is synthesized mainly in cardiomyocytes in response to ventricular stretch and pressure overload (40), but it may also result from concomitant coronary artery disease or left ventricular dysfunction (41). Studies have indicated that the degree of BNP elevation in patients with CKD may predict left ventricular function and future cardiac events (40–42). In a previous study (18), we also found an increase in cardiovascular morbidity and mortality in HD patients with higher BNP plasma levels. In this study, as reported in other studies, mean BNP plasma levels were high (compared with the general population) as a result of renal failure and in some patients simultaneous cardiac disease, but its levels were significantly decreased after cholecalciferol supplementation.

Vitamin D is also important in the regulation of BP through inhibition of the renin-angiotensin pathway (42), and lower circulating vitamin D levels correlated with increased BP and LVMI (15,43). Our study has shown that the correction of vitamin D deficiency with vitamin D precursors, namely cholecalciferol, can decrease LVMI. This reduction in BNP plasma levels and LVMI with supplementation was independent of PP and of the use of antihypertensive therapy, namely ACEIs and ARBs, and statins.

The main limitation of this study is the absence of a control group. Because the general care and therapeutic approach to these patients did not change during the study, the results were assumed to be due to cholecalciferol supplementation. Other limitations are the short duration of cholecalciferol supplementation, the small number of patients studied, and some drawbacks of echocardiography in evaluating LVMI.

Conclusions
Cholecalciferol supplementation corrected vitamin D deficiency without evident toxicity. It increased mineral metabolism control with less use of active vitamin D, decreased inflammatory parameters with reduction of erythropoiesis-stimulating agent consumption, and improved cardiac dysfunction (reflected by lower BNP levels and decreased LVMI). These effects may be related to the direct action of 25(OH)D on target cells and/or to persistent renal or extrarenal 1α-hydroxylation.

This supplementation was simple, apparently safe, and cost-effective. More important, it seems to be a treatment that positively affects surrogate markers and may potentially improve clinical outcomes. On the basis of this study, we propose cholecalciferol supplementation for patients with stage 5D CKD, although the effects on morbidity and mortality still need to be confirmed in randomized, controlled, and longer follow-up studies.

Disclosures
None.

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![Figure 4. Correlations between the percentage of change in 25(OH)D and the percentage of change in BNP and LVMI with cholecalciferol supplementation.](image-url)
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