Inverse Association of Serum 25-Hydroxyvitamin D With Markers of Inflammation and Suppression of Osteoclastic Activity in Hemodialysis Patients

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Introduction. In hemodialysis patients, 25-hydroxyvitamin D conversion to active 1,25-dihydroxyvitamin D by the kidneys is very limited. The expression of both vitamin D receptor and 1α-hydroxylase in cells of the immune system and in both osteoblasts and osteoclasts makes it possible that 25-hydroxyvitamin D could play an important role in both inflammation and bone metabolism acting in an autocrine and/or paracrine way in these patients.

Materials and Methods. Thirty-three hemodialysis patients not under vitamin D receptor agonist treatment were enrolled into the study. Serum levels of 25-hydroxyvitamin D, C-reactive protein (CRP), interleukin-6 (IL-6), receptor activator of nuclear factor-κB ligand (RANKL), and osteoprotegerin, as well as intact parathyroid hormone (PTH) were assessed by immunoassays.

Results. Regarding inflammation, 25-hydroxyvitamin D inversely correlated with both CRP and IL-6. Regarding bone metabolism, 25-hydroxyvitamin D was positively related to osteoprotegerin, but negatively to the RANKL. The latter could be the result of PTH suppression by 25-hydroxyvitamin D, since 25-hydroxyvitamin D negatively correlated with PTH, which in turn was positively related to RANKL.

Conclusions. Serum 25-hydroxyvitamin D is inversely correlated with markers of inflammation and may suppress osteoclastic activity in hemodialysis patients.

Keywords. hemodialysis, inflammation, bone metabolism, vitamin D

INTRODUCTION

According to the central dogma about vitamin D metabolism and action, vitamin D is produced in the skin or absorbed from dietary sources. After its conversion to 25-hydroxyvitamin D in the liver, vitamin D is transported to the kidneys where it is converted by 1α-hydroxylase to its active form, 1,25-dihydroxyvitamin D. Active vitamin D is indeed not a vitamin, it is a hormone that plays a central role in bone metabolism by increasing calcium and phosphorus absorption in the gastrointestinal tract, stimulating calcium reabsorption in the distal tubule and stimulating osteoclastic activity.1 Vitamin D metabolism is heavily disturbed in hemodialysis patients. Its reduced activation by the kidneys results in very low circulating levels of 1,25-dihydroxyvitamin D, whereas 25-hydroxyvitamin D deficiency is also a common finding.2-4 Because of the central dogma about vitamin D metabolism and action, much attention is being paid to active vitamin D receptor (VDR) agonists administration in hemodialysis patients primarily for the treatment of secondary
hyperparathyroidism.\(^5\) On the contrary, and despite the Kidney Disease Outcomes Quality Initiative guidelines that recommend supplementation with vitamin D2, if the serum level of 25-hydroxyvitamin D is less than 30 ng/mL our sense is that 25-hydroxyvitamin D insufficiency is frequently overlooked.\(^6\)

The significance of serum 25-hydroxyvitamin D in patients with chronic kidney failure has been supported by clinical data. In patients with chronic kidney failure, low serum levels of 25-hydroxyvitamin D and not of 1,25-dihydroxyvitamin D was a negative prognostic factor for both progression to end-stage renal disease and death.\(^7\) In hemodialysis patients, 25-hydroxyvitamin D deficiency was associated with sudden cardiac death and overall mortality.\(^8\) The same has been confirmed in peritoneal dialysis patients and in the general population, as well.\(^9,10\) Such findings challenge the central dogma about vitamin D metabolism and action. It seems that vitamin D does not act exclusively in bone metabolism, which is also supported by the presence of the VDR in a wide variety of tissues. The expression of 1α-hydroxylase in extrarenal cells also challenges the central dogma regarding the exclusive endocrine action of vitamin D, suggesting that autocrine and/or paracrine actions are also possible through local 25-hydroxyvitamin D activation.\(^1,11\)

Among the cells that express VDR are dendritic cells, macrophages, and stimulated T cells and B cells.\(^12-15\) The same cells also express the enzyme 1α-hydroxylase. Various mediators of inflammation have been shown to increase 1α-hydroxylase expression, and consequently in case of inflammation very high concentrations of 1,25-dihydroxyvitamin D can be produced locally.\(^16-19\)

At the bone level, 1,25-dihydroxyvitamin D increases the expression of the receptor activator of nuclear factor-κB ligand (RANKL) in osteoblasts.\(^20-22\) The RANK, which is the receptor for the RANKL on preosteoclasts, induces them to become mature osteoclasts and start bone resorption.\(^23\) On the other hand, 1,25-dihydroxyvitamin D also induces osteoblasts to produce osteoprotegerin,\(^20-22\) which acts as a decoy receptor that binds and neutralizes RANKL and thus inhibits osteoclastogenesis and osteoclastic activity and induces osteoclast apoptosis.\(^23\) The finding that both osteoblasts and osteoclasts express 1α-hydroxylase supports that 25-hydroxyvitamin D can act in the bone in an autocrine and/or paracrine way, without the need of previous activation by the kidneys.\(^24,25\)

The aim of the present study was to evaluate the role of serum 25-hydroxyvitamin D in inflammation that characterizes hemodialysis patients,\(^26\) as well as its possible effect on bone metabolism that is heavily disturbed in this population.\(^27\)

**MATERIALS AND METHODS**

**Patients**

Thirty-three hemodialysis patients (mean age, 62.8 ± 9.4 years; 20 men and 13 women) were enrolled into this observational laboratory study. The cause of end-stage renal disease was diabetes mellitus in 13 patients, primary glomerulonephritis in 5, interstitial nephritis in 3, hypertension in 3, obstructive nephropathy in 2, autosomal dominant polycystic kidney disease in 2, and unknown in 5. The patients underwent regular hemodialysis via an arteriovenous fistula. Polysulfone low-flux dialysis membranes and a bicarbonate dialysis solution containing 1.25 mmol/L or 1.5 mmol/L of calcium were used. The low calcium dialysis solution was used in case of high serum calcium or very low serum parathyroid hormone (PTH) levels. Hemodialysis sessions (4 hours) had been performed 3 times a week and for at least 1 year prior to the study. The KT/V according to the second-generation natural logarithmic formula, based on the single-pool urea kinetic model, was 1.21 ± 0.19. At the time of blood collection, serum calcium level was 9.48 ± 0.56 mg/dL, serum phosphorous was 5.97 ± 1.90 mg/dL, and serum albumin was 3.94 ± 0.32 g/dL. None of the patients was a smoker or suffered from infection, malignancy, or autoimmune disease and none had a history of parathyroidectomy. Because our aim was to investigate the role of serum 25-hydroxyvitamin D in inflammation and bone metabolism, patients treated with VDR activators were excluded from the study.

Additionally, none of the patients were receiving corticosteroids, cytotoxic drugs, warfarin, anticonvulsants, antidepressants, hormone replacement therapy, bisphosphonates, or cinacalcet for at least 6 months prior to the study. Sevelamer hydrochloride (Renagel, Genzyme Pharmaceuticals, Naarden, Netherlands) or lanthanum carbonate (Fosrenol, Shire Pharmaceuticals Group, Chineham,...
Methods

Blood samples were drawn in the morning before the onset of the second dialysis session of the week and the serum was stored at -80°C. Immunoassays for measuring 25-hydroxyvitamin D and intact PTH were performed in an ELECSYS 2010 automatic analyser (Roche Diagnostics GmbH, Mannheim, Germany). C-reactive protein (CRP) was measured using the Cobas Integra 400 automatic analyzer (Roche Diagnostics GmbH, Mannheim, Germany). Interleukin-6 (IL-6) was assessed by means of an enzyme-linked immunosorbent assay (Biosource Europe SA, Nivelles, Belgium). The RANKL and osteoprotegerin were also measured with an enzyme-linked immunosorbent assay using two commercially available kits (Human sRANKL [total] enzyme-linked immunosorbent assay, BioVendor GmbH, Heidelberg, Germany and Osteoprotegerin enzyme-linked immunosorbent assay, Biomedica Medizinprodukte GmbH & Co KG, Wien, Austria).

Statistical Analyses

Mean values and standard deviations were calculated for each continuous variable. The normal distribution of the evaluated variables was assessed using the 1-sample Kolmogorov-Smirnov test; 25-hydroxyvitamin D, osteoprotegerin, IL-6, and intact PTH values were normally distributed, but not the CRP and RANKL levels. For evaluating relationship among normally distributed variables, the Pearson correlation coefficient was calculated, whereas the Spearman rho was calculated in case of nonparametric data. A relationship was considered significant when the 2-sided P value was less than .05.

RESULTS

The mean serum 25-hydroxyvitamin D level was 23.45 ± 5.34 ng/mL. Twenty-eight of the 33 patients (84.8%) had serum 25-hydroxyvitamin D levels less than 30 ng/mL. The mean CRP level was 11.69 ± 18.60 mg/L and the mean IL-6 level was 15.53 ± 12.45 pg/mL. Of the 33 patients, 15 (45.5%) had increased CRP values, ie, greater than 5 mg/L.

The mean serum values for were 494.55 ± 672.06 pmol/L for the RANKL, 14.27 ± 3.96 pmol/L for osteoprotegerin, and 211.95 ± 141.31 pg/mL for intact PTH.

Serum 25-hydroxyvitamin D inversely correlated with CRP (r = -0.360, P = .04). An inverse relationship was also detected between 25-hydroxyvitamin D and IL-6 (r = -0.452, P = .008; Figure 1). As expected, serum CRP correlated with serum IL-6 (r = 0.576, P < .001). Serum 25-hydroxyvitamin D inversely correlated with serum intact PTH (r = -0.398, P = .02), but directly correlated with serum osteoprotegerin (r = 0.366, P = .04; Figure 2).
An inverse correlation was found between serum 25-hydroxyvitamin D and serum RANKL ($r = -0.479$, $P = .005$). Serum intact PTH was not significantly related with serum osteoprotegerin ($r = -0.038$, $P = .834$), but it correlated with RANKL ($r = 0.439$, $P = .01$).

**DISCUSSION**

In the present study, the associations of serum 25-hydroxyvitamin D with markers of inflammation and with the two major proteins that control osteoclastic activity, the RANKL and the osteoprotegerin, were investigated. These questions are of particular interest in hemodialysis patients because inflammation is common in this population, which is also characterized by heavily disturbed bone metabolism. The expression of both VDR and 1α-hydroxylase in the cells of the immune system and in both osteoblasts and osteoclasts, combined with the drastic decreased conversion of 25-hydroxyvitamin D to active 1,25-dihydroxyvitamin D by the kidneys of hemodialysis patients, means that the autocrine and paracrine action of 25-hydroxyvitamin D in these two systems could be important.

Not surprisingly, 25-hydroxyvitamin D insufficiency was very common in the cohort of our patients, since its serum level was below the recommended limit of 30 ng/mL (85% of the patients). The very high incidence of 25-hydroxyvitamin D insufficiency in hemodialysis patients was detected by previous studies as well. Regarding inflammation, increased serum CRP levels in approximately half of our patients reconfirmed that inflammation is a common finding in hemodialysis patients. Besides their central role in the treatment of secondary hyperparathyroidism, the beneficial effect of active vitamin D agonists on various other aspects, and particularly on inflammation in patients with chronic kidney failure, has been confirmed. In vitro studies showed that active VDR agonists decrease cytokine production by human blood mononuclear cells after various inflammatory stimuli, and in the clinic, administration of an active VDR agonist decreased CRP in patients with kidney failure.

However, the available data about the association of serum 25-hydroxyvitamin D with inflammation in hemodialysis patients are scarce. The present study showed that serum 25-hydroxyvitamin D was negatively correlated with the markers of inflammation, CRP and IL-6, in hemodialysis patients. This capacity of 25-hydroxyvitamin D to attenuate inflammation in hemodialysis patients is very important because inflammation has been incriminated for malnutrition, erythropoietin resistance, atherosclerosis, impaired immune response, and death in this population.

Regarding the effect of serum 25-hydroxyvitamin D on bone metabolism, the present study supports that 25-hydroxyvitamin D may suppress osteoclastic activity, since it is positively related to osteoprotegerin, but negatively to RANKL. The positive relationship of serum 25-hydroxyvitamin D with serum osteoprotegerin was expected, but the negative relationship of serum 25-hydroxyvitamin D with serum RANKL is against to what is known from in vitro studies. The answer could be in the effect of 25-hydroxyvitamin D on PTH.

As expected, serum intact PTH level was increased in hemodialysis patients. In addition, serum intact PTH positively correlated with serum RANKL. It is known that PTH increases bone resorption by increasing RANKL expression in osteoblasts. Additionally, in the cohort of our patients, serum 25-hydroxyvitamin D was negatively related with serum intact PTH. The suppression of PTH by 1,25-dihydroxyvitamin D is established. However, the expression of 1α-hydroxylase in the parathyroid glands supports that 25-hydroxyvitamin D could suppress PTH by acting in an autocrine way. The latter has also been confirmed by clinical studies and could be responsible for the negative correlation between 25-hydroxyvitamin D and RANKL detected in the present study. It is possible that in vivo 25-hydroxyvitamin D suppresses RANKL expression indirectly through the suppression of intact PTH expression. Additionally, there are in vivo experimental data suggesting that vitamin D suppresses RANKL independently of its effect on PTH production. In parathyreoidectomized rats constantly infused with PTH, pharmacological or toxic doses of 1,25-dihydroxyvitamin D stimulate bone resorption by inducing RANKL, but a certain range of physiological doses of the vitamin inhibits PTH-induced bone resorption possibly by suppressing the PTH/PTHrP receptor-mediated signaling. Furthermore, it has been experimentally confirmed that 25-hydroxyvitamin D depletion
induces RANKL-mediated osteoclastogenesis and bone loss.48

The design of the present study could not exclude the possibility that the negative relationship between serum 25-hydroxyvitamin D and markers of inflammation could result from decreased vitamin D intake due to inflammation-induced malnutrition.32 However, our patients were stable, did not suffer from other than the hemodialysis inflammatory conditions, and mostly had normal serum albumin values. Additionally, there are plenty of clinical and experimental data, already mentioned in the present text, that support the anti-inflammatory properties of VDR agonists.

Another problem with the interpretation of the results of the present study is that besides osteoblasts, other cell types, particularly of the immune system, are also sources of serum osteoprotegerin and RANKL. However, these molecules, even if are expressed in immune cells, affect osteoclastic activity. In inflammatory diseases the participation of activated lymphocytes in pathological bone erosion, mainly through RANKL expression by activated T cells, is established.49 Another study showed that both B and T lymphocytes play a significant role in basal bone turnover, since T cells help B cells to produce the 65% of the total osteoprotegerin in bone marrow in mice. B-cell knockout mice were found to be osteoporotic and deficient in bone marrow osteoprotegerin, phenomena that are reversed by B-cell reconstitution.50

The last limitation of this study to be discussed here is that hemodialysis patients with very high serum intact PTH levels were not present in this study, because those treated with VDR agonists for secondary hyperparathyroidism were excluded. Nonetheless, this was the only way to isolate the effect of serum 25-hydroxyvitamin D on the evaluated parameters. Unfortunately, this exclusion criterion limited the number of the patients enrolled in the study.

CONCLUSIONS

The associations detected in the present study suggest that in hemodialysis patients, 25-hydroxyvitamin D, acting in an autocrine and/or paracrine way, ameliorates inflammation. In addition, regarding bone metabolism, 25-hydroxyvitamin D exerts an anabolic effect. Correction of 25-hydroxyvitamin D insufficiency, which is very common in this population, is expected to offer great benefit. However, larger, prospective, interventional studies, based on the administration of 25-hydroxyvitamin D in hemodialysis patients, are required for definite conclusions.

CONFLICT OF INTEREST

None declared.

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