Vitamin D and Kidney Disease. What we know and what we do not know

Vitamina D e doença renal. O que nós sabemos e o que nós não sabemos

ABSTRACT

Vitamin D deficiency is common in the chronic kidney disease (CKD) population. CKD has been recognized as a significant public health problem and CKD patients are at increased risk of total and cardiovascular morbidity and mortality. There are increasing epidemiological data suggesting that vitamin D deficiency may play a role in overall morbidity and mortality associated with CKD. The vitamin D hormonal system is classically implicated in the regulation of calcium homeostasis and bone metabolism but there is ample evidence to support the claim that extra renal conversion of 25(OH)D to 1,25(OH)₂ has significant biological roles beyond those traditionally ascribed to vitamin D. Based on the current state of evidence this review intends to give an update on novel biological and clinical insights with relevance to the steroid hormone vitamin D specifically in patients with kidney disease.

Keywords: 25-hydroxyvitamin D₂; kidney failure, chronic; vitamin D deficiency.

INTRODUCTION

Vitamin D deficiency/insufficiency is an increasingly recognized public health problem in the general population. Chronic kidney disease (CKD) has been known to be a risk factor for development of vitamin D deficiency/insufficiency and is associated with increased morbidity and mortality. The vitamin D hormonal system is implicated in the regulation of calcium homeostasis and bone metabolism but also potentially has extra-mineral metabolism functions through activation of non-renal vitamin D receptors (VDR) and over the last decade, interest in the therapeutic potential of vitamin D has grown. The active form of vitamin D are present in many tissues which are not associated with calcium or bone metabolism. Indeed, 1,25-dihydroxyvitamin D regulates cell proliferation, differentiation and apoptosis in many normal and cancer cells. Epidemiologic studies have shown that vitamin D deficiency increases the risk of cancer, cardiovascular disease, autoimmune disease, type 2 diabetes mellitus, and infectious disease; however, in this review we will focus our attention on highlighting new information from recent
studies on vitamin D deficiency/insufficiency and pathogenesis in renal disease, and vitamin D replacement therapy and outcomes in renal disease.

Sources and metabolism of vitamin D
In nature, vitamin D can be obtained in two forms - dietary ingestion or sunlight-induced endogenous synthesis by the skin (Figure 1). Humans derive vitamin D mostly from skin-exposure to sunlight and, to a lesser extent, from the diet and dietary supplements. Few foods naturally contain or are fortified with vitamin D. Therefore, without daily regular consumption of naturally-rich or fortified foods, individuals may be deficient in vitamin D. In the absence of daily exposure to sunlight, or with the use of sunscreens, this deficiency will be more pronounced. Solar ultraviolet B radiation converts 7-dehydrocholesterol in the epidermis to pre-vitamin D$_3$, which is immediately converted to biologically inactive vitamin D$_3$ in a heat-dependent process.

Vitamin D refers to two biologically inert precursors or prohormones: vitamin D$_2$ (ergocalciferol) and vitamin D$_3$ (cholecalciferol). They behave in a similar manner and are subsequently referred here only as 25-hydroxyvitamin D (25(OD)D). Vitamin D in these forms must be converted to the active hormone to be able to exert biological influence affecting mineral metabolism and other physiological functions. Vitamin D is transported in the blood by the vitamin D-binding protein (DBP) to the liver. In the liver vitamin D is hydroxylated at the C-25 position by one or more cytochrome P450 vitamin D 25 hydroxylases, resulting in the formation of 25(OH)D. 25-hydroxyvitamin D is the main storage form of vitamin D. In the proximal renal tubule (PCT), the enzyme 1-α-hydroxylase catalyzes the hydroxylation of 25(OH)D (Figure 1) at the position of carbon 1 of the A ring resulting in the hormonally active form of vitamin D$_1$. 1,25-dihydroxyvitamin D (1,25(OH)$_2$D$_2$). Also called calcitriol, this is the biologically active form of vitamin D that acts on receptors in different target organs.

The renal synthesis of 1,25(OH)$_2$D$_2$ is a tightly regulated step in itself, given its potent activity in calcium homeostasis (Figure 1). Dietary calcium can regulate vitamin D directly through changes in serum calcium and indirectly by altering parathyroid hormone (PTH) levels. 1-alpha hydroxylase can be suppressed by other factors such as phosphorus and chronic metabolic acidosis. However, high circulating calcium and fibroblast growth factor-23 (FGF-23) levels directly suppress renal 1-α-hydroxylase activity, via regulation of 1-α-hydroxylase gene transcription and indirectly through PTH suppression via cAMP-mediated changes. FGF-23 is a hormone produced by osteocytes and is a critical circulating hormone involved in phosphate metabolism.

Vitamin D in the extra-renal milieu
Evidence suggests that the physiological significance of vitamin D extends beyond regulating the classical calcium-phosphorus-PTH axis domain. Research in the last few years has demonstrated the expression of 1-α-hydroxylase in tissues and organs other than kidneys. Evidence suggests that 1,25(OH)$_2$D exerts a wide range of non-classical actions, including effects on modulation of the immune system, regulation of cellular differentiation, programmed cell death, inhibition of cell growth, control of the central nervous system, and other physiological functions.
system, regulation of cardiomyocyte hypertrophy, regulation of insulin secretion and regulation of blood pressure via the renin angiotensin aldosterone system (RAAS). Laboratory experiments have demonstrated the expression of 1-α-hydroxylase in non-renal tissues and therefore the synthesis of 1.25(OH)₂D in extra-renal sites. Also, it has been shown that intracellular VDR are expressed such as breast, skin, prostate, lymph nodes, colon, pancreas, adrenal medulla, brain, and placenta. It is likely that 1.25(OH)₂D is locally produced in these sites to mediate local biological effects in the immediate cellular environment.

FGF-23 AND VITAMIN D
The discoveries of FGF-23 and Klotho have added an interesting dimension to our understanding of vitamin D homeostasis. FGF-23 is an approximately 32-kD (251 amino acids) protein and its N-terminal region contains the FGF homology domain. FGF-23 induces phosphaturia and has been shown to directly suppress the activity and expression of 1-α-hydroxylase. Also, FGF-23 production induces the expression of 24-hydroxylase the enzyme responsible for the degradation of 1.25(OH)₂D thus decreasing vitamin D bioavailability.

Klotho is a type 1 membrane protein, with a single transmembrane domain near its C-terminus that is hypothesized to anchor the protein to the membrane. The FGF-23 receptor, Klotho, is involved in aging and both the klotho knock-out mouse and the FGF-23 knock-out mouse experience rapid aging and vascular calcification. Further research has suggested that Klotho can bind to multiple FGF receptors, and that the Klotho-FGF receptor complex binds to FGF-23 with much higher affinity than either the FGF receptor or Klotho alone. The binding of this complex can then activate downstream signaling events.

The phosphate-lowering effect of FGF-23 is partly mediated through the reduced expression of NaPi-2a and 1α hydroxylase in the proximal tubular epithelial cells. The interaction of proximal and distal tubules to facilitate FGF-23-Klotho-mediated functions is an important and unsolved issue, and an active area of research.

VITAMIN D METABOLISM IN CKD
The kidney is the key organ involved in production of bioactive forms of vitamin D from inert precursors. Consequently, chronic kidney disease is an important risk factor for development of vitamin D deficiency. There are several mechanisms by which 1.25(OH)₂D are lowered during the course of CKD, starting with decreased availability of the 25(OH)D substrate for the production of 1.25(OH)₂D (Figure 2). A reduction in glomerular filtration rates (GFR) limits the delivery of 25(OH)D to the 1-α-hydroxylase enzyme in the proximal renal tubule, and therefore restricts the ability of the kidney to produce 1.25(OH)₂D. Levels of phosphaturic hormone FGF-23 also increase early in CKD, presumably in response to phosphate retention, which also suppresses production of 1.25(OH)₂D. In addition to these factors, there may be additional contribution from potential suppressive effects of Carboxyl (C)-terminal fragments of PTH on 1.25(OH)₂D synthesis.

**Figure 2.** The mechanism contributing to the progressive decrease in the levels of 1,25-dihydroxyvitamin D (calcitriol).
The Workshop Consensus for Vitamin D Nutritional Guidelines, published in 2010, estimated that more than 50% of the older populations in the world do not have satisfactory vitamin D status. The situation in younger subjects and pediatric population seems to be not different. Epidemiological studies have demonstrated a high prevalence of insufficient or deficient vitamin D status in the general population, CKD patients in pre- and chronic dialysis.

The Study for the Evaluation of Early Kidney disease (SEEK) investigated more than 1800 CKD patients over a wide range of renal function and detected 1.25(OH)₂D deficiency (< 22.0 pg/mL) in more than 60% in those with eGFR < 30 ml/min/1.73 m² and 25(OH)D deficiency (< 15 ng/mL) in 12% of patients overall. In a cross-sectional study, LaClair et al. measured serum 25(OH)D levels in 201 patients with a mean estimated GFR of 27 ± 11 ml/min/1.73 m² (GFR range 6-69 ml/min/1.73 m²). The overall mean serum levels of 25(OH)D were 19 ± 14 ng/mL. Only 29% of the 65 patients with stage 3 CKD and only 17% of the 113 patients with stage 4 CKD had a serum 25(OH)D level above 30 ng/mL. Furthermore, 14% of patients with stage 3 CKD and 26% of those with stage 4 CKD had 25(OH)D level below 10 ng/mL.

Even in a sunny country like Brazil, a cross-sectional study including 144 nondialyzed CKD patients at stage 2 to 5 found 57 patients (39.6%) 25(OH)D insufficient (16 to 30 ng/mL). However, while this one study suggests that vitamin D status in Brazil may be better than in other countries, it still needs to be addressed due to the present knowledge of the proposed non-calcemic effects of vitamin D and vitamin D's potential beneficial role in vascular health, insulin resistance and immune function.

The high prevalence of 25(OH)D deficiency/insufficiency in CKD patients can be partially explained by poor sunlight exposure in chronically ill patients, decreased skin synthesis of cholecalciferol in response to sunlight, diminished ingestion of food that are natural sources of vitamin D, and urinary loss of 25(OH)D and DBP in proteinuric nephropathies. Moreover, renal megalin, the binding protein for 25(OH)D in the proximal tubule, decreases as GFR falls, thus reducing 25(OH)D tubular reabsorption. Additionally, renal retention of phosphorus in early stages of CKD may contribute the impaired production of 1.25(OH)D directly and by increasing FGF-23.

Recently, in a cross-sectional study, Figuiredo-Dias et al. analyzed 120 patients with CKD at stages 2 to 5. They found diabetes mellitus (Odds Ratio (OR): 3.8; 95% CI: 1.2 to 11.7; p = .02) and BMI ≥ 30 Kg/m² (OR: 4.3; 95% CI: 1.2 to 15.3; p = 0.02) as independent risk factors for hypovitaminosis D in nondialyzed patients even after adjustment for sex, skin color, and season of the year.

**Vitamin D in CKD and Mortality**

CKD with or without hemodialysis has been consistently shown to be an independent risk factor for all-cause and cardiovascular mortality across diverse clinical populations. Within the CKD population, evidence gathered from epidemiological studies point to a potential role the levels as well as supplementation of vitamin D play in survival outcomes in CKD patients regardless of dialysis status. A recent meta-analysis of prospective studies by Pilz et al. estimated a significant decrease by 14% in mortality risk [RR 0.86, 95% CI (0.82-0.91)] per of 10 ng/ml higher 25(OH)D levels. Current evidence also suggests a survival benefit with vitamin D supplementation that is independent of changes in serum calcium, phosphorus or PTH levels, and supports a growing body of literature on non-classical effects of vitamin D beyond regulation of bone and mineral metabolism. While there is no direct explanation for the apparent survival benefits associated with vitamin D, indirect supportive evidence can be derived from studies showing an association of low levels of 25(OH)D and 1.25(OH)₂D with cardiovascular risk factors, including increased renin activity, hypertension, left ventricular hypertrophy (LVH), inflammation, insulin resistance, diabetes mellitus, and albuminuria.

**Vitamin D in CKD and Cardiovascular Disease**

25-hydroxyvitamin D deficiency/insufficiency has been recently associated with higher risk of cardiovascular disease (CVD) in CKD and in the general population. Evidence suggests that the non-traditional risk factors arising from CKD mediate vascular damage with activation of inflammatory pathways. In CKD patients not on dialysis with a mean eGFR 38 ml/min/1.73 m² (SD 15), using non-invasive brachial artery flow-mediated dilatation (FMD) methods to evaluate endothelial function, Chitalia et al. found a correlation between decreasing 25(OH)D levels and worsening FMD (r = 0.44,
This association between Vitamin D levels and FMD persisted in further analysis using regression models adjusting for traditional cardiovascular risk factors. Endothelial dysfunction is posited to be a marker of atherosclerosis and thereby surrogate for cardiovascular disease. These findings should encourage future clinical trials to further investigate the impact of Vitamin D supplementation on endothelial function in an attempt to establish a biological mechanism. Despite of small number of patients studied, the absence of a control group, and a short time of supplementation, two recent studies have shown that cholecalciferol supplementation on hemodialysis patients decrease inflammatory parameters represented by increased serum albumin and reduction of both C-reactive protein and interleukin-6.58,59 Also, they showed improve cardiac dysfunction reflected by lower BNP levels (58) and left ventricular mass index decrease.49

Animal models have shown that there are others pathways by which Vitamin D may ameliorate or prevent cardiovascular disease. Decreased VDR activity increases circulating renin levels and blood pressure and causes left ventricular and myocyte hypertrophy in genetically manipulated mouse models.51,53 The 1α-hydroxylase knockout mice develop hypertension, cardiac hypertrophy, and deprived cardiac function, but the use of calcitriol seems to ameliorate hypertension and improve cardiac function in these animals.60 These findings suggest that vitamin D is a regulator of the renin-angiotensin-aldosterone system (RAAS), and suppress the RAAS axis.

The Paricalcitol benefits in Renal Failure Induced Cardiac Morbidity (PRIMO) multi-center trial was designed to evaluate the beneficial effects of paricalcitol on left ventricular hypertrophy in pre-dialysis patients. However, results from the trial failed to demonstrate a reduction in ventricular mass index at the end of 48 weeks of daily paricalcitol supplementation in CKD patients with mild to moderate left ventricular hypertrophy.61 There was, however, a decrease in hospitalizations, a secondary end-point. These results are contrary to multiple animal experimental and human observational data. One potential explanation for the results is that the paricalcitol may have increased FGF-23 levels and multiple studies have shown a link between FGF-23 and LVH.62-64 More work is need in this area.

**Vitamin D and Albuminuria**

Albuminuria is an important marker and major risk factor for progressive decline in renal function and is considered by many to be the first step in an inevitable progression to nephropathy and renal failure. Multiple studies have shown an inverse relationship between the level of vitamin D and degree of albuminuria and an anti-albuminuric effect of vitamin D analogs.67 Isakova et al. showed that lower levels of 25(OH)D and 1.25(OH)2D were associated with albuminuria in patients with CKD, independent of age, sex, race, blood pressure and diabetes mellitus. In this cross-sectional study of 1847 patients, they estimated 2-3 times increased odds of albuminuria for those in lowest tertiles of 25(OH)D [OR 3.0; 95% CI (1.3-7.0)] and 1.25(OH)2D [OR 2.6; 95% CI (1.7-3.9)]. The linear relationship between 25(OH)D and albuminuria persisted after controlling for different eGFR levels and use of angiotensin converting enzyme inhibitors (ACEI) and angiotensin receptor blockers (ARB). Additionally, in a subgroup analysis in 387 patients with data on inflammatory markers, they found a significantly higher plasma concentration of IL-6, IL-10 and TNF-α in the presence of albuminuria.

Findings from the VITAL Study67 provide interesting clues to the renoprotective effects of vitamin D. This placebo-controlled randomized trial evaluated the anti-albuminuric effect of paricalcitol 1 or 2 µg/day versus placebo in 281 diabetic nephropathy patients with CKD stages 2-4, using standard therapy to control blood pressure and proteinuria. The primary outcome of reduced albuminuria (albumin/creatinine ratio) only showed a small trend but, significant reversible reductions in secondary outcomes at 24 weeks were seen in those randomized to receive high-dose 2 µg/day paricalcitol, including reductions in 24 h albuminuria of -28% (95% CI -43 to -8; p = 0.009), eGFR (range -3 to -5 mL/min per 1.73 m2; p = 0.001 vs. placebo), and blood pressure (range -3 to -9 mm Hg; p = 0.033 vs. placebo). The reduction of eGFR observed could be possibly explained by the effect of VDR activation on increased creatinine generation and, consequently, increased serum creatinine in response to paricalcitol as has been shown by Agarwal et al.68

**Vitamin D and Progression of CKD**

Reduction of albuminuria is a major target in renoprotective therapy and vitamin D has demonstrated...
a positive effect.\textsuperscript{67} At the same time, there exists supportive evidence that vitamin D therapy may have the potential to alter the progression of kidney disease. Vitamin D may possibly modify podocyte hypertrophy, podocyte function as a filtration barrier, expression of TGF-β, expression of MCP-1, recruitment of macrophage-like cells, and the T effector lymphocytes.\textsuperscript{69-73} Animal models\textsuperscript{71-73} and human clinical studies\textsuperscript{74,75} have reported findings in support of vitamin D induced attenuation of kidney disease progression. Using data from NHANES III, Melamed et al.\textsuperscript{75} reported 2.6 fold higher incidence of ESRD among individuals with 25(OH)D levels < 15 ng/mL compared to those with higher levels after adjusting for demographic, socioeconomic, and clinical and laboratory factors including diabetes mellitus, hypertension, estimated GFR, and albuminuria [IRR 2.64; 95% CI (1.00 to 7.05)]. We still do not have clearly what is the role of FGF-23 in the progression of CKD but some studies have shown it as a significant independent predictor of CKD progression in diabetic nephropathy patients and in patients with nondiabetic CKD.\textsuperscript{76,77} Important contributions to the understanding of the role of vitamin D will come up in the next few years with the results from on-going clinical trials (NCT01214356); (NCT00552409); (NCT01029002) evaluating the following aspects, respectively: the effectiveness of vitamin D supplementation on progression of kidney disease in African Americans adults type 2 diabetes stage 1 or 2 CKD; the change in urine albumin excretion in adults type 2 diabetes stages 1-2 CKD, and the albuminuria in patients stages 3-4 CKD from various causes. Also, important contributions to the understanding of the role of FGF-23 on progression of kidney disease and to the effects of different forms of vitamin D on FGF-23 levels are coming from the on-going observational (NCT01317173), and interventional (NCT00957879) studies, respectively.

**Vitamin D and Immune System**

Vitamin D deficiency/insufficiency may predispose to infectious disease by impairing innate immunity. Cells of the innate and adaptive immune system including macrophages, lymphocytes and dendritic cells express the VDR and respond to stimulation by 1,25(OH)\textsubscript{2}D.\textsuperscript{78,79} Vitamin D treatment \emph{in vitro} has also been demonstrated to modulate levels of systemic inflammatory cytokines such as TNF-α and IL-6, and to inhibit LPS-induced activation vasodilation of the vascular endothelium.\textsuperscript{80,81} Recently, two studies showed a significant reduction in IL-6,\textsuperscript{82} IL-8, and TNF\textsubscript{2} after cholecalciferol supplementation in ESRD patients were on hemodialysis. Therefore, it is reasonable to believe that a deficiency/insufficiency of nutritional 25(OH)D in ESRD may contribute to an altered immune response, predisposing to early morbidity and mortality from infection. Results from an ongoing randomized, placebo-controlled trial (NCT 00892099) evaluating cathelicidin, cytokine levels, and the incidence of infections in hemodialysis patients randomized to high and low dose of ergocalciferol will provide further clarity when they are published.

**Vitamin D in CKD - What we need to answer**

Given the high prevalence of vitamin D insufficiency and deficiency in CKD patients, the first step in the correction of vitamin D abnormalities in these patients would be to correct the levels of 25(OH)D levels in order to facilitate and maintain the production of 1,25(OH)\textsubscript{2}D. Current K/DOQI guidelines recommend treatment of CKD stage 3 and 4 patients with nutritional forms (ergocalciferol or cholecalciferol) of vitamin D when levels of 25(OH)D are below 30 ng/mL.\textsuperscript{31} Our current understanding and experience with vitamin D mainly come from various observational and laboratory experimental studies. Epidemiological studies, even with rigorous methodology, are limited by their biases and the inability to prove causality. Many issues remain to be clarified such as an understanding of whether vitamin D therapy improves defined clinical end points in CKD patients, whether nutritional vitamin D plays a role in improving CKD outcome; the appropriate time to start vitamin D treatment; the frequency and dose of vitamin D administration (daily or bolus); the benefit of nutritional vitamin D supplementation in stage 5 CKD patients; whether vitamin D can slow the progression of CKD; understanding the role of non-classical effects of vitamin D and whether local production in non-classical tissues is critical for these actions; whether systemic 1,25(OH)\textsubscript{2}D alone is sufficient to improve survival. Large rigorous randomized clinical trials (RCTs) are needed to answer at least some of these questions.

**Conclusion**

Vitamin D deficiency/insufficiency is a common condition affecting the general population and special
populations such as CKD patients. Aside from effects on bone and mineral metabolism, low serum levels of 25(OH)D have been associated with all-cause and cardiovascular mortality as well as increased risk of comorbidities such as cardiovascular disease, infections, and kidney dysfunction in general and CKD populations. The widespread expression of VDR in many organ systems constitutes the biological basis for the hypothesized pleiotropic and non-skeletal actions of vitamin D. These properties include RAAS inhibition, endothelial protection, immune modulation and anti-inflammatory actions. In this regard, vitamin D deficiency is associated with insulin resistance, left ventricular hypertrophy, proteinuria, atherogenicity, decreased thrombolyis, immune imbalances, susceptibility to infections and perpetuation of inflammation. More RCTs are needed to determine whether vitamin D supplementation could reduce future CVD events, the rate of progression of kidney disease and mortality risk in individuals with CKD as well more accurately define the precise therapeutic agent, dose, timing, monitoring parameters and indications for vitamin D therapy.

Referências

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