

ORIGINAL ARTICLE

Severe hypovitaminosis D in chronic kidney disease: association with blood pressure and coronary artery calcification

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Hypovitaminosis D occurs early in the course of chronic kidney disease (CKD), and its association with cardiovascular morbidity and mortality is well known. In this study, we aimed to evaluate whether the degree of hypovitaminosis D may differently affect blood pressure (BP) and coronary artery calcification (CAC) in nondialyzed CKD patients. This study included 80 CKD patients with a creatinine clearance between 15 and 60 ml/min/1.73 m² and serum 25 hydroxvitamin D [25(OH)D] level < 30 ng/ml. Patients underwent 24-h ambulatory BP monitoring, evaluation of CAC (multi-slice computed tomography), and laboratory evaluation. Two groups, based on the degree of hypovitaminosis D, were defined according to the median 25(OH)D value. Patients with severe hypovitaminosis D [25(OH)D < 17.2 ng/ml; S-group] exhibited a higher systolic BP at all time periods (24-h, nighttime, daytime) when compared to patients with mild hypovitaminosis D [25(OH)D > 17.2 ng/ml; M-group]. No differences were found between the S and M-group in terms of diastolic BP and the presence of coronary calcification. In the multiple linear regression analysis, severe hypovitaminosis D was a predictor of 24-h, daytime and nighttime BP after controlling for a number of confounders. The severity of hypovitaminosis D was associated with increased BP in nondialyzed CKD patients. The degree of hypovitaminosis D was not related to CAC, which was equally elevated in both the severe and mild hypovitaminosis D groups. *Hypertension Research* (2013) 36, 428–432; doi:10.1038/hr.2012.230; published online 31 January 2013

Keywords: blood pressure; hypovitaminosis D; nondialysis patients; vascular calcification

INTRODUCTION

Vitamin D is an essential micronutrient with important implications for human health.¹ There is evidence that beyond its actions on mineral metabolism, vitamin D may also have pleiotropic effects on the cardiovascular system.² Worldwide, approximately 1 billion people have hypovitaminosis D,³ which is characterized by a serum 25-hydroxvitamin D (25(OH)D) level < 30 ng ml⁻¹. Notably, a large number of studies have demonstrated an association between lower vitamin D levels and an increased risk of fatal and nonfatal cardiovascular events in the general population.^{4–6}

Cardiovascular disease is the leading cause of death in patients with chronic kidney disease (CKD). In the last few years, the association of low 25(OH)D levels with higher cardiovascular mortality was confirmed in dialysis as well as in nondialysis CKD patients.^{7–9}

These findings are in agreement with the increasing body of evidence that supports the important role of vitamin D in the pathogenesis of cardiovascular diseases.¹⁰ Matias *et al.*¹¹ found that 25(OH)D was strongly associated with brain natriuretic peptide, pulse pressure and the presence of vascular calcification in hemodialysis patients. The inverse and independent association between serum 25(OH)D levels and the development of coronary calcification in

patients with kidney dysfunction has been corroborated by some authors,^{12,13} but not by others.¹⁴ Finally, although not consistent across all studies, a number of reports of the general population support the hypothesis that suboptimal vitamin D status is associated with an increased risk of hypertension.^{15,16} A loss of regulation of the renin-angiotensin-aldosterone system, inflammation and proliferation and hypertrophy of the myocardial cells are among the potential mechanisms involved.¹⁷ The putative relationship between hypovitaminosis D and hypertension in CKD, however, has been rarely investigated.¹⁸

Hypovitaminosis D occurs early in the course of CKD, and its association with cardiovascular morbidity and mortality has been recognized. In this study, we aimed to evaluate whether the degree of hypovitaminosis D affects blood pressure (BP) and coronary artery calcification (CAC) in nondialyzed CKD patients.

METHODS

Patients

Eighty patients with a creatinine clearance between 15 and 60 ml min⁻¹ per 1.73 m² and serum 25(OH)D < 30 ng ml⁻¹ were recruited from the outpatient clinic of the Federal University of São Paulo, Brazil. Exclusion criteria included

age < 18 years and > 85 years, nephrotic syndrome, immobilization in the last 3 months, liver disease, autoimmune disease, infection, neoplasia and positive serology for HIV. Additional exclusion criteria included supplementation with vitamin D or analogs, the use of corticosteroids and immunosuppressive drugs in the last three months, and hypercalcemia (ionized calcium > 1.40 mmol l⁻¹) and/or a serum parathormone (PTH) level > 500 pg ml⁻¹. Seventy-nine patients (99%) were on regular antihypertensive therapy, 27 (34%) were using three different classes of drugs and 20 (25%) were using more than three different classes of drugs. Angiotensin-converting enzyme inhibitors were used by 52 patients (65%).

Written informed consent was obtained from all participants, and the study was approved by the University Ethical Advisory Committee. This study is part of an ongoing protocol on cholecalciferol supplementation and is in accordance with the ethical principles of the Declaration of Helsinki. The study is registered at The Brazilian Clinical Trials Registry (REBEC—Registro Brasileiro de Ensaios Clínicos, RBR-6pngwz), which belongs to the International Clinical Trial Registry Platform of the World Health Organization (ICTRP/WHO: U1111-1126-2741).

Laboratory measurements

Blood samples were drawn in a 12-hour fasting state for the evaluation of serum creatinine, hemoglobin, albumin (bromocresol green), bicarbonate, ionized calcium, phosphorus, alkaline phosphatase, intact PTH (reference values: 10–65 pg ml⁻¹; chemiluminescence, DPC, Medlab, San Antonio, TX, USA), 25(OH)D (reference values: 18–62 ng/dl; radioimmunoassay, DiaSorin, MN, USA), intact fibroblast growth factor-23 (FGF-23, ELISA Kainos Laboratories, Tokyo, Japan; reference range 27.8–30 pg ml⁻¹), lipid profile, high-sensitivity C-reactive protein (reference value: < 0.11 mg dl⁻¹; immunonephelometry) and renin (HPLC, high-performance liquid chromatography). Angiotensin-converting enzyme activity was determined fluorometrically using Z-Phe-His-Leu as a substrate. Urinary protein and phosphorus were measured by 24-h urine collection. The glomerular filtration rate was estimated using the Modification of Diet in Renal Disease (MDRD) Study equation. Hyperphosphatemia and hyperparathyroidism were considered when serum phosphorus and iPTH levels were higher than the upper limit of the normal range (> 4.6 mg dl⁻¹ and > 65 pg ml⁻¹, respectively), as proposed by the Kidney Disease Improving Global Outcomes (KDIGO) Guideline on Mineral and Bone Disorder of Chronic Kidney Disease.¹⁹ Dyslipidemia was defined according to the NCEP III guidelines (National Cholesterol Education Program—Adult Treatment Panel (ATP) III).²⁰

Blood pressure recording

Twenty-four hour ambulatory BP monitoring (ABPM) was performed using Dyna—MAP (Cardios, São Paulo, Brazil) equipment, which was approved by the Association for the Advancement of Medical Instrumentation. Blood pressure measurements were obtained at 20-minute intervals during the day and 30-minute intervals during the night. The participants were instructed to keep their habitual routine during the 24-h period and to pause momentarily during each BP measurement. Dipping (%) was defined as the percent decrease in nighttime systolic-diastolic BP compared with daytime systolic-diastolic BP. When patients exhibited dipping of < 10%, they were defined as non-dippers. Hypertension was defined as a BP > 140/90 mm Hg or the use of antihypertensive medication.

Coronary artery calcification

Patients underwent coronary artery calcification (CAC) quantification with a multislice computed tomography scanner (LightSpeed Pro 16—GE Healthcare, Milwaukee, WI, USA), using a gantry rotation of 0.4 s, collimation of 2.5 mm (slice thickness) and reconstruction time of 6 frames per second. A calcium threshold of ≥ 130 HU (Hounsfield units) was used. The images were scored by a single radiologist who was blinded to the clinical and biochemical information of the patients. As described by Agatston, the calcium score was determined by multiplying the area of each calcified lesion by a weighting factor corresponding to the peak pixel intensity for each lesion.²¹ The sum of each lesion of all the coronary arteries was used for the analysis. The presence

of calcification was defined as a CAC score > 10 AU (Agatston units), and severe calcification was defined as a CAC score of ≥ 400 AU.

Statistical analysis

Data are reported as the mean and s.d., median (interquartile range) or frequency. Groups of hypovitaminosis D were defined according to the median 25(OH)D value. Thus, a 25(OH)D level > 17.2 ng ml⁻¹ was defined as the mild hypovitaminosis D group (M-group) and a 25(OH)D level < 17.2 ng ml⁻¹ was defined as the severe hypovitaminosis D group (S-group). Comparisons of continuous variables were performed using a Student's independent *t*-test and Mann–Whitney *U*-test for normal and skewed data, respectively. Comparisons of proportions were performed using χ^2 analysis or a Fischer's exact test, as appropriate. Multiple linear regression analyses (stepwise) were performed to evaluate the association of the degree of hypovitaminosis D with BP. A *P*-value < 0.05 was considered to be statistically significant. All statistical analyses were performed using SPSS (Statistical Package for the Social Sciences) for Windows version 16.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Table 1 depicts the characteristics of the study participants. The population is mostly composed of elderly patients with a high prevalence of cardiovascular risk factors. Hypertension was found in the majority of the patients (99%), and diabetes was found in almost 60% of the patients. Overweight (BMI 25–29.9 kg m⁻²) and obesity (BMI ≥ 30 kg m⁻²) were found in 33% and 27% of the cohort, respectively. Forty-seven patients (59%) were classified as CKD stage 3, and 33 (41%) were classified as stage 4. Proteinuria was found in 34%, and a CRP > 0.11 mg dl⁻¹ was found in 85% of the patients. At least one lipid profile abnormality was observed in 82% of the patients. Hyperphosphatemia and hyperparathyroidism were observed in 6 and 75% of the patients, respectively.

Although the mean values of both systolic and diastolic pressures were within the normal range, 36% of the patients had high BP. A non-dipper pattern was found in 46% of the patients. The presence of CAC (calcium score > 10 AU) was observed in 57 patients (71%), and 24 (30%) of these patients had severe CAC (calcium score ≥ 400 AU). Compared with the patients without calcification, those with CAC were older (70 ± 9 years versus 54 ± 15 years; *P* < 0.01), and no clinical or laboratorial differences were found between the groups.

Comparisons of the patients according to the degree of hypovitaminosis D are shown in Table 1. The S-group had a higher prevalence of diabetes, proteinuria, serum glucose, iPTH and alkaline phosphatase, and a lower ionized calcium concentration. Additionally, there was a trend towards a higher phosphorus level in this group. As can be seen in Figure 1, the S-group exhibited a higher systolic BP at all time periods (24-hour, nighttime, daytime). No differences were found between the S- and M-group in terms of the diastolic BP and non-dipper pattern. The type and number of antihypertensive drugs in both groups were similar. The prevalence of coronary calcification was 70% in the S-group and 72.5% in the M-group (*P* = 0.80). The calcium score and other clinical and laboratory parameters did not differ between the S and M-group.

In the multiple linear regression analysis, severe hypovitaminosis D was a predictor of 24-h BP, daytime BP and nighttime BP after including a number of confounders, such as age, diabetes, renal function, phosphorus, calcium, iPTH and renin. The final regression models are depicted in Table 2.

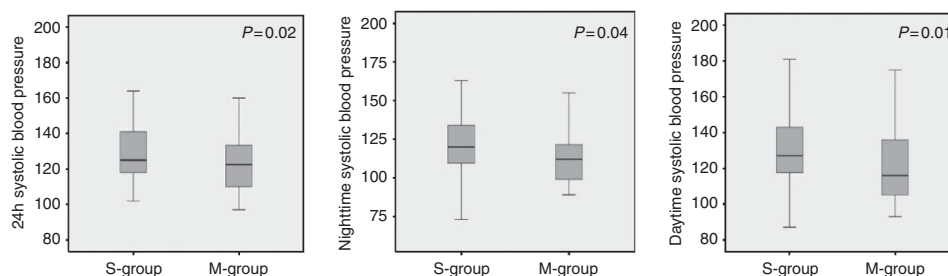
DISCUSSION

This study demonstrated the independent association of the severity of hypovitaminosis D with increased BP in nondialyzed CKD patients. Considering the burden of cardiovascular risk factors

Table 1 Characteristics of the patients according to the degree of hypovitaminosis D

	All (n = 80)	S-group 25 (OH) D < 17.2 (n = 40)	M-group 25 (OH) D > 17.2 (n = 40)	P-value
Age (years)	65 ± 13	64 ± 14	66 ± 12	0.34
Gender (females), n (%)	40 (50)	21 (52)	19 (47)	0.65
Dark skin color, n (%)	35 (48)	18 (45)	17 (42)	0.82
CKD etiology				
Diabetic nephropathy, n (%)	21 (26)	14 (35)	7 (17)	0.14
Hypertensive nephropathy, n (%)	20 (25)	6 (15)	14 (35)	
Unknown, n (%)	17 (21)	7 (17)	10 (25)	
Other, n (%)	22 (28)	13 (33)	9 (23)	
Hypertension, n (%)	78 (97.5)	39 (97.5)	39 (97.5)	1.00
Diabetic, n (%)	39 (58)	25 (62)	14 (35)	0.02
Dyslipidemia, n (%)	49 (61)	35 (87)	31 (77)	0.23
Sedentarism, n (%)	49 (61)	25 (62.5)	24 (60)	0.81
BMI (kg m ⁻²)	27 ± 4.7	27 ± 5.3	27 ± 4.1	0.79
Laboratory parameters				
eGFR (ml min per 1.73 m ²)	28 ± 9	28 ± 11	29 ± 8	0.93
Proteinuria (mg per 24 h)	0.20 (0–0.63)	0.4 (0–1.23)	0 (0–0.4)	0.01
Hemoglobin (g dl ⁻¹)	13.2 ± 1.86	13.1 ± 1.87	13.3 ± 1.87	0.58
Blood glucose (mg dl ⁻¹)	91 (83–111)	100 (85–151)	90 (80–98)	0.02
HDL (mg dl ⁻¹)	44 ± 10.55	45 ± 11	44 ± 10	0.59
LDL (mg dl ⁻¹)	97 (76–122)	97 (74–122)	97 (77–121)	0.88
Triglycerides (mg dl ⁻¹)	143 (103–197)	159 (96–235)	141 (112–174)	0.62
CRP (mg l ⁻¹)	0.36 (0.15–0.91)	0.26 (0.14–0.89)	0.41 (0.16–0.98)	0.45
Ionized calcium (mmol l ⁻¹)	1.30 ± 0.04	1.29 ± 0.05	1.31 ± 0.03	0.01
Phosphorus (mg dl ⁻¹)	3.5 ± 0.63	3.6 ± 0.67	3.3 ± 0.57	0.06
Alkaline phosphatase (UI ⁻¹)	86 ± 29.4	95 ± 33	76 ± 21	0.003
iPTH (pg ml ⁻¹)	100 (70–154)	109 (83–162)	88 (56–142)	0.05
25(OH)D (ng dl ⁻¹)	17.20 ± 5.85	12 ± 2.89	22 ± 3.59	NA
Phosphaturia (mg per 24 h)	419 (323–517)	426 (341–517)	405 (316–519)	0.51
FGF23 (pg ml ⁻¹)	66.3 (43–101)	64 (40–117)	68 (45–102)	0.96
ACE (nmo ml min ⁻¹)	106.5 ± 44.7	111.2 ± 50.9	101.8 ± 37.5	0.35
Renin (nmol ml h ⁻¹)	0.22 ± 0.11	0.22 ± 0.10	0.21 ± 0.12	0.58
Blood pressure (mm Hg)				
24-h systolic	127 ± 17	131 ± 17	123 ± 15	0.02
24-h diastolic	72 ± 10	72 ± 10	71 ± 11	0.68
Nighttime systolic	114 (105–129)	123 (109–135)	112 (99–122)	0.01
Nighttime diastolic	66 ± 12	66 ± 11	65 ± 12	0.51
Daytime systolic	126 ± 22	131 ± 23	121 ± 20	0.04
Daytime diastolic	72 ± 14	73 ± 12	72 ± 16	0.73
Non-dipper, n (%)	36 (46)	18 (50)	18 (50)	0.91
Calcium Score (AU)	98 (1–458)	84 (1–498)	106 (0–427)	0.88
CAC (≥ 10 AU), n (%)	57 (71%)	28 (70%)	29 (72.5%)	0.80

Abbreviations: ACE, Angiotensin converting enzyme; BMI, body mass index; CAC, coronary artery calcification; CKD, chronic kidney disease; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; FGF23, fibroblast growth factor 23; iPTH, parathyroid hormone.

**Figure 1** Systolic blood pressure in patients with severe hypovitaminosis D and with mild hypovitaminosis D.

associated with poor vitamin D status that has been evidenced in the past years, understanding the cardiovascular consequences promoted by severe hypovitaminosis D in the early stages of CKD is important.

Hypovitaminosis D is very common within the general population and is highly prevalent among CKD patients, even in earlier stages of the disease. Previous studies have demonstrated that the prevalence of hypovitaminosis D varies from 40 to 86% among nondialyzed patients^{22–24} and from 77 to 100% among patients on dialysis.^{24–26} A recent study from NECOSAD observed that 87% of dialysis patients

had hypovitaminosis D, and ~30% of these patients had severe hypovitaminosis D, which was defined in the study as a 25(OH)D level < 10 ng ml⁻¹.⁷ Similar proportions of severe hypovitaminosis D have been found among patients in the nondialysis stages.²⁷ Some groups are more susceptible to vitamin D deficiency, including the elderly, diabetics and obese patients.²⁸ Elderly patients usually have reduced exposure to sunlight, reduced mobility and lower cutaneous synthesis of vitamin D. Diabetics have a higher degree of proteinuria with subsequent loss of the vitamin D carrier. In addition, autonomic

Table 2 Multiple linear regression analysis with systolic blood pressure as a dependent variable

	β	95% CI	P-value
<i>Panel A: 24-h SBP</i>			
S-group	-9.53	-16.93–-2.13	0.012
Age	0.35	0.07–0.62	0.013
Constant	109.44	91.23–127.64	<0.001
<i>Panel B: Daytime SBP</i>			
S-group	-16.35	-28.88–-3.82	0.011
Constant	135.53	117.74–153.31	<0.001
<i>Panel C: Nighttime SBP</i>			
S-group	-9.98	-18.89–-1.08	0.028
Age	0.44	0.11–0.77	0.008
Constant	92.60	70.67–114.52	<0.001

Abbreviations: CI, confidence interval; SBP, systolic blood pressure.

neuropathy, which may lead to a deficit in absorption, contributes to a deficiency of vitamin D.²⁹

One of the most accepted theories that associates obesity with hypovitaminosis D is that vitamin D is a fat-soluble substance that can easily be sequestered and stored in the adipose tissue.³⁰ Accordingly, a recent study indicated that obesity and diabetes are the most important risk factors for hypovitaminosis D in nondialyzed CKD patients.³¹ In the present study, although age and the prevalence of overweight patients did not differ between the S- and M-group, the frequency of diabetes was substantially higher in the former group.

Increasing evidence exists supporting the premise that low 25(OH)D levels result in the development of cardiovascular complications in the general population as well as in CKD patients.¹⁰ Our study is in agreement with the available studies in non-CKD subjects and shows the association between low vitamin D levels and high BP.³² The Third National Health and Nutrition Examination Survey (NHANES III) included >12 000 participants, found an inverse correlation with 25(OH)D levels and BP.¹⁶ In a prospective fashion, the Health Professionals Follow-up Study and The Nurses' Health Study showed that women with 25(OH)D levels <15 ng ml⁻¹ had three-fold higher risk of hypertension compared with women with levels \geq 30 ng ml⁻¹. In men, the risk was almost six times higher.³³

Although the exact pathways bridging hypovitaminosis, vitamin D and hypertension are not completely established, some mechanisms have been suggested. The first and most important mechanism is the regulation of the renin-angiotensin-aldosterone system. Blood pressure is mainly controlled by the renin-angiotensin-aldosterone system, which is known to affect blood vessel tone, extracellular fluid volume and electrolyte homeostasis. Vitamin D acts as a negative endocrine regulator of renin biosynthesis. Vitamin D receptor-knockout mice were found to have a high production of renin and angiotensin II and developed hypertension and cardiac hypertrophy.³⁴ The suppression of the renin gene is independent of calcium metabolism. In normal rats, the deficiency of vitamin D stimulates the expression of renin, whereas the injection of 1,25(OH)D₂ reduces the synthesis of renin.³⁴ In a cohort study with patients undergoing coronary angiography, the multivariate analysis showed that plasma renin values increased steadily with declining levels of 25(OH)D.³⁵ In the present study, renin was not a predictor of BP.

Notably, the independent relationship between vitamin D and hypertension is not a homogeneous finding.^{36,37} In the Tromsø study,

no difference was found in the risk of hypertension when comparing extreme serum 25(OH)D levels in subjects who were normotensive.³⁸ Some factors related to the study design and, most importantly, to the methods of hypertension diagnosis are among the potential reasons for the divergent findings.³⁹ In the majority of previous studies on this topic, BP was assessed by a simple measurement or was self-reported. In the present study, BP was assessed by ABPM, which includes diurnal variations in BP and better information on the levels of mean arterial pressure during daily life activities. In addition, ABPM correlates best with hypertension-induced target organ damage.⁴⁰ Data focusing on this issue are scarce in nondialyzed CKD population. Apart from our data, only one observational study found an inverse association of 25(OH)D levels with systolic BP by ABPM in nondialyzed patients.²⁷

In the current study, we also aimed to investigate the association of 25(OH)D with CAC. However, contrary to some authors^{11,12} and in agreement with others,^{14,23} we did not find any link between 25(OH)D and CAC. Calcification is very common in CKD patients and is associated with high mortality in this group.⁴¹ The pathogenesis of calcification in CKD is not completely understood, but it is certainly multifactorial. It is an active process that involves promoting and inhibiting factors of calcification. The use of some vitamin D analogs often results in elevated calcium and phosphorus levels, which favors vascular calcification.⁴² On the other side, vitamin D deficiency could lead to calcification.¹¹ Some studies have suggested that some of the actions of vitamin D may protect vessels against vascular calcification, such as the downregulation of pro-inflammatory cytokines and the upregulation of anti-inflammatory cytokines.⁴³ In our study, no difference in the prevalence of CAC was observed between the S- and M-group. Of note, the prevalence of CAC was equally elevated in our patients regardless of the degree of hypovitaminosis D. London *et al.*¹⁴ observed that 25(OH)D was negatively correlated with aorta stiffness, but not with vascular calcification in hemodialysis patients. Interestingly, Barreto *et al.*⁴⁴ evaluated nondialysis patients and found that low 25(OH)D levels affected mortality independently of vascular calcification. Other negative effects of vitamin D depletion on the cardiovascular system have also been described, including the development of congestive heart failure and left ventricular hypertrophy. Unfortunately, in our study, those examinations were not performed.

Our study has some limitations, such as the cross-sectional design of the study, which does not provide cause-effect conclusions. In addition, our findings are based on a selected sample of patients with hypovitaminosis D and do not include patients with higher levels of 25(OH)D. However, the impact of severe hypovitaminosis D on increased BP was observed by demonstrating the impact of the severity of poor vitamin D status on an important cardiovascular risk factor in the earlier stages of CKD.

In conclusion, our study of nondialyzed CKD patients demonstrated that severe hypovitaminosis D is associated with increased systolic BP, which was measured by a gold-standard method. The degree of hypovitaminosis D was not related to the presence of coronary artery calcification, which was equally elevated in both the severe and mild hypovitaminosis D groups. Studies are necessary to evaluate the benefits of vitamin D supplementation on cardiovascular morbidity and mortality in the earlier stages of CKD. More clarity will come from the ongoing cholecalciferol supplementation study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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