

Title

Vascular function and cholecalciferol supplementation in CKD: A self-controlled case series

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Abstract

Vitamin D deficiency is common and associated with mortality in chronic kidney disease (CKD) patients. Cardiovascular disease (CVD) is the commonest cause of mortality in CKD patients. In a randomized, double blind, placebo controlled trial, we have recently reported favorable effects of vitamin D supplementation on vascular & endothelial function and inflammatory biomarkers in vitamin D deficient patients with non-diabetic stage 3-4 CKD (J Am Soc Nephrol 28: 3100–3108, 2017). Subjects in the placebo group who had still not received vitamin D after completion of the trial received two oral doses 300000 IU of oral cholecalciferol at 8 weeks interval followed by flow mediated dilatation (FMD), pulse wave velocity (PWV), circulating endothelial and inflammatory markers (E-Selectin, vWF, hsCRP and IL-6), 1,25 (OH)₂D, iPTH and iFGF-23 assessment at 16 weeks. 31 subjects completed this phase of the study. Last values recorded in the preceding clinical trial were taken as baseline values. Serum 25(OH)D and 1,25(OH)₂D increased and FMD significantly improved after cholecalciferol supplementation [mean change in FMD%: 5.8% (95% CI: 4.0-7.5%, p<0.001]. Endothelium independent nitroglycerine mediated dilatation, PWV, iPTH, iFGF-23 and IL-6 also showed favorable changes. The data further cement the findings of beneficial effects of correction of vitamin D deficiency on vascular function.

Highlights

- Cholecalciferol supplementation in patients with CKD and vitamin D deficiency improved endothelial function (FMD) and vascular stiffness (PWV)
- NMD also improved which is a novel finding and needs to be explored further
- PTH decreased with cholecalciferol supplementation
- FGF-23 also decreased with cholecalciferol supplementation unlike previously reported increase with use of activated vitamin D

Keywords

Vitamin D, chronic kidney disease, cardiovascular disease, flow mediated dilatation, pulse wave velocity

Abbreviations

1,25(OH)₂D 1,25-Dihydroxyvitamin D

25(OH)D	25-Hydroxyvitamin D
AKI	Acute kidney injury
BAP	Bone-specific alkaline phosphatase
CKD	Chronic kidney disease
CVD	Cardiovascular disease
CTX-1	Serum C-terminal cross-linked collagen type I telopeptides
EIA	Enzyme immunoassay
ELISA	Enzyme linked immunosorbent assay
FMD	Endothelium dependent flow mediated dilatation
hs-CRP	High sensitivity C reactive protein
iFGF-23	Intact fibroblast growth factor-23
IL-6	Interleukin-6
iPTH	Intact parathormone
NMD	Endothelium independent nitroglycerine mediated dilatation
PWV	Pulse wave velocity
RAAS	Renin angiotensin aldosterone system
SAP	Serum total alkaline phosphatase
vWF	Von willebrand factor

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1. Introduction

Vitamin D deficiency is common in patients with chronic kidney disease (CKD) [1]. Unlike normal individuals in which vitamin D deficiency is ascribed to reduced sun exposure or intake, patients with CKD have an additional risk factor as kidney is the major site for final conversion of various dietary forms of vitamin D to active form i.e. 1,25-dihydroxyvitamin D [1,25(OH)₂D]. Cardiovascular disease (CVD) is the most common cause of mortality in patients with CKD and has been associated with vitamin D deficiency [2, 3]. Till recently, the main therapeutic target of replenishing vitamin D in CKD has been maintenance of altered bone and mineral health. However, observational data linking vitamin D deficiency to hypertension, CVD and mortality in CKD [4] has prompted investigations into effect of vitamin D replenishment on cardiovascular health. Recently we published the results of a randomized, double blind, placebo controlled clinical trial in which 120 adult subjects with nondiabetic CKD stage G3–4 and vitamin D deficiency (serum 25(OH)D ≤20 ng/ml) were randomized to receive either two directly observed oral doses of cholecalciferol (300,000 IU) or matching placebo at baseline and 8 weeks [5]. High bolus doses ensure compliance and have been shown to be effective in increasing circulating 25(OH)D levels [6, 7]. The intervention significantly improved vascular function as shown by increased endothelium-dependent brachial artery flow-mediated dilation (FMD) in the cholecalciferol group [between-group difference in mean change (5.49%; 95% confidence interval, 4.34% to 6.64%; P,0.0010 [5]. The results were concordant with the improvement in FMD that has been reported earlier with use of paricalcitol in patients with CKD stage 3-4 in Paricalcitol and Endothelial Function in Chronic Kidney Disease (PENNY) trial [8]. In addition, endothelium-independent nitroglycerine mediated dilatation (NMD) and pulse wave velocity (PWV) also improved after cholecalciferol supplementation. As cholecalciferol supplementation significantly improved vascular function in our previous clinical trial, we decided to investigate whether it would similarly affect vascular function in subjects who were enrolled in the placebo arm and had not received it.

In this paper, we report the change in FMD, PWV, NMD and biomarkers in subjects who were enrolled in the placebo arm and received cholecalciferol supplementation after completion of clinical trial and removal of blinding.

2. Material and methods

2.1 Study setting, design, population and enrolment

The present study is a self-controlled case series which reports follow up of subjects who were enrolled in the placebo arm of the randomized, double blind, placebo controlled clinical trial (CTRI/2013/05/003648) and who subsequently received vitamin D supplementation in similar manner after completion of this clinical trial [5]. After completion of clinical trial and removing of blinding, subjects in the placebo group who were clinically stable and had still not received any form of vitamin D supplementation after exit from the clinical trial were eligible for enrolment.

2.2 Study intervention and follow up

All enrolled subjects prospectively received two directly observed oral doses of cholecalciferol (300,000 I.U. each) 8 weeks apart. This was followed by measurements at 16 weeks as described below.

2.3 Study measurements

The last values recorded in clinical trial were extracted from trial database and taken as baseline values for the purpose of this study [5]. One time measurements done as part of this study (after cholecalciferol supplementation) were taken as follow up measurements. FMD and NMD were measured as described previously [5] in fasting state using Philips IU22 xMatrix ultrasound system (Philips, Cambridge, MA), Philips IU22 L12–5 linear transducer with extended operating frequency range of 5–12 MHz. Recordings were analyzed in a random order by a blind independent investigator, using Brachial Analyzer for Research 6 (Vascular Research Tools 6; Medical Imaging Applications LLC, Coralville, IA), an automated continuous edge detection and wall tracking software. Carotid-femoral PWV were calculated using the SphygmoCor CPV system (AtCor Medical, Inc., Itasca, IL) on the same day as the brachial artery FMD assessment.

Plasma and serum samples at follow up visit were stored at -80°C. Serum 1,25(OH)₂D [Enzyme immunoassay (EIA); Immunodiagnostic Systems], serum intact parathormone (iPTH) (EIA; Calbiotech Inc., El Cajon, CA), serum intact fibroblast growth factor-23 (iFGF-23) [second-generation enzyme linked immunosorbent assay

(ELISA; Immutopics, Inc., Athens, Ohio], serum E-selectin (Quantikine solid-phase sandwich ELISA; R&D Systems, Minneapolis, MN), serum high sensitivity C reactive protein (hs-CRP) (Quantikine solid-phase sandwich ELISA; R&D Systems), serum interleukin-6 (IL-6) (Quantikine solid-phase sandwich ELISA; R&D Systems), and plasma von Willebrand factor (vWF) (IMUBIND ELISA; Sekisui Diagnostic, Lexington, MA) were analyzed in all samples.

2.4 Study outcomes

The main outcome was change in brachial artery FMD. Other outcome measures were changes in PWV and levels of serum 1,25(OH)₂D, serum iPTH, serum FGF-23, serum E-selectin, serum hs-CRP, serum IL-6, and plasma vWF.

2.5 Statistical considerations

All enrolled patients with non-missing outcome data were included in the analysis. Categorical data were expressed as frequencies and continuous data were expressed as mean (\pm standard deviation) or median (interquartile range) whichever was appropriate. Paired t test or Wilcoxon signed-rank test were used as appropriate for comparing baseline and follow up measurements. Two-tailed P values ≤ 0.05 were considered statistically significant. An analysis was conducted using SPSS software for Macintosh, version 21.0 (IBM SPSS, Chicago, IL).

Data from clinical trial (mean change in FMD%: -0.07 ± 2.45 and 5.42 ± 3.70 in the placebo and cholecalciferol groups, respectively) has shown that a sample size of 6 subjects would have been adequate to detect any significant difference of cholecalciferol supplementation on FMD in crossover or self-controlled design with 80% power and two-sided alpha of 0.05.

3. Results

3.1 Study subjects

Between October-December 2015, 59 subjects who had completed the trial protocol in placebo group were called. A total of 52 subjects responded, out of whom 18 had received cholecalciferol after completing the study and one had developed AKI associated with tropical acute febrile illness and hence were excluded. Remaining 33 eligible subjects consented and received intervention. However, only 31 subjects

completed study measurements at follow up. The mean duration between baseline and follow up measurements was 42.7 ± 10.6 (range 22-56) weeks.

3.2 Baseline and follow up measurements

The demographic characteristics of study population are shown in table 1. Two thirds were males and cause of CKD was unknown in 50% of subjects. Around 94% were hypertensive and 13% had family history of kidney disease. Table 2 shows selected biochemical parameters, biomarkers and vascular function measurements at baseline and follow up in study subjects. Serum creatinine remained similar over the study period. Serum calcium showed an increasing trend but the results did not reach statistical significance [mean change in serum calcium: 0.61 mg/dl (95% CI: -0.02 to 1.24 mg/dl), $p=0.06$]. Serum 25-Hydroxyvitamin D [25(OH)D] and 1,25(OH)₂D levels increased significantly after cholecalciferol supplementation. FMD significantly improved after cholecalciferol supplementation [mean change in FMD%: 5.8% (95% CI: 4.0-7.5%), $p<0.001$]. Similarly, NMD [mean change in NMD%: 5.1% (95% CI: 2.8 to 7.5%), $p<0.001$] and PWV [mean change in PWV: -0.76 m/s (95% CI: -1.3 to -0.25m/s), $p=0.005$] also changed favorably. Serum iPTH [mean change in iPTH: -92.3 ng/ml (95% CI: -172.9 to -11.7 ng/ml), $p=0.019$], IL-6 [mean change in IL-6: -1.7 pg/ml (95% CI: -2.8 to -0.5 pg/ml), $p=0.002$] and iFGF-23 [mean change in iFGF-23: -21.5 pg/ml (95% CI: -46.8 to 3.8 pg/ml), $p=0.005$] significantly decreased after supplementation.

4. Discussion

The improvement in endothelial function and vascular stiffness after cholecalciferol supplementation as shown by favorable changes in FMD, NMD and PWV in this self-controlled phase is concordant with the results reported in the preceding clinical trial [5]. Reproduction of similar results is a major advantage of the present study. We also observed a decrease in iPTH and IL-6 levels, as well as decrease in iFGF-23 levels which is in line with the trend towards lower iFGF-23 levels after cholecalciferol supplementation in the intervention group in the preceding clinical trial [5]. Serum calcium and phosphorus levels remained similar though serum calcium levels did show an increasing trend which emphasizes the need of monitoring serum calcium levels while on cholecalciferol therapy.

Since our study was published, two other randomized controlled trials have been published which explored the impact of various forms of vitamin D supplementation on short term surrogate cardiovascular outcomes. In the randomized, placebo controlled trial by Levin *et al.* that evaluated the effect of calcifediol (5000 IU thrice weekly) or calcitriol (0.5 µg) thrice weekly on PWV in 119 stable subjects with CKD stage 3b-4 on renin angiotensin aldosterone system (RAAS) blocking drugs irrespective of circulating 25(OH)D levels, PWV increased in placebo group, remained similar in calcitriol group and decreased in calcifediol group at 6 months [9]. When calcifediol and calcitriol groups were combined together, PWV significantly declined in combined vitamin D intervention group as compared to the placebo [9]. In another randomized controlled trial. that investigated the effect of oral cholecalciferol (2000 IU daily) or calcitriol (0.5 µg daily) on FMD in subjects with CKD stage 3b-4 and vitamin D levels <30 ng/ml, no significant difference in changes in FMD were observed either within or between groups at 6 months [10]. The lack of a placebo arm in this trial prevents a meaningful comparison with our results [5]. Racial differences, relatively younger subjects, exclusion of subjects with diabetes, higher mean dose of cholecalciferol (approximately 5000 IU daily), selection of subjects with relatively severe vitamin D deficiency [25(OH)D levels ≤20 ng/ml] and shorter duration of follow up in our studies might be responsible for the observed differences. All these aforementioned studies had small sample sizes, different designs and interventions, but together with previously published small studies and clinical trials, these results do suggest possible short term favorable modulation of vascular function by vitamin D supplementation [8] [11] [12] [13] [14].

Vitamin D supplementation in subjects with CKD is usually advocated in the form of activated vitamin D compounds like calcitriol or paricalcitol as it is presumed that native forms of vitamin D would not get converted to 1,25(OH)₂D, on account of decreased functioning renal parenchymal mass in CKD. However, our results suggest that even in patients with CKD stage G3-4 and vitamin D deficiency there is a significant increase in circulating 1,25(OH)₂D levels after cholecalciferol supplementation [serum 25(OH)D levels at baseline and follow up: 15.7±9.8 versus 34.7±14.5 ng/ml, respectively, p<0.001; serum 1,25(OH)₂D levels at baseline and follow up: 21.4±20.2 versus 40.1±18.7 pg/ml, respectively, p<0.001]. At present, it is difficult to determine whether

the effects after cholecalciferol supplementation in our current study and preceding clinical trial are independent or mediated through increase in $1,25(\text{OH})_2\text{D}$.

We have also shown that cholecalciferol supplementation decreased iPTH and bone turnover markers. In a secondary analysis of our clinical trial, we have shown that serum total and bone-specific alkaline phosphatase (SAP, BAP) and serum C-terminal cross-linked collagen type I telopeptides (CTX-1) were significantly reduced in cholecalciferol group [15]. This suggests that just cholecalciferol supplementation was able to suppress secondary hyperparathyroidism and improve bone turnover. The magnitude of decrease in iPTH in our study and preceding clinical trial was similar to what has been reported with use of activated forms of vitamin D by [8]. However, the decrease in iFGF-23 in our study is in contrast to what has been observed with use of activated vitamin D compounds. In the trial by Kendrick *et al.*, FGF-23 significantly increased in both calcitriol and cholecalciferol groups at 6 months [10]. The authors did not relate this to vitamin D repletion and suggested that this could be reflective of increase in FGF-23 with time in CKD population. Paricalcitol use (2 $\mu\text{g}/\text{day}$) was associated with increase in FGF-23 levels at 12 weeks in randomized, placebo controlled PENNY trial that showed improvement in FMD in subjects with CKD stage 3-4 and PTH >65 pg/ml [8] [16]. These observations need further exploration as increasing levels of FGF-23 have been associated with adverse cardiovascular outcomes in CKD [17]. Also, this suggests that there could be mechanistic differences between vitamin D supplementation by native or activated vitamin D compounds in CKD.

A novel and potentially interesting finding of our RCT was the increase in NMD, which was also noted in this phase. As vascular endothelium and smooth muscle cell functions are intricately linked and vitamin D receptor expression is well known in both types of tissue, it would be interesting to investigate whether these results are due to independent modulation of vascular smooth muscle cell function over and above the endothelium.

Long term and well-designed studies are needed to investigate the effect of vitamin D supplementation on long term cardiovascular risk reduction, examination of the magnitude of effect across various patient subgroups, differences between effects of

activated and native forms of vitamin D in CKD, effect modification due to degree of vitamin D deficiency and the possible mechanisms of improvement in vascular function beyond modulation of endothelial function.

5. Conclusions

The present study reaffirms the favorable effects of cholecalciferol supplementation on vascular function in subjects with CKD and vitamin D deficiency. Though uncontrolled observational design is a major limitation, the fact that it replicates the effects seen in the original clinical trial does add to the limited evidence that we have in this regard.

Tables

Table 1: Baseline and demographic characteristics of study population (n=31)

Parameter	Value
Gender (M/F)	21/10
Age (years)	45.39±11.80
Body mass index (kg/m ²)	24.64±4.40
SBP (mmHg)	128.30±12.34
DBP (mmHg)	80.90±14.37
Fasting blood sugar (mg/dl)	88.06±13.08
Duration of CKD (months)	37.8±46.8
Smoking history, N (%)	4 (12.9)
Hypertension, N (%)	29 (93.5)
Family history of kidney disease	4 (12.9)
Family history of diabetes	9 (29.0)
Family history of hypertension	12 (38.7)
Cause of CKD	
Unknown	16 (51.6)
Chronic interstitial nephritis	6 (19.4)
Chronic glomerulonephritis	4 (12.9)
Polycystic kidney disease	3 (9.6)
Others	2 (6.5)
Medications	
ACE inhibitor/ARB	19 (61.3)
Beta Blockers	7 (22.6)
Statins	9 (29.0)
Hypouricemic agents	9 (29.0)
Calcitriol	1 (3.2)
Calcium based phosphate binders	1 (3.2)
Sodium bicarbonate	20 (64.5)
Calcium-channel blockers	20 (64.5)

Data presented as mean ± SD or number (percentage). SBP, systolic BP; DBP, diastolic BP; ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blockers.

Table 2: Change in biochemical parameters, blood pressure, vascular function and biomarkers in study population (n=31)

Parameter	Baseline	Follow up	P value
SBP (mmHg)	128.30±12.34	126.20±19.43	0.496
DBP (mmHg)	80.90±14.37	80.06±11.50	0.749
FMD (%)	8.0±2.7	13.8±4.3	<0.001
NMD (%)	11.0±3.2	16.1±5.9	<0.001
FMD/NMD	0.77±0.31	0.93±0.42	0.107
PVW (m/sec)	8.0±1.3	7.3±1.3	0.005
AI (%)	16.4±12.6	18.0±13.8	0.417
Serum Urea (mg/dl)	62.06±27.53	61.08±27.40	0.903
Serum creatinine	2.36±1.73	2.24±0.96	0.678
Uric Acid (mg/dl)	7.47±2.11	7.12±1.86	0.195
Serum total protein (mg/dl)	7.32±0.71	6.96±1.46	0.155
Serum albumin (mg/dl)	4.42±0.68	4.09±0.87	0.079
Calcium (mg/dl)	8.42±1.20	9.00±0.59	0.056
Inorganic phosphate mg/dl)	3.40±1.02	3.35±0.87	0.752
Alkaline phosphatase (mg/dl)	124.8±47.6	121.6±39.5	0.647
Total Cholesterol (mg/dl)	160.50±43.38	159.20±43.64	0.784
Triglyceride (mg/dl)	145.50±66.64	175±102.3	0.241
25(OH) Vit D (ng/ml)	15.7±9.8	34.7±14.5	<0.001
1,25(OH) ₂ Vit D (pg/ml)	21.4±20.2	40.1±18.7	0.001
iPTH (ng/ml)*	186 (101, 293)	119 (87-172)	0.019
E-selectin (ng/ml)	54.2±22.0	51.6±18.0	0.316
IL-6 (pg/ml)*	3.3 (2.3, 5.5)	2.7 (1.9-4.5)	0.002
hs-CRP (mg/L)	3.0±2.9	3.3±2.5	0.775
iFGF-23 (pg/ml)*	52.3 (42.9, 78.4)	32.0 (27.4-50.6)	0.005
vWF (mU/ml)	1076±379	1225±253	0.103

Data presented as mean±SD or median (25th, 75th percentile). 25(OH)D, 25-hydroxyvitamin D; 1,25(OH)₂D, 1,25-dihydroxyvitamin D; AI, Augmentation index; FMD, endothelium dependent flow-mediated dilation; hs-CRP, high sensitivity C reactive protein; IL-6, Interleukin 6; iFGF-23, intact fibroblast growth factor 23; iPTH,

intact para- thyroid hormone; NMD, endothelium independent nitroglycerine mediated dilation; vWF, von Willebrand factor. *Compared using Wilcoxon signed-rank test

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