Original Article

Effect of Vitamin D Supplementation on Serum Hepcidin Levels in Non-Diabetic Chronic Kidney Disease Patients

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Abstract

Introduction: Vitamin D deficiency and anemia frequently coexist. Moreover, vitamin D deficiency is found to play a role in chronic kidney disease (CKD)-associated anemia. We investigated the effect of cholecalciferol on serum hepcidin levels in vitamin D–deficient, non-diabetic individuals with CKD in a randomized, double-blind, placebo-controlled trial. **Methods:** This study was performed on stored samples of our previously published randomized, double-blind, placebo-controlled trial of cholecalciferol supplementation in non-diabetic patients with stage III–IV CKD and vitamin D deficiency. Stable patients of either sex, aged 18–70 years, with non-diabetic stage III–IV CKD (estimated glomerular filtration rate between 15 and 60 ml/min/1.73 m^2), and having serum 25-hydroxyvitamin D_3 [25(OH) D] levels ≤20 ng/ml were included. Participants received either two directly observed oral doses of cholecalciferol (300,000 IU) or matching placebo at baseline and at eight weeks. Follow-up was done at 16 weeks. Serum hepcidin levels were analyzed at baseline and at 16 weeks. **Results:** A total of 120 CKD patients were enrolled. Serum 25(OH) D levels were similar in the placebo and cholecalciferol groups at baseline (13.21 ± 4.78 ng/ml and 13.40 ± 4.42 ng/ml; P = 0.88). After 16 weeks, the serum 25(OH) D levels were found to be increased in the cholecalciferol group but not in the placebo group (between-group difference in mean change 23.40 ng/ml; 95% CI: 19.76 to 27.06; P < 0.001). Serum hepcidin levels were similar at baseline (median [IQR]: 33.6 [8.6–77.8] ng/ml vs. 24.6 [9.3–70.7] ng/ml, P = 0.903) and did not vary between groups at 16 weeks (median [IQR]: 41.5 [10.9–75.0] ng/ml vs. 34.8 [12.3–63.75] ng/ml, P = 0.703). **Conclusion:** Our study provides preliminary data based on which a larger adequately powered clinical trial can be conducted to conclusively assess the impact of vitamin D supplementation on hepcidin levels and anemia in patients with CKD and vitamin D deficiency.

Keywords: *Cholecalciferol, chronic kidney disease, hepcidin*

Introduction

Iron deficiency is commonly associated with anemia in patients with chronic kidney disease (CKD). Hepcidin, a small cysteine-rich cationic peptide and an important regulator of iron metabolism, was elevated in patients with CKD. Encoded by the hepcidin antimicrobial peptide (HAMP) gene, hepcidin is produced in the liver and negatively regulates iron uptake by suppressing the post-translational expression of cellular iron transporter ferroportin by causing its internalization. Elevated levels of hepcidin block iron absorption in the gut and iron efflux from macrophages and hepatocyte stores, leading to reduced iron bioavailability for erythropoiesis. Given its importance, new treatment strategies[1] that target the hepcidin–ferroportin axis are being developed for the treatment of anemia in CKD.

Vitamin D deficiency has been postulated to play a role in CKD-associated anemia.[2] Experimental data suggest that insufficient 25-hydroxyvitamin D_3 [25(OH)D] levels leads to decreased production of local calcitriol in the bone marrow, limiting erythropoiesis. Vitamin D may also directly affect the proliferation of burst forming unit–erythroid cells. The immunomodulatory effect of vitamin D is key to the systemic production of cytokines that suppress the anemia-specific inflammatory pathways.[3–7] Vitamin D has been shown to reduce hepcidin levels in healthy adults and in people with CKD.[8,9] This mechanism involves regulation of hepcidin and ferroportin expression, as well as pro-hepcidin cytokines, interleukin-6 (IL-6), and interleukin-1 beta (IL-1β) release by

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vitamin D.[10] Hence, treatment with vitamin D may reduce serum hepcidin levels, and thereby have a salutary effect on the anemia in CKD. Studies till date, however, have shown that vitamin D does not reduce serum hepcidin concentrations in participants with CKD.[11,12] Based on this background, we investigated the effect of vitamin D (cholecalciferol) on serum hepcidin levels in vitamin D–deficient, non-diabetic individuals with CKD in a randomized, double-blind, placebo-controlled trial.

Methods

Study design and study participants

This study was performed on stored samples of our previously published randomized, double-blind, placebo-controlled trial of cholecalciferol supplementation in non-diabetic patients with stage III–IV CKD and vitamin D deficiency.[13] The study was performed at the Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, India. It was approved by the institute's ethics committee and registered with the Clinical Trials Registry of India (CTRI/2013/05/003648). Stable patients of either sex aged 18–70 years, with non-diabetic stage III–IV CKD [estimated glomerular filtration rate (eGFR) between 15 and 60 ml/min/1.73 m^2] and vitamin D deficiency [serum 25(OH)D ≤20 ng/ml] were included in the study. Patients with heart failure, experiencing pregnancy, having present or past malignancy, or who had taken vitamin D supplementation within the past 30 days were excluded. Informed consent was taken from each participant before enrollment and randomization in 1:1 ratio by a computer-generated Bernoulli random number table. Each participant received either two directly observed oral doses of cholecalciferol (300,000 IU) or matching placebo at baseline and at eight weeks. Follow-up was done 16 weeks after baseline.

Serum 25(OH)D levels at baseline and at 16 weeks were measured by enzyme immunoassay (EIA, Immunodiagnostic Systems, UK). Investigations including serum creatinine, calcium, inorganic phosphorous, lipid profile, uric acid, and blood hemoglobin were done at baseline and at 16 weeks. Serum levels of hepcidin were analyzed at baseline and at 16 weeks by enzyme-linked immunosorbent assay (ELISA; Quantikine® ELISA kit, R&D, Minneapolis, MN, USA). Intra-assay and inter-assay coefficient of variation of this kit was 3.2%–4.3% and 6.2%–11%, respectively.

Outcomes

The outcome of this secondary analysis was change in serum hepcidin levels between the groups. Secondary outcome of interest was the between-group change in serum hemoglobin levels.

Statistical analysis

Characteristics between the groups were compared by using student's *t* test for normally distributed continuous variables or using the Mann–Whitney *U* test if the distribution was skewed. Changes in serum hepcidin levels and other clinical parameters within the group after 16 weeks were compared using paired *t* test or the Wilcoxon signed-rank test. Chi-squared test or Fisher's exact test was used to compare categorical variables. All data are presented as mean ± standard deviation, mean change (95% confidence interval), and median (25^{th}, 75^{th} percentile), as appropriate. Two-tailed *P* value less than 0.05 were considered statistically significant. The IBM SPSS Statistics software for Macintosh, version 21.0 (IBM Corp., Armonk, NY, USA) was used for the analysis.

Results

A total of 120 CKD patients were enrolled in the clinical trial. There were no between-group differences in the baseline characteristics with respect to demographic details and causes of CKD [Supplementary Table 1].[13] Serum 25(OH) D levels were similar in the placebo and the cholecalciferol group at baseline (13.21 ± 4.78 ng/ml and 13.40 ± 4.42 ng/ml; *P* = 0.88). After 16 weeks, the serum 25(OH) D levels increased in the cholecalciferol group but not in the placebo group (between-group difference in mean change was 23.40 ng/ml (95% CI: 19.76 to 27.06; *P* < 0.001) [Table 1]. Serum hepcidin levels were similar at baseline (median [IQR]: 33.6 (8.6–77.8) ng/ml vs. 24.6 [9.3–70.7] ng/ml, *P* = 0.903) and did not vary between groups after 16 weeks (median [IQR]: 41.5 (10.9–75.0) ng/ml vs. 34.8 [12.3–63.75] ng/ml, *P* = 0.703) [Figure 1a]. The between-group difference in mean change at 16 weeks was 2.71 ng/ml (95% CI: −17.06 to 11.64; *P* = 0.709) [Table 1]. Hemoglobin levels were similar at baseline (12.02 ± 1.94 mg/dl vs. 11.97 ± 1.69 mg/dl, *P* = 0.947) and did not change after 16 weeks in either group (between-group difference in mean change; 0.21 mg/dl, 95% CI: −0.22 to 0.63, *P* = 0.340) [Figure 1b]. Within-group difference and between-group difference of other biochemical parameters are shown in Table 1.

Discussion

In the current study, cholecalciferol supplementation did not lead to any significant change in serum levels of hepcidin and hemoglobin in non-diabetic patients with stage III–IV CKD and vitamin D deficiency despite there being a significant elevation in circulating vitamin D levels. As shown previously,[16] the increase was physiologically relevant and corrected the markers of bone metabolism.

Hepcidin is a potent regulator of iron–ferroportin axis as it suppresses the expression of ferroportin post translationally, which is the only exporter of intracellular iron, thus leading to anemia by making iron unavailable

Table 1: Change in serum biomarkers and biochemical parameters at 16 weeks

	Placebo (*n*=59)		Vitamin D (*n*=58)		Between group difference	*P*
	Mean change (95% CI)	*P*	Mean change (95% CI)	*P*	Difference in mean change (95% CI)	
Hepcidin (ng/ml)	0.35 (−9.47 to 10.18)	0.943	−2.36 (−13.04 to 8.32)	0.66	−2.71 (−17.06 to 11.64)	0.709
Hemoglobin (g/dl)	−0.22 (−0.53 to 0.10)	0.177	−0.01 (−0.30 to 0.28)	0.937	0.13 (−0.14 to 0.39)	0.340
25(OH) D (ng/ml)	1.51 (−0.46 to 3.48)	0.131	24.91 (21.77 to 28.06)	<0.001	23.40 (19.76 to 27.06)	<0.001
Calcium (mg/dl)	−0.48 (−0.76 to−0.19)	0.001	0.21 (−0.05 to 0.46)	0.113	0.17 (0.08 to 0.26)	0.001
Inorganic phosphate (mg/dl)	−0.30 (−0.67 to 0.08)	0.116	0.19 (−0.19 to 0.59)	0.311	0.16 (−0.12 to 0.33)	0.070
Alkaline phosphate (mg/dl)	9.40 (−2.08 to 20.89)	0.106	−10.85 (−20.70 to−1.01)	0.031	−20.25 (−35.14 to−5.38)	0.008
eGFR (min/ml/1.73 m^2)	1.57 (−0.93 to 4.01)	0.214	1.42 (−0.55 to 3.40)	0.153	−0.15 (−3.31 to 3.01)	0.920
Uric acid (mg/dl)	−0.51 (−0.67 to 0.08)	0.086	−0.60 (−1.12 to−0.03)	0.039	−5.36 (−52.94 to 42.82)	0.832
Total cholesterol (mg/dl)	−3.03 (−13.14 to 7.09)	0.550	−8.15 (−17.22 to 0.92)	0.077	−5.12 (−21.56 to 5.46)	0.291
Triglyceride (mg/dl)	0.56 (−20.77 to 21.89)	0.958	−8.38 (−24.41 to 7.65)	0.299	−8.94 (−35.13 to 17.23)	0.501

Data presented as mean change (95% confidence interval). 25(OH) D: 25-hydroxyvitamin D_3, eGFR: Estimated glomerular filtration rate

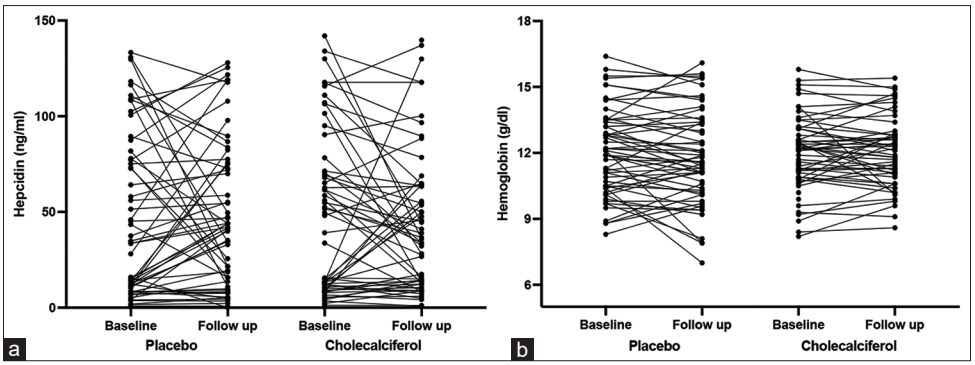

Figure 1: Line plots showing serum level of (a) hepcidin and (b) hemoglobin at baseline and at 16 weeks in placebo and cholecalciferol for individual patients

for erythropoiesis.[17] Several studies have highlighted the effect of oral vitamin D supplementation on iron homeostasis by lowering the levels of serum hepcidin.[8,18,19] This is due to anti-inflammatory effects of vitamin D on reduction of serum IL-6.[20] Although there was a reduction in IL-6 after vitamin D supplementation,[13] there was no associated reduction in hepcidin levels. In a study of 28 healthy adults, a high dose vitamin D significantly reduced plasma hepcidin levels one week post dosing.[8] Bacchetta *et al*.,[15] in a pilot study of seven healthy individuals, showed that a single oral dose of 100,000 IU vitamin D_2, decreased the levels of hepcidin by 34% after 24 hours and by 33% after 72 hours. *In vitro* and *in vivo* studies have shown the inverse relation between vitamin D and hepcidin stimulatory cytokines and the direct effect of vitamin D on lowering hepcidin mRNA expression through the *HAMP* gene. Studies on animal models have also shown the positive effect of anti-hepcidin therapy on inflammation-induced anemia. Treatment with 25(OH) D or 1,25-dihydroxyvitamin D led to a 0.5-fold reduction in expression of hepcidin mRNA in cultured hepatocytes or monocytes.[15]

Our study showed no effect of high-dose cholecalciferol on serum hepcidin and blood hemoglobin levels. A similar randomized, placebo-controlled, double -blinded trial of 40 participants with mild-to-moderate kidney disease failed to find a significant effect of oral calcitriol on hepcidin levels after six weeks of administering 0.25 mcg oral calcitriol daily or matching placebo.[11] A pilot study

Table 2: Various studies showing the effect of vitamin D supplementation on circulating hepcidin levels

Study	Characteristics (no. of patients)	Intervention	Findings
Panwar *et al*.[11]	CKD stage G3–4 (*n*=40)	Oral calcitriol 0.5 mcg daily or identically-matched placebo for 6 weeks	No significant di fferences in the change in serum hepcidin, iron parameters, or hemoglobin levels between the two groups in over 6 weeks of follow-up
Atkinson *et al*.[12]	Children with non-dialysis CKD stage G2–G5 (*n*=34)	Oral cholecalciferol of 4000 IU versus 400 IU daily for 12 weeks	Nutritional vitamin D supplementation did not modify serum hepcidin levels in children with CKD
Obi *et al*.[14]	Thrice-weekly maintenance hemodialysis patients receiving erythropoiesis-stimulating agents (*n*=96)	Participants assigned in 2:2:1:1 ratio to either (1) thrice-weekly 3,000 IU cholecalciferol, (2) monthly cholecalciferol (equivalent to 9,000 IU/week), (3) thrice-weekly placebo, or (4) monthly placebo.	Serum hepcidin-25 levels increased in the short term with cholecalciferol supplementation
Smith *et al*.[8]	Healthy adults (*n*=28)	Oral vitamin D_3 of 250,000 IU once or matching placebo	High-dose vitamin D_3 significantly reduced plasma hepcidin concentrations in healthy adults 1-week post dosing.
Bacchetta *et al*.[15]	Healthy volunteers (*n*=7)	Single dose of oral ergocalciferol of 100,000 IU	Serum hepcidin levels decreased by 34% 24 hours after supplementation

on the effect of cholecalciferol on hepcidin in children with CKD also suggested no correlation between these two parameters.[12] Similarly, recent studies involving pregnant women[21] and patients with digestive tumors[22] also did not show any association between vitamin D and hepcidin levels. In a recent placebo-controlled, double-blind randomized trial on 96 hemodialysis patients, cholecalciferol supplementation did not change the serum hepcidin levels after three and six months.[14] The mixed results were likely due to differences in dosage, the form of vitamin D administered, and the type of study population [Table 2].

There may be several reasons for a lack of effect of high-dose cholecalciferol on serum hepcidin in the present study. While other studies have shown the effect of 25(OH) D on participants with early-stage CKD or on healthy individuals, we enrolled patients with stage III–IV CKD. In later CKD stages, factors like reduced GFR, treatment with iron, increased inflammation may collectively contribute to increased serum hepcidin levels, which may override the effect of cholecalciferol supplementation.

An important strength of this study is that it was a randomized, placebo-controlled, double-blind trial. However, this was a secondary analysis. Therefore, the limited sample size of the study population could also have affected the outcome parameters. Furthermore, we did not collect data on iron, ferritin, and iron therapy.

To conclude, our study provides preliminary data based on which a larger, adequately powered clinical trial can be conducted to conclusively assess the impact of vitamin D supplementation on hepcidin and anemia in patients with CKD and vitamin D deficiency.

Disclosures

VJ has research grants from Baxter, GSK and Consultancy and Advisory Board honoraria from Baxter Healthcare, and AstraZeneca, outside the published work. All other authors reported no conflict.

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Conflicts of interest

There are no conflicts of interest.

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Supplementary Material

Supplementary Table 1: Baseline clinical and biochemical characteristics of CKD patients

	Placebo (*n*=59)	Vitamin D (*n*=58)	*P*
Male sex	40 (67.80)	41 (70.69)	0.845
Age (years)	45.16±11.52	43.36±11.79	0.400
Body mass index (kg/m^2)	24.22±5.19	24.18±4.90	0.970
Systolic blood pressure (mmHg)	128.10±15.36	128.20±14.28	0.971
Diastolic blood pressure (mmHg)	82.67±10.43	82.90±10.25	0.902
Fasting blood sugar (mg/dl)	93.27±11.6	92.17±13.04	0.660
Hemoglobin (g/dl)	12.02±1.93	11.99±1.69	0.947
eGFR (min/ml/1.73 m^2)	34.33±12.35	35.8±12.16	0.507
Serum albumin (mg/dl)	4.62±0.62	4.74±0.54	0.242
Serum calcium (mg/dl)	9.09±0.93	9.00±0.73	0.622
Serum inorganic phosphate (mg/dl)	4.04±0.62	3.67±0.91	0.089
Serum uric acid (mg/dl)	7.74±2.41	7.89±2.83	0741
Serum alkaline phosphatase (mg/dl)	135.24±59.60	134.55±57.73	0.951
Serum total cholesterol (mg/dl)	168.09±54.20	166.34±42.11	0.848
Serum triglyceride (mg/dl)	157.19±81.02	154.01±69.63	0.823
Serum 25(OH) D (ng/ml)	13.31±4.81	13.40±4.41	0.903
Serum hepcidin (ng/ml)	33.60 (8.60–77.80)	41.50 (10.90–75.00)	0.903

Data presented as mean±standard deviation, median (25th, 75th percentile) and number (percentage). eGFR: Estimated glomerular filtration rate; 25(OH) D: 25-hydroxyvitamin D_3