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# Effect of vitamin D supplementation on serum sclerostin levels in chronic kidney disease

**Running head:** Vitamin D supplementation and serum Sclerostin in CKD

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## Highlights:

- Serum sclerostin level correlated with 25(OH) D at baseline in CKD Subjects.
- Cholecalciferol supplementation did not lead to change in serum sclerostin.
- Change in serum sclerostin level at 16 weeks correlated with change in eGFR and change in serum uric acid.

## Abstract

Vitamin D deficiency, cardiovascular disease and abnormal bone mineral metabolism are common in chronic kidney disease (CKD). Abnormal bone mineral metabolism has been linked to vascular calcification in CKD. Sclerostin has emerged as an important messenger in cross talk between bone-vascular axis. We analyzed sclerostin in subjects who participated in the randomized, double blind, placebo controlled trial investigating the effect of cholecalciferol supplementation on vascular function in non-diabetic CKD stage G3-4 and vitamin D  $\leq 20$ ng/ml [CTRI/2013/05/003648]. Patients were randomized (1:1) to receive either two directly observed oral doses of 300,000 IU of cholecalciferol or matching placebo at baseline

and 8 weeks. Of the 120 subjects enrolled, 58 in the cholecalciferol group and 59 in the placebo group completed the study. At baseline, serum levels of sclerostin were similar in both groups (cholecalciferol - median;190pg/ml, IQR;140-260pg/ml and placebo - median;180pg/ml, IQR; 140-240pg/ml,  $p=0.67$ ). 16 weeks after cholecalciferol supplementation, there was no change in level of sclerostin (mean change;1.10 pg/ml, 95%CI; -27.34 to 29.34 pg/ml,  $p=0.25$ ). However, a significant decrease in sclerostin level was noted in the placebo group (mean change; -31.94pg/ml, 95%CI; -54.76 to -9.13 pg/ml,  $p=0.002$ ). Change ( $\Delta$ ) in sclerostin level at 16 weeks correlated negatively with  $\Delta$  eGFR ( $r=-0.20$ ,  $p=0.03$ ) and positively with  $\Delta$ uric acid ( $r=0.37$ ,  $p<0.001$ ) but not with  $\Delta$ 25(OH) D ( $r=0.06$ ,  $p=0.54$ ),  $\Delta$  iPTH ( $r=-0.03$ ,  $p=0.78$ )  $\Delta$ FGF23 ( $r=-0.08$ ,  $p=0.38$ ) and  $\Delta$ 1,25 (OH)<sub>2</sub> D ( $r=-0.04$ ,  $p=0.65$ ). In conclusion, high dose cholecalciferol supplementation did not change sclerostin levels in non-diabetic stage 3-4 CKD subjects.

**Keywords:** Sclerostin, vitamin D, parathyroid, chronic kidney disease, cardiovascular disease

## Background

Chronic kidney disease-mineral bone disorder (CKD-MBD), is the name given to abnormalities in mineral and hormone metabolism which lead to declining bone health, cardiovascular disease and high mortality[1-3]. Abnormal circulation level of bone biomarkers including fibroblast growth factor 23 (FGF-23)[4], bone-specific alkaline phosphatase[5, 6] and parathyroid hormone (PTH)[7] have been shown to be associated with adverse clinical outcomes, leading to suggestion that CKD-MBD represents a state of crosstalk between bone and vascular axis in CKD.

In recent years sclerostin, a 190-amino acid glycoprotein produced mainly by osteocytes, has emerged as new player in this crosstalk. A soluble Wnt inhibitor, sclerostin binds to low density

lipoprotein related receptor 5 and 6 (LRP5/6) and frizzled co-receptor to inhibit bone formation by osteocytes[8, 9]. Patients with CKD exhibit high sclerostin levels[10]. Sclerostin interacts with fibroblast growth factor 23 (FGF23) [11], vitamin D [11, 12], and parathyroid hormone (PTH) [13-15].

Vitamin D modulates the secretion of sclerostin from osteocytes, and could represent a way to modulate its levels. A recent report showed an inverse correlation between levels of the two compounds in healthy postmenopausal women [16]. Correction of vitamin D deficiency led to decline in sclerostin [17, 18]. In contrast, other studies showed an increase in sclerostin following supplementation with native [12, 19] or activated forms of vitamin D in non-CKD and CKD subjects [20].

We evaluated sclerostin levels and its association with mineral bone parameters, and the effect of cholecalciferol supplementation on sclerostin levels in subjects with non-diabetic CKD G3-4 and vitamin D deficiency.

### **Subjects and Methods:**

The study was done on existing samples (stored in biobank) and data of a randomized, double blind, placebo-controlled trial to examine the effect of correction of vitamin D deficiency on vascular function in subjects with early CKD done at the Postgraduate Institute of Medical Education and Research, Chandigarh, India after approval of Institute Ethics Committee [21] with only addition of measurement of serum sclerostin. The detailed study protocol has been published[21]. In brief, 120 stable non-diabetic, CKD stage G3-4 subjects between ages of 18 and 70 years and serum 25(OH)D  $\leq$ 20 ng/ml were randomized in a 1:1 ratio to receive two doses of either 300,000 IU of cholecalciferol or matching placebo at baseline and 8 weeks.

Biochemical parameters were measured at baseline and at after 16 weeks. Serum 25(OH)D and 1,25(OH)<sub>2</sub>D were measured by enzyme immunoassay [EIA; Immunodiagnostic Systems (IDS), UK]. Serum sclerostin was analysed by Quantikine ELISA (R&D System, USA). Serum intact parathyroid hormone (iPTH; EIA, Calbiotech Inc., USA), serum intact fibroblast growth factor-23 [FGF-23; second generation enzyme linked immunosorbent assay (ELISA), Immutopics, Diagnostic Hybrids Inc., USA], were also analyzed.

#### *Statistical analysis*

Data are presented as mean  $\pm$  standard deviation, mean (95% confidence interval) and median (interquartile range) as appropriate. Continuous variables were compared with independent samples Student's t test if normally distributed, or with Mann-Whitney U test if the distribution was skewed. Categorical variables were analyzed by Chi Square test or Fisher's Exact test as appropriate. Paired Student's t-test and Wilcoxon signed-rank test were used for within-group comparisons. Correlation analysis was performed using Pearson correlation or Spearman's rank correlation as appropriate. Two tailed p-values  $<0.05$  were considered statistically significant. Analyses were conducted using the Statistical Package for the Social Sciences (SPSS) software for Macintosh, version 21.0 (IBM Corp., Armonk, NY, USA).

#### **Results:**

Baseline characteristics of the study subjects are shown in table 1. Biochemical parameters and circulating biomarker levels did not differ between the groups. The demographic details, causes of CKD and use of medications were also similar[21].

#### *Baseline analysis*

Baseline sclerostin levels were similar in the two groups (cholecalciferol - median; 190 pg/ml, IQR;140-260 pg/ml and placebo - median;180 pg/ml, IQR; 140-240 pg/ml,  $p=0.67$ ). Sclerostin levels correlated positively with age ( $r=0.44$ ,  $p<0.001$ ), 25(OH) vitamin D ( $r=0.24$ ,  $p=0.01$ ), serum uric acid ( $r= 0.27$ ,  $p=0.004$ ) and FGF23 ( $r=0.20$ ,  $p=0.02$ ), and inversely with BMI ( $r=-0.21$ ,  $p=0.03$ ), iPTH ( $r=-0.23$ ,  $p=0.01$ ) and SAP ( $r=-0.25$ ,  $p=0.009$ ) (Figure1). eGFR was not significantly correlated with sclerostin level but a trend of negative association was noted ( $r=-0.13$ ,  $p=0.16$ ) (Figure1). Levels were higher in males (median 190 pg/ml, IQR; 160-260 pg/ml) compared to females (median 160 pg/ml, IQR; 130-210 pg/ml,  $p=0.04$ ).

#### *Follow-up analysis*

After 16 weeks sclerostin levels remained unchanged in the cholecalciferol treated subjects (mean change;1.10 pg/ml, 95%CI; -27.34 to 29.34 pg/ml,  $p=0.25$ ), whereas a significant decrease was noted in the placebo group (mean change; -31.94 pg/ml, 95%CI; -54.76 to -9.13 pg/ml,  $p=0.002$ ). The between group difference in mean change, however, remained non-significant (Table 2). Response to cholecalciferol or placebo remained similar in both males and females (data not shown). Change ( $\Delta$ ) in sclerostin level correlated negatively with  $\Delta$  eGFR ( $r=-0.20$ ,  $p=0.03$ ) and positively with  $\Delta$ uric acid ( $r=0.37$ ,  $p<0.001$ ) but not with  $\Delta$ 25(OH) D ( $r=0.06$ ,  $p=0.54$ ),  $\Delta$ iPTH ( $r=-0.03$ ,  $p=0.78$ )  $\Delta$ FGF23 ( $r=-0.08$ ,  $p=0.38$ ) and  $\Delta$ 1,25 (OH)<sub>2</sub> D ( $r=-0.04$ ,  $p=0.65$ ). As shown in table 2, the serum 25(OH)D and 1,25(OH)<sub>2</sub>D levels increased in the cholecalciferol group, but not in the placebo group. There was a decline in iPTH in the cholecalciferol group but rise in the placebo group.

#### **Discussion**

In this randomized double-blind placebo controlled trial setting, supplementation of cholecalciferol did not affect the sclerostin level in non-diabetic CKD G3-4 subjects. Although

level declined in the control group, the intergroup difference was not significant. We also show that serum level of sclerostin is associated with demographic characteristic like age, gender and BMI as well as with mineral metabolism markers including 25(OH) vitamin D, iPTH, SAP and FGF23.

The consequences of increased sclerostin levels on outcomes in CKD are not clear. Sclerostin has been associated with decline in GFR[10], progression of renal osteodystrophy[22], hyperparathyroidism and bone turnover[23]. In CKD stage 3-5, increased sclerostin levels are associated with abnormality in parameters of CKD-MBD and vascular function. Notably, in predialysis CKD cohort, sclerostin levels are associated with increased incidence in mortality and severe cardiovascular events [24].

There is interest in understanding whether manipulation of sclerostin represents a worthwhile therapeutic goal, and if so how this might be achieved. Studies of native vitamin D supplementation have produced conflicting results. Correction of vitamin D deficiency has been shown to decrease sclerostin in vitamin D deficient adult females[18] and vitamin D deficient general population[17]. By contrast, Sankaralingam et al showed increase in sclerostin level after vitamin D<sub>2</sub> supplementation in vitamin D deficient patients[19]. An increase in sclerostin was also shown in CKD subjects after paricalcitol treatment[20]. In a large placebo controlled trial, 700 IU per day vitamin D and 500 mg per day calcium in men and women age 65 years and older produced an increase of sclerostin level in men, whereas it remained unchanged in women [12]. The reason for the differential sclerostin responses to vitamin D and calcium supplementation in the men and the women is unclear. In our study, sclerostin level remained unchanged after cholecalciferol supplementation in spite of decline in PTH. Vitamin D supplementation decrease serum PTH levels[21, 25], and being negative

regulator of sclerostin expression [14, 26] decline in PTH should increase serum sclerostin. Cross-sectional studies have identified inverse associations of serum sclerostin with a variety of biochemical markers of bone turnover [27-29] and the same has been confirmed in the present study.

Our study has strengths and limitations. The strength is the setting of randomized double-blinded, placebo-controlled trial, homogenous study population, and adequate sample size. The limitation of the study was short duration. Serum calcium level should be monitored as it may rise with vitamin D therapy.

### **Conclusion**

In conclusion, high dose cholecalciferol supplementation did not alter serum sclerostin in CKD patients despite decline in other mineral markers such as PTH and SAP. Further studies are needed to fully elucidate the effect of vitamin D on sclerostin level in subjects with CKD.

### **Disclosures**

None

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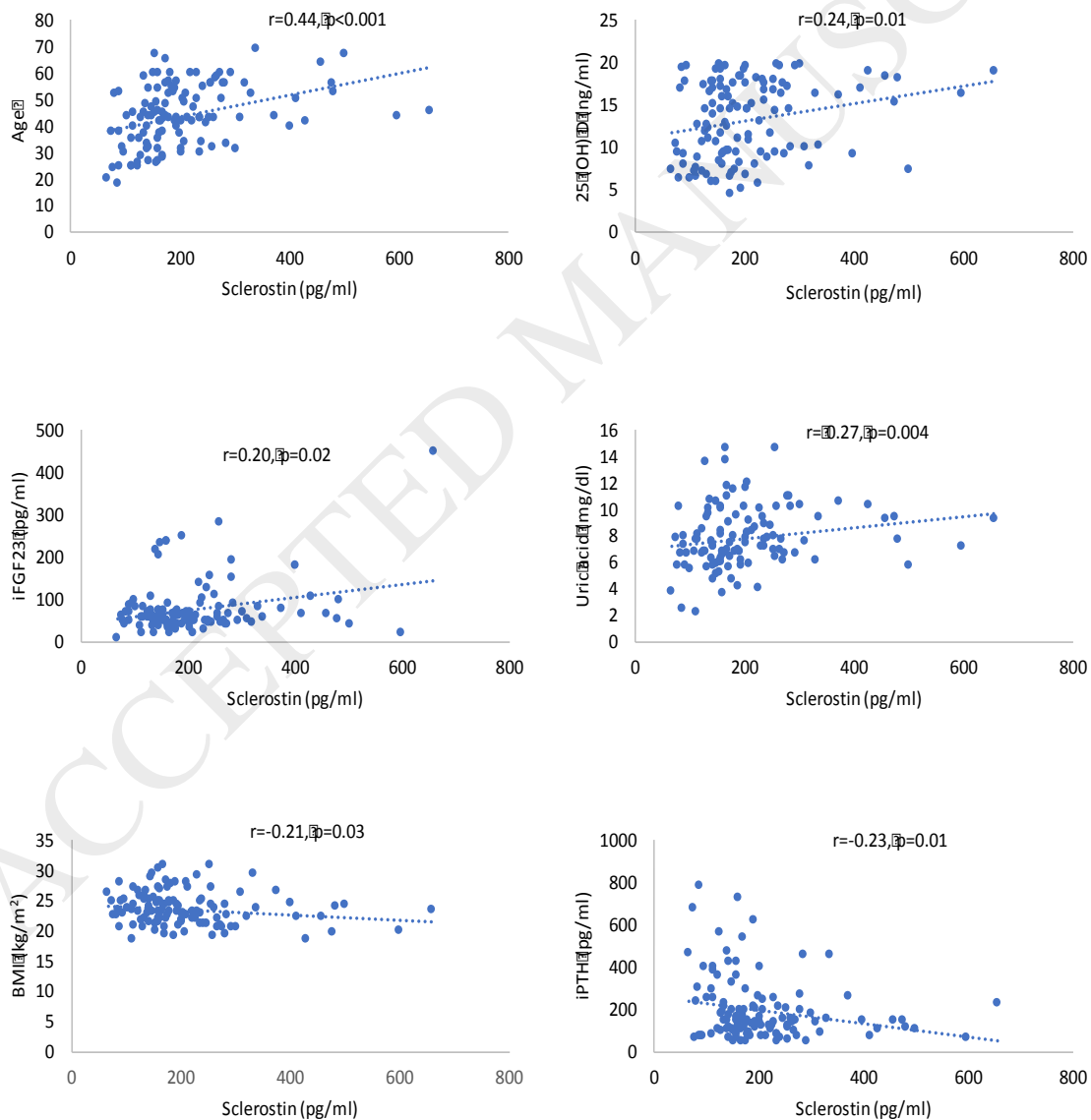
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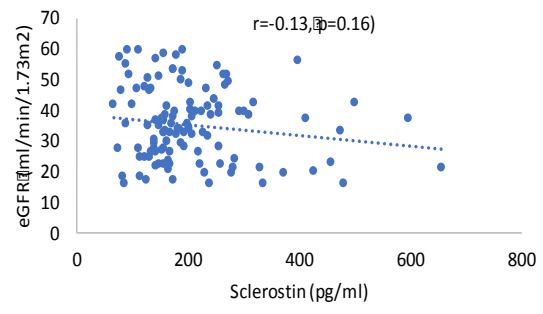
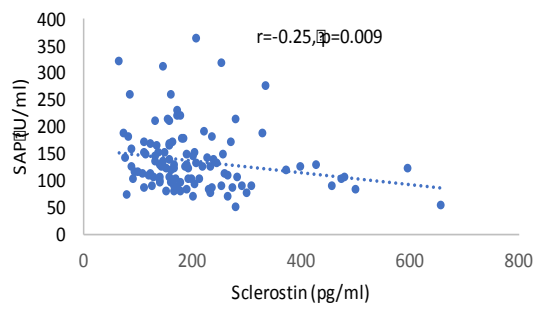
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**Figure legend:**

Figure 1: Scatter plot showing association of serum level of sclerostin with anthropometric and serum biomarkers. eGFR, estimated glomerular filtration rate; 25(OH)D, 25 hydroxy vitamin D; iPTH, intact parathyroid hormone; iFGF-23, intact fibroblast growth factor-23; SAP, total serum alkaline phosphatase; BMI, body mass index

Figure 1





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**Table 1:** Baseline characteristics of study subjects

Parameter	Placebo (n=59)	Cholecalciferol (n=58)
Gender (M/F)	40/19	41/17
Age (years)	45.20±11.61	43.17±11.79
Body mass index (kg/m <sup>2</sup> )	23.45±2.91	23.57±2.67
Hemoglobin (g/dl)	12.02±1.94	11.97±1.69
eGFR (ml/min/1.73m <sup>2</sup> )	34.63±12.25	35.77±12.37
Albumin (g/dl)	4.62±0.63	4.74±0.54
Calcium (mg/dl)	9.09±0.94	9.01±0.73
Inorganic Phosphorus (mg/dl)	4.03±1.39	3.65±0.91
Uric Acid (mg/dl)	7.66±2.37	8.01±2.32
SAP (U/L)	136.07±59.83	135.17±58.32
25(OH)D (ng/ml)	13.21±4.78	13.40±4.42
1,25 (OH) <sub>2</sub> D (pg/ml)	20.11±12.54	19.10±10.69
iPTH (pg/ml)*	150 (100, 250)	140(80, 210)
iFGF-23 (pg/ml)*	57.66 (44.48, 88.90)	57.88 (41.42, 69.04)
Sclerostin (pg/ml)*	190 (140,260)	180 (140,240)

Abbreviations: eGFR, estimated glomerular filtration rate; 25(OH)D, 25 hydroxy vitamin D; 1,25 (OH)<sub>2</sub> D, 1,25 di-hydroxy vitamin D; iPTH, intact parathyroid hormone; iFGF-23, intact fibroblast growth factor-23; SAP, total serum alkaline phosphatase;

Data presented as mean± standard deviation and median (25<sup>th</sup>, 75<sup>th</sup> percentile)

\* Compared using Mann-Whitney U test

**Table 2:** Change in biochemical parameters and markers of bone turnover between baseline and 16 weeks

	Placebo (n=59)		Cholecalciferol (n=58)		Between group difference	
	Mean change (95% CI)	P value	Mean change (95% CI)	P value	Difference of Mean change (95% CI)	P value
<b>25(OH)D (ng/ml)</b>	1.51 (-0.46 to 3.48)	0.13	24.91 (21.77 to 28.06)	<b>&lt;0.001</b>	23.40 (19.76 to 27.06)	<b>&lt;0.001</b>
<b>1,25 (OH)<sub>2</sub> D (pg/ml)</b>	0.48 (-4.65 to 5.62)	0.85	15.46 (5.42 to 25.50)	<b>0.003</b>	14.98 (4.48 to 27.18)	<b>0.007</b>
<b>i-PTH (pg/ml) *</b>	47.36 (6.63 to 88.10)	<b>0.05</b>	-53.37 (-82.37 to -24.36)	<b>&lt;0.001</b>	-100.73 (-150.50 to -50.95)	<b>&lt;0.001</b>
<b>iFGF-23 (pg/ml) *</b>	-7.33 (-24.10 to 9.45)	0.27	-14.71 (-28.45 to -0.97)	0.33	-7.38 (-28.92 to 14.15)	0.96
<b>SAP (U/L)</b>	9.40 (-2.08 to 20.89)	0.11	-10.85 (-20.70 to -1.01)	<b>0.03</b>	-20.25 (-35.14 to -5.38)	<b>0.008</b>
<b>Sclerostein (pg/ml)*</b>	-31.94(-54.76 to-9.13)	0.002	1.1(-27.34 to 29.34)	0.25	-33.04 (-69.14 to 3.05)	0.07
<b>Uric Acid (mg/dl)</b>	-0.51 (-0.67 to 0.08)	0.09	-0.60 (-1.12 to -0.03)	<b>0.04</b>	-0.09 (-0.89 to 0.72)	0.83
<b>Calcium (mg/dl)</b>	-0.48 (-0.76 to -0.19)	<b>0.001</b>	0.21 (-0.05 to 0.46)	0.11	0.69 (0.31 to 1.06)	<b>0.001</b>
<b>Inorganic Phosphorus (mg/dl)</b>	-0.30 (-0.67 to 0.08)	0.12	0.19 (-0.19 to 0.59)	0.31	0.49 (-0.37 to 1.03)	0.07

Abbreviations: eGFR, estimated glomerular filtration rate; 25(OH)D, 25 hydroxy vitamin D; 1,25 (OH)<sub>2</sub> D, 1,25 di-hydroxy vitamin D; iPTH, intact parathyroid hormone; iFGF-23, intact fibroblast growth factor-23; SAP, total serum alkaline phosphatase;

\*Compared using Willcoxon-signed rank test within group and Mann-Whitney U test between group