

Serum sclerostin is decreased following vitamin D treatment in young vitamin D-deficient female adults

Muharrem Cidem¹ · İlhan Karacan¹ · Neval Bozok Arat¹ · Oguzhan Zengi² · Murat Ozkaya¹ · Saadet Pilten Guzel² · Cansu Ozkan¹ · Ozan Beytemur³

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Abstract Sclerostin is produced almost exclusively by osteocytes, which also express receptors for 1,25 dihydroxyvitamin D₃. The aim of this study was to investigate the effects of vitamin D₃ treatment on serum sclerostin levels in young adult females with severe vitamin D deficiency. A total of 26 subjects were treated orally with calcium (1.200 mg/day for 2 months) and vitamin D₃ (300.000 IU/week for 1 month). Serum 25-hydroxyvitamin D (25(OH)D) and sclerostin levels were measured before and after treatment. Baseline serum 25(OH)D and sclerostin levels were at 5.7 ± 2.4 ng/mL and 39.1 ± 14.4 pg/mL, respectively. Serum 25(OH)D was significantly increased, to 62.4 ± 18.7 ng/mL, following treatment; serum sclerostin was significantly decreased, to 29.3 ± 8.8 pg/mL. We conclude that serum sclerostin level is decreased following vitamin D₃ treatment in patients with vitamin D deficiency.

Keywords Sclerostin · 25-Hydroxyvitamin D · Osteomalacia

Introduction

Vitamin D is a steroid hormone involved in the regulation of musculoskeletal function. Vitamin D deficiency

results in increased bone resorption, skeletal mineralization defects, muscle weakness, and an increased risk of falling [1, 2].

Sclerostin, a SOST gene protein, potently inhibits Wnt canonical signaling by binding to low-density lipoprotein receptors and is also a potent inhibitor of bone formation [3–5]. SOST gene mutation or deletion increases bone formation and hyperostosis [6]. However, bone formation can be inhibited when SOST gene expression or serum sclerostin levels increase. SOST gene expression increases under immobilization conditions, and serum sclerostin increases during the postmenopausal period; in both cases, osteoporosis may develop such that anti-sclerostin antibodies represent important targets of investigation for the treatment for osteoporosis [3, 4, 7, 8].

Sclerostin is produced almost exclusively by osteocytes, which also express receptors for 1,25 dihydroxyvitamin D₃ [3–5]. Osteocytes are abundant in bone and can control bone formation by modulating the Wnt signaling pathway [9]. Wnt-activated pathway efficiency is modulated by co-stimulatory signals from pathways activated by 1,25 dihydroxyvitamin D₃, or by Wnt inhibitors such as sclerostin [10]. Although vitamin D and sclerostin exert opposite effects on the Wnt pathway in vitro, few studies have investigated the in vivo effects of vitamin D on serum sclerostin. We hypothesized that vitamin D₃ treatment would decrease serum sclerostin levels in vitamin D-deficient young adult females.

Materials and methods

This clinical study used a prospective, open-label single-group design. Ethical approval was obtained from the Institutional Review Board. All procedures were explained

✉ İlhan Karacan
ilhankaracan@hotmail.com

¹ Physical Medicine and Rehabilitation Department, Bağcılar Training and Research Hospital, İstanbul, Turkey

² Biochemistry Department, Bağcılar Training and Research Hospital, İstanbul, Turkey

³ Orthopaedics and Traumatology Department, Bağcılar Training and Research Hospital, İstanbul, Turkey

Table 1 Inclusion and exclusion criteria (the numbers of cases excluded were expressed in parentheses)

Inclusion criteria	
Pre-menopausal	
Serum 25(OH)D < 10 ng/mL	
Exclusion criteria	
Anemia (1)	
Tumor-induced osteomalacia (hypophosphatemia) (0)	
Hypothyroidism (0)	
Hepatic/renal disease (0)	
Primary hyperparathyroidism (0)	
Fibromyalgia (33)	
Polymyalgia rheumatica (0)	
Serum 25(OH)D > 10 ng/mL (24)	
Vitamin D and calcium treatment commenced prior to study participation (0)	

prior to enrollment, and all participants provided written informed consent. Unique protocol IDs were obtained during trial registration at www.clinicaltrials.gov.

Subjects

A total of 84 young adult females with body-wide pain, weakness and fatigue (e.g., tiring after traversing stairs or standing up from a seated position), and marked diffuse bone pain and tenderness when pressed during physical examination were assessed for eligibility. Informed consent was obtained from 26 patients who met the inclusion and exclusion criteria. The inclusion and exclusion criteria are listed in Table 1. We included patients with severe vitamin D deficiency (serum concentration of 25(OH)D < 10 ng/mL). The mean age of participants was 32.5 ± 7.2 years, and mean body mass index (BMI) was 27.3 ± 4.9 kg/m².

Laboratory analyses

Blood samples were collected before vitamin D3 treatment for hemogram analysis and assessment of serum sclerostin, 25(OH)D, calcium, phosphorus, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, TSH, free T3, and intact parathormone (PTH) levels. Serum 25(OH)D, sclerostin, and PTH levels were measured again following treatment. Blood samples for the second measurements were collected within 3 days after treatment. The primary outcome measure was serum sclerostin level. The secondary outcome measures were serum 25(OH)D, alkaline phosphatase, inorganic phosphorus, calcium, and PTH levels.

Blood samples were collected between 8.00 A.M. and 10.00 A.M., following fasting for ≥ 8 h, during any phase

of the menstrual cycle (because serum sclerostin levels do not differ according to menstrual cycle phase) [11]. Blood samples were obtained from the antecubital vein and centrifuged for 15 min, at $1000 \times g$, <30 min after collection. Serum samples were frozen and stored at -80 °C until assayed.

Serum sclerostin levels were measured using a Human Sclerostin ELISA kit (Cusabio™, Catalog No: CSB-E13146 h; Newark, DE, USA). All assays were performed according to the manufacturer's instructions. The minimum detectable concentration of human sclerostin is typically <7.8 pg/mL; no samples were below this threshold in our sample. To determine intra-assay precision (CV), serum sclerostin levels were twice analyzed and measured in the same serum samples. Mean and standard deviation of difference in serum sclerostin levels between two measurements was calculated. The CV was <8 %.

Serum 25(OH)D levels were measured using the ADVIA Centaur and ADVIA Centaur XP systems (Siemens Healthcare Diagnostics). PTH was analyzed in EDTA plasma samples using an electrochemiluminescence immunoassay (Elecsys PTH; Roche Diagnostics, Mannheim, Germany) and the Cobas 601 analysis system (Roche Diagnostics).

Treatment

Based on our medical experience, all patients were treated with oral calcium (1,200 mg/day for 2 months) and vitamin D3 (300,000 IU/week for 1 month). The first dose of vitamin D3 was given the first day of treatment (day 0). Following doses of vitamin D3 were given on day 7, day 14, and day 21. All patients fully complied with the treatment protocol.

Power analysis

The primary outcome measure was change in serum sclerostin levels following treatment. For the given effect size (mean of difference = 9.8 ± 13.9), sample size (26 pairs), and alpha (0.05, two-tailed), power was calculated at 0.93. β (type II error level) was 0.07.

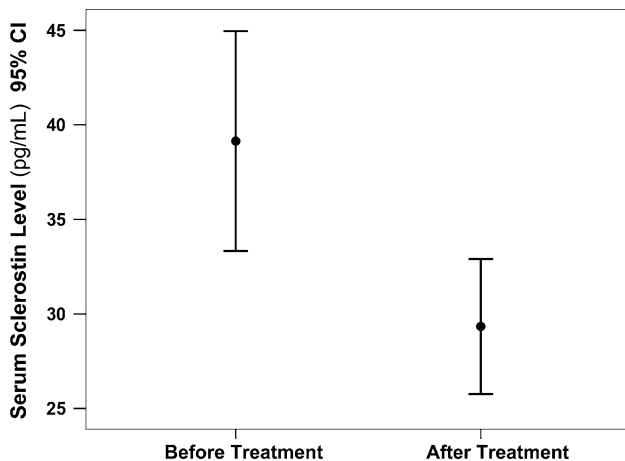
Statistical analysis

Means and standard deviations were computed for continuous variables. The Kolmogorov–Smirnov test was used to confirm that all data were normally distributed. The paired *t* test was used to assess differences between pre- and post-treatment period biochemical marker serum concentrations. A value of $p < 0.05$ was taken to indicate statistical significance. The Pearson test was used to analyze the correlation between the sclerostin levels and 25(OH)D levels. A correlation coefficient (*r*) value of more than 0.30 and a *p*

Table 2 Changes in biochemical marker serum concentrations following treatment ($n = 26$)

Serum level	Baseline	After treatment	<i>p</i> value
25(OH)D (ng/mL)	5.7 ± 2.4	62.4 ± 18.7	<0.0001
PTH (pg/mL)	116.6 ± 42.7	39.5 ± 16.2	<0.0001
Sclerostin (pg/mL)	39.1 ± 14.4	29.3 ± 8.8	0.001
Alkaline phosphatase (U/L)	67.2 ± 18.2	60.1 ± 19.4	0.002
Inorganic phosphorus (mg/dL)	3.5 ± 0.5	3.5 ± 0.7	0.971
Calcium (mg/dL)	9.4 ± 0.4	9.9 ± 0.4	<0.0001

Data are given as mean ± SD

**Fig. 1** Changes in serum sclerostin level following treatment (with 95 % confidence interval)

value of less than 0.05 were considered statistically significant. Coefficient of variation (CV) was calculated by using following formula: $CV = \text{Standard deviation} \times 100/\text{mean}$. Type II error level (β) was calculated by using following formula: $\beta = 1 - \text{Power}$. The analyses were performed using the PASW Statistics software package (SPSS Inc., Chicago, USA). Power analysis was performed using the G*Power software package (version 3.1.2).

Results

Serum 25(OH)D, sclerostin, PTH, alkaline phosphatase, and calcium levels significantly changed following treatment (Table 2; Fig. 1). Serum 25(OH)D was increased by 308.5 %; serum sclerostin was decreased by 17.5 %; and PTH was decreased by 63.8 %. Serum inorganic phosphorus levels did not significantly change following treatment. There was no statistically significant correlation between sclerostin levels and 25(OH)D levels at baseline

($r = 0.090$, $p = 0.662$). The changes in 25(OH)D levels were not correlated with the changes in 25(OH)D levels ($r = 0.133$, $p = 0.517$).

Discussion

This study reported for the first time that vitamin D3 treatment reduces circulating sclerostin, thereby confirming our hypothesis that vitamin D3 treatment would decrease serum sclerostin levels in vitamin D-deficient young adult females. Studies of the in vivo effects of vitamin D3 on serum sclerostin levels are lacking; to our knowledge, this is the first to evaluate the in vivo effect of vitamin D3 treatment on serum sclerostin levels in vitamin D-deficient patients.

In a previous study, the prevalence of vitamin D deficiency was found to be higher in younger and female patients in our country population; the mean age of patients with vitamin D deficiency was 45.7 ± 14.5 years, and the mean age of patients without vitamin D deficiency was 50.0 ± 15.4 years. Based on these data, the present study was conducted among the premenopausal women [12].

Dawson-Hughes et al. [13] demonstrated that vitamin D (and calcium) supplementation does not alter serum sclerostin levels in healthy elderly females, which is not consistent with the present findings. This inconsistency may be attributable to methodological differences. Dawson-Hughes et al. recruited postmenopausal elderly females, in whom structural and functional bone tissue regression may occur. Therefore, such patients may not be suitable to investigate the effects of vitamin D on serum sclerostin; furthermore, their serum vitamin D levels were normal. Vitamin D-deficient young adult females may be better able to demonstrate the effects of vitamin D on serum sclerostin levels: Vitamin D deficiency affects osteocytes and may also alter serum sclerostin levels. Therefore, we recruited premenopausal females exhibiting clinical symptoms of severe vitamin D deficiency. Vitamin D3 treatment significantly decreased serum sclerostin levels.

Vitamin D and sclerostin exert opposing effects on osteocyte mechanotransduction function. The Wnt pathway, which drives osteocyte mechanotransduction function, is activated by 1,25 dihydroxyvitamin D3 and blocked by sclerostin [10, 14, 15]. Given the opposing effects of vitamin D and sclerostin, alterations in their serum levels, in opposite directions, represent a notable finding.

There is no previous study that provides relative data for us to use to calculate sample size. Although our sample size was relatively small, the statistical power of the study was high (0.93). Placebo-controlled studies are required to validate the serum sclerostin-lowering effect of vitamin D.

In the present study, the secondary outcome measures were serum alkaline phosphatase, inorganic phosphorus,

calcium, and PTH levels. It was found that serum alkaline phosphatase, calcium, and PTH levels were significantly changed after vitamin D3 and calcium treatment. It is well known that serum alkaline phosphatase and PTH levels increase in patients with vitamin D deficiency. PTH increases the activity of 1- α -hydroxylase enzyme, which converts 25(OH)D to 1,25(OH)₂D, the active form of vitamin D. This activated form of vitamin D increases the absorption of calcium by the intestine [16]. It might be hypothesized that vitamin D affects serum sclerostin level directly or via PTH or other mechanisms.

In conclusion, the current study suggests that serum sclerostin levels decrease following vitamin D3 treatment in vitamin D-deficient patients. Understanding the effects of vitamin D on serum sclerostin may improve knowledge of bone physiology. The physiological mechanism underlying the serum sclerostin-lowering effect of vitamin D is not known; future studies should further investigate this mechanism.

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Conflict of interest The authors declare that they have no conflict of interest.

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