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Effect of Vitamin D Treatment on Serum Sclerostin Level

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Key words
- sclerostin
- vitamin D deficiency
- parathyroid hormone

Abstract

Objective: Sclerostin is an osteocyte-secreted endogenous inhibitor of Wnt signaling. Several systemic and local factors have been suggested as possible regulators of sclerostin expression by osteocytes. In this study, we examined the effect of vitamin D treatment on sclerostin levels.

Subject and Methods: 44 patients with diagnosis of vitamin D deficiency (25(OH)D ≤ 20 ng/ml) were involved in the study. Patients had monthly intramuscular injection of 300.000 IU cholecalciferol for 3 consecutive months. Sclerostin, 25(OH)D, parathyroid hormone (PTH), calcium, phosphorus and alkaline phosphatase (ALP) levels were measured during the diagnosis and after the replacement of vitamin D.

Results: 8 male and 36 female patients were enrolled in the study. Minimum age, maximum age and average age were 21, 55 and 32.02 ± 9.26 years, respectively. A statistically significant difference was observed between the pre-treatment and post-treatment values in 25(OH)D levels (p:0.001, 10.27 ± 4.62 ng/ml and 51.40 ± 14.62 ng/ml, respectively), PTH levels (p:0.001, 50.32 ± 19.05 pg/ml and 33.97 ± 13.12 pg/ml, respectively) and sclerostin levels (p:0.002, 858.98 ± 351.63 pg/ml and 689.52 ± 197.92 pg/ml, respectively). No statistically significant difference, however, was found between the pre-treatment and post-treatment calcium, phosphorus and ALP levels. Correlation analysis made on pre-treatment and post-treatment sclerostin levels and 25(OH)D, PTH, calcium, phosphorus and ALP levels revealed no statistically significant correlation.

Conclusion: Our findings show that the sclerostin level of patients with vitamin D deficiency decreases considerably through treatment.

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Introduction

Osteoporosis is a systemic disease characterized by low bone mineral density (BMD), reduced bone strength and increased risk of fractures [1]. Wnt signaling is a regulator of bone formation and regeneration. Modulation of Wnt signaling can be beneficial in the treatment of skeletal disorders such as osteoporosis [2]. Sclerostin is a SOST gene product and is an endogenous inhibitor of Wnt signaling. It is expressed by osteocytes and articular chondrocytes [2]. When sclerostin binds to the low-density lipoprotein receptor-related protein-5 and -6 (LRP5/6) and frizzled co-receptors on the cell surface of osteoblasts, Wnt/β-catenin signaling is inhibited [3]. This inhibits osteoblast differentiation, proliferation, and activity, resulting in reduced osteoblastic bone formation [4,5]. Antibodies against sclerostin have been shown to have beneficial effects on fracture healing and bone repair in rodents, with acceleration of bone repair, increased bone strength and increased callus density [6,7].

Vitamin D is very important for bone metabolism. Its deficiency can results in abnormalities in calcium and phosphorus levels. In vitamin D deficiency especially the efficiency of intestinal calcium and phosphorus absorption is affected, resulting in an increase in parathyroid hormone (PTH) levels [8]. The increased PTH secretion mobilizes calcium from the skeleton and increases the phosphorus wasting in the kidneys in order to maintain normal serum calcium levels. The PTH-mediated increase in osteoclastic activity creates local foci of bone weakness and causes a generalized decrease in BMD and an increase risk of osteoporotic fractures [8].

In this study, we examined the effect of vitamin D treatment on sclerostin levels and the relationship between sclerostin and the pre-treatment, post-treatment parameters of serum 25(OH)D, PTH, calcium (Ca), phosphorus (P) and alkaline phosphatase (ALP) levels in patients with vitamin D deficiency.

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Materials and Methods

44 patients, diagnosed with vitamin D deficiency at our University Hospital were included in this study. The study was approved by the local ethical committee of our university. All of the patients had 25(OH)D levels lower than or equal to 20 ng/ml. Patients with prior diagnosis of osteoporosis, patients who are taking medications that can interfere with vitamin D metabolism and patients with high creatinine levels were excluded. All patients had monthly 300 000 IU intramuscular injections of vitamin D for 3 months.

Sclerostin, 25(OH)D, PTH, calcium, phosphorus and ALP levels were measured before and at the end of the treatment period. All of the measurements were performed in the same biochemistry laboratory of our hospital. Creatinine, calcium, phosphorus and ALP were analyzed using the spectrophotometric method on the Beckman Coulter LX-20 device, PTH measurements were conducted using the enzyme immunoassay method on the Cobas e 601(Roche Hitachi) device and 25(OH)D was analyzed using the micro particle immunoassay method on the Architect i 2000 SR device.

Sclerostin levels were measured using Sclerostin Aviscera Bioscience branded test kits. This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for human soluble sclerostin. The capture antibody can bind to the human soluble sclerostin in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against human soluble sclerostin is added to the wells. After another washing of the plate, Streptavidin-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution (TMB) is added to the wells and color develops in direct proportion to the amount of human soluble sclerostin bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve. Intra-assay precision of the test is 4–6%, Inter-assay precision of the test is 8–10% Analysis were done using a fully automatic Chemwell branded ELİSA device. For measurements the taken blood samples were centrifuged for 5 min under 4000 rpm using the 'Hettich universal 32' centrifuge. Serum samples were placed into 1.4 ml eppendorf tubes and stored at −80 C. During the study, all samples were adjusted to room temperature and their sclerostin levels were measured.

Statistical analysis were done using SPSS 14.0 program. A parametric test hypothesis was achieved during the evaluation of data, Wilcoxon test and correlation analysis were applied since a significance test of peer-to-peer difference was unable to be developed. The bias level was set at 0.05.

Results

44 patients, 8 males and 36 female participated in our study. Minimum, maximum and average age were 21 years, 55 years and 32.02 ± 9.26 years, respectively. Pre-treatment, post-treatment laboratory values and demographic characteristics of the patients are shown in **Table 1**.

A statistically significant difference (p:0.001) was observed between the pre-treatment and post-treatment values in 25(OH) D levels (10.27 ± 4.62 ng/ml and 51.40 ± 14.62 ng/ml, respectively) and PTH levels (50.32 ± 19.05 pg/ml and 33.97 ± 13.12 pg/ml, respectively) (**Table 1**).

Table 1 Pre-treatment, post-treatment laboratory values and demographic characteristics of the patients.

	Pre-treatment Mean ± SD	Post-treatment Mean ± SD	
Age (year)	32.02 ± 9.26		
Height	163.09 ± 7.92		
Weight	66.31 ± 9.83	66.34 ± 9.80	P:0.868
BMI (kg/m^2)	24.89 ± 2.99	24.91 ± 3.06	P:0.720
25 (OH)D3 (ng/ml)	10.27 ± 4.62	51.40 ± 14.62	P:0.001
PTH (15–68.3 pg/ml)	50.32 ± 19.05	33.97 ± 13.12	P:0.001
Ca (8.6–10.2 mg/dl)	9.24 ± 0.41	9.31 ± 0.43	P:0.340
P (2.5–4.5 mg/dl)	3.31 ± 0.63	3.37 ± 0.54	P:0.545
ALP (45–129 U/L)	62.84 ± 18.59	60.17 ± 14.10	P:0.061
Sclerostin (pg/ml)	858.98 ± 351.63	689.52 ± 197.92	P: 0.002
Creatinine (0.6–1.2 mg/dl)	0.84 ± 0.11	0.85 ± 0.08	P:0.588

PTH: Parathormone; Ca: Calcium; P: Phosphorus; ALP: Alkaline phosphatase; BMI: Body mass index

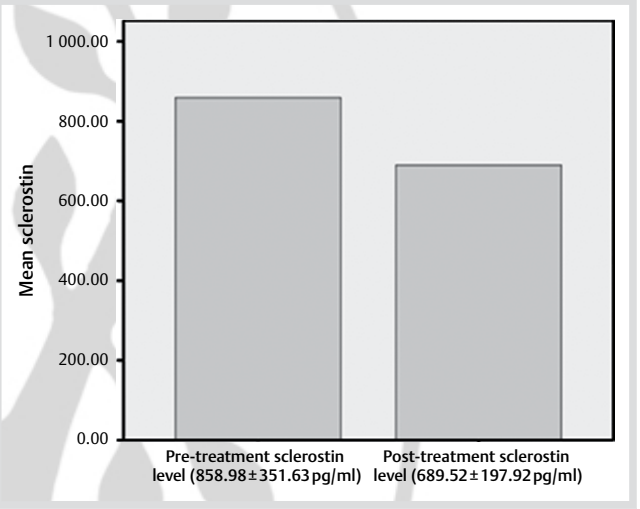


Fig. 1 Pre-treatment and post-treatment serum sclerostin levels.

No statistically significant difference was found between the pre-treatment and post-treatment calcium levels (p:0.340; 9.24 ± 0.41 mg/dl and 9.31 ± 0.43 mg/dl, respectively), phosphorus levels (p:0.545; 3.31 ± 0.63 mg/dl and 3.37 ± 0.54 mg/dl, respectively) and ALP levels (p:0.061, 62.84 ± 18.59 u/l and 60.17 ± 14.10 u/l, respectively) (**Table 1**).

A statistically significant difference (p: 0.002) was found between the pre-treatment sclerostin level (858.98 ± 351.63 pg/ml) and the post-treatment sclerostin level (689.52 ± 197.92 pg/ml) (**Table 1**) (**Fig. 1**).

No statistically significant difference was found when a correlation analysis was made on pre-treatment sclerostin levels and 25(OH)D, PTH, calcium, phosphorus and ALP (p > 0.05, r:0.29, r: −0.11, r:0.22, r:0.04, r:0.31, respectively) (**Table 2**).

Similarly, no statistically significant difference was found when a correlation analysis was made on post-treatment sclerostin levels and 25(OH)D, PTH, calcium, phosphorus and ALP (p > 0.05, r: −0.03, r: −0.11, r:0.09, r: −0.12, r: −0.04, respectively) (**Table 2**).

No statistically significant difference was found when a correlation analysis was made on deltasclerostin level and delta25(OH)D, deltaPTH (p:0.291, r:0.163 and p:0.632, r: −0.074, respectively).

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Table 2 Correlation analysis between pre- and post-treatment sclerostin and other parameters.

		Pre-treatment				
		25(OH)D	PTH	Ca	P	ALP
Sclerostin	p	0.053	0.442	0.136	0.771	0.052
	r	0.29	−0.11	0.22	0.04	0.31
		Post-treatment				
		25(OH)D	PTH	Ca	P	ALP
Sclerostin	p	0.855	0.471	0.552	0.417	0.799
	r	−0.03	−0.11	0.09	−0.12	−0.04

PTH: Parathormone; Ca: Calcium; P: Phosphorus; ALP: Alkaline phosphatase

Discussion

Sclerostin is almost entirely produced by osteocytes and plays a major role in suppression of new bone formation via inhibition of Wnt signaling [3]. Sclerostin deficient mice have been shown to have increased bone mass and bone strength [5]. Overexpression of normal human SOST alleles (the gene for sclerostin) in mice have been shown to causes osteopenia [9]. Monoclonal antibodies against sclerostin have been shown to increase the bone formation markers and decrease the bone resorption markers leading to an increase in bone mass [2]. Further, sclerostin monoclonal antibody therapy has been shown to enhance fracture healing in rats and cynomolgus monkeys [10]. However present understanding of the regulation of SOST/sclerostin expression and sclerostin metabolism is, to date, incomplete. Several systemic and local factors have been suggested as possible regulators of SOST/sclerostin expression by osteocytes [11,12]. For example sclerostin levels are increased in long-term immobilized patients and negatively correlate with bone formation markers [13]. In this study, we examined the effect of vitamin D on sclerostin level and found a statistically significant decline in the serum sclerostin level of patients after treatment due to vitamin D deficiency. However, we were unable to find a significant correlation between 25(OH)D level and sclerostin level before and after vitamin D treatment.

Parathyroid hormone has both anabolic and catabolic skeletal effects, however the underlying molecular mechanism is not fully understood. Intermittent PTH administration leads to marked increases in bone mass [14], whereas continuous exposure to PTH is associated with low bone mass [15]. Recently, it has been proposed that sclerostin, may mediate, at least in part, the anabolic effects of PTH [16]. In vivo, the PTH administration resulted in a decrease in SOST mRNA and sclerostin expression in mice and rats [17–20]. Both chronic and acute injections of PTH have been shown to decrease the SOST gene expression in mice by Bellido et al. [17].

In a study conducted on postmenopausal women, a statistically significant negative correlation was determined between PTH levels and serum sclerostin levels that was independent of serum estrogen levels in the multivariate analysis. The same study was unable to demonstrate any correlation between serum sclerostin levels and serum PTH levels in premenopausal women [21]. In some cohorts of osteoporotic and nonosteoporotic subjects, serum PTH levels were shown to be inversely correlated with sclerostin levels [21–23], whereas low sclerostin concentrations were found in patients with primary hyperparathyroidism [24–26]. Sclerostin levels of the patients with chronic renal failure have been shown to be increased despite a concomitant increase of PTH. This discrepancy can be explained, by the fact that chronic renal failure ng is a state of PTH resistance [27].

Secondary hyperparathyroidism due to calcium or vitamin D deficiency may accelerate bone loss and cause osteoporosis [21]. In secondary hyperparathyroidism bone resorption is increased, bone formation is impaired [21]. This imbalance leads to deterioration of the skeletal microarchitecture and causes height loss, skeletal deformities and fractures [21].

Positive and negative correlations between PTH and sclerostin levels were found in previous studies. However, these previous studies did not explore the mentioned relationship in secondary hyperparathyroidism caused by vitamin D deficiency. We consider that secondary hyperparathyroidism and also elevated sclerostin levels may be responsible for bone loss due to vitamin D deficiency. Thereby we also consider that a positive relationship between sclerostin and PTH, negative relationship between sclerostin and vitamin D may exist. In this study, we examined the relationship between pre-treatment and post-treatment PTH, calcium, phosphorus, ALP and 25(OH)D levels and the sclerostin levels of patients with vitamin D deficiency. We were unable to find a statistically significant correlation between these parameters. This can be due to the low number of patients involved. Also including both male and female patients and pre and postmenopausal patients together could have been another obstacle in reaching a significant correlation.

To conclude, we demonstrated that sclerostin levels of patients with vitamin D deficiency decrease considerably after vitamin D replacement, although we were not able to find a relationship between sclerostin levels and PTH, vitamin D. Sclerostin could be a factor in the progress of osteoporosis caused by vitamin D deficiency, but further studies in more homogenous groups including larger number of patients are needed to explain the relationship between vitamin D deficiency and sclerostin.

Conflict of interest: None.

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