Serum Sclerostin Level in Patients of Type 2 Diabetes Mellitus and Its Correlation with HbA1c and Bone Turnover Markers

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ABSTRACT

Aims and objectives: The aims were to observe the circulating level of sclerostin in type 2 diabetes mellitus patients and its relationship with glycemic control and markers of bone turnover. **Material and methods:** The study was an observational study conducted at JNMCH, Aligarh, Uttar Pradesh, with 50 male patients between 40 and 60 years of age, who were diabetic as per the ADA criteria. It excluded patients having diseases affecting bone metabolism (Paget disease, liver dysfunction, vitamin D deficiency, renal insufficiency, hematological disorder) or patients who had or were receiving treatment with drugs altering bone metabolism (calcium, vitamin D, calcitonin, thiazide, steroids, anticonvulsant). After obtaining the approval by Institutional Ethics Committee and the consent of patients, the subjects underwent investigations to assess for glycemic control, along with estimation of the serum levels of calcium, phosphate, 25-hydroxyvitamin D [25(OH)D], bone-specific alkaline phosphatase (BSAP) and sclerostin. Bone mineral density (BMD) was measured at L2-L4 by DEXA scan. **Results:** The mean level of serum sclerostin in our study was 79.84 pmol/L. The mean values of serum calcium, serum phosphate, 25(OH) D and BSAP were 8.75 mg/dL, 3.35 mg/dL, 24.66 pg/mL and 28.8 U/L, respectively. There was inverse correlation between sclerostin and BSAP ($r = –0.225$, $p < 0.004$) and levels of vitamin D ($r = –0.638$, $p < 0.001$). The serum sclerostin levels were negatively correlated with BMD ($r = –0.701$, $p < 0.001$) and positively with HbA1c ($r = 0.846$, $p < 0.001$). **Conclusion:** The circulating sclerostin level is increased in poorly controlled diabetes and is correlated with BMD and BSAP. It may be contributing to the deranged bone metabolism in diabetics. Additional studies are needed to evaluate the role of sclerostin on bone metabolism in this population.

Keywords: Type 2 diabetes mellitus, sclerostin, bone turnover markers

The incidence of type 2 diabetes mellitus (T2DM) and osteoporosis is increasing day by day and when present simultaneously, result in additive effect on morbidity and mortality of the patient. Osteoporosis is characterized by decreased bone mineral density (BMD) and deterioration of bone microarchitecture. Dual-energy X-ray absorptiometry (DEXA) is currently the criterion standard for the evaluation of BMD. DEXA provides the patient's T-score, which is the BMD value compared with that of control subjects who are at their peak BMD. The World Health Organization (WHO) criteria define a normal T-score value as within 1 standard deviation (SD) of the mean BMD value in a healthy young adult. Values lying farther from the mean are stratified as follows:

- T-score of -1 to -2.5 SD indicates osteopenia
- T-score of -2.5 SD or lower indicates osteoporosis
- T-score of -2.5 SD or lower with fragility fracture(s) indicates severe osteoporosis.

T2DM is associated with poor quality of bone due to impaired blood glucose, poor glycemic control, decreased levels of insulin-like growth factor, impaired vitamin D and calcium metabolism, possible vascular abnormalities, associated neuropathy, acidosis, ketosis, associated abnormalities of sex hormone levels and history of repeated falls in patients of diabetes mellitus.

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Sclerostin is a glycoprotein coded by *SOST* gene, located on chromosome 17 locus q11.2 with C terminal cysteine-like domain, with a length of 213 residues. It was previously considered as nonclassical bone morphogenic protein but recent studies reveal that it competitively binds to low-density lipoprotein receptor-related protein (LRP)-5 or -6 and inhibits Wnt signaling pathways. Wnt signaling pathway leads to expansion of osteoprogenitor cells as well as reduced apoptosis of osteoblast, leading to anabolic effects on bone. So, sclerostin by inhibiting this pathway, hinders bone formation. Sclerostin is secreted almost exclusively by osteocytes in adults and its levels are increased by calcitonin and decreased by parathormone, mechanical loading and cytokines.

AIMS AND OBJECTIVES

The objective of our study was to evaluate serum sclerostin levels in a cohort of T2DM patients and to analyze its relationships with bone turnover markers, BMD and glycated hemoglobin (HbA1c).

MATERIAL AND METHODS

The study was an observational, cross-sectional study. It was conducted in 50 male type 2 diabetes patients who were attending Medicine OPD of Jawaharlal Nehru Medical College and Hospital or Rajiv Gandhi Centre for Diabetes and Endocrinology, Aligarh, Uttar Pradesh. The study had Institutional Ethics Committee permission, and the procedures followed in the study were in accordance with institutional guidelines. All the participants were enrolled in the study after obtaining informed consent.

The study included only male patients, between 40 and 60 years of age, who were type 2 diabetic as per the American Diabetes Association (ADA) 2014 guideline. The patients having other co-existing diseases which affect bones like Paget disease, rheumatoid arthritis, hyperparathyroidism, hypercortisolism, renal bone disease, malignancy, liver dysfunction, hematological disorder, etc. were excluded from the study. The participant should not have received drugs which alter bone metabolism like calcium supplementation, vitamin D preparation, calcitonin, thiazide, steroids, anticonvulsant, at the time or prior to enrollment in the study.

All the participants were assessed as per a pre-designed proforma. After history, physical examination and routine laboratory investigations, they underwent specific investigations for bone metabolism and turnover like serum calcium, serum phosphate, bone-specific alkaline phosphatase (BSAP) and 25-hydroxyvitamin D [25(OH)D] assay. The serum level of sclerostin was measured by Sandwich enzyme-linked immunosorbent assay (ELISA) technique. The BMD of subjects at lumbar spine L2-L4, was assayed by DEXA scan. The measurements were compared to the normal range for bone density in a healthy young adult of same gender and ethnicity (T-score), with normal range between -1 to +1, T-score between -1 to -2.5 denoting osteopenia and T-score of <2.5 implying osteoporosis.

Statistical Analysis

The data for continuous variables was expressed as mean ± SD and categorical variables were expressed as numbers or percentages. The association between continuous variables was described by Pearson's correlation coefficients. Statistical analysis was performed using SPSS version 10. Statistical significance was set at $p < 0.05$.

OBSERVATION AND RESULTS

All the subjects in the study were males in the age group of 40-60 years, with mean age of 47.63 ± 6.88 years. The mean value of serum calcium in the study was 8.75 ± 0.348 mg/dL and that of serum phosphorus was 3.35 ± 0.6 mg/dL. The serum level of BSAP was 28.8 ± 9.2 U/L. 25(OH)D was also estimated in all the 50 subjects enrolled in the study with the mean value of 24.66 ± 3.18 pg/mL. The mean BMD was observed as 1.239 gm/cm^2 with SD of 0.0619. The mean value for serum sclerostin was 79.84 ± 20.04 pmol/L (Table 1).

Most of the participants had poorly controlled diabetes with 15 (30%) having HbA1c 6.5-8%, 26 (52%) having HbA1c 8.1-10% and 9 subjects (18%) having HbA1c more than 10% (Fig. 1). On correlating the HbA1c level with serum sclerostin levels, the correlation coefficient r was 0.846 with a p value of <0.001, indicating that poor glycemic control may contribute to increased sclerostin levels which has antianabolic effect on bone metabolism (Fig. 2).

The BMD of the subjects were correlated with sclerostin level and a negative correlation was observed ($r = –0.70$), which was significant ($p < 0.001$) (Fig. 3). BSAP is a marker for positive bone growth and was observed to be negatively correlated with serum sclerostin levels ($r = –0.225$, $p < 0.004$) (Fig. 4). In the study, on correlating the levels of 25(OH)D, it was seen that it

Table 1. The Values of Glycemic Indices and Bone Turnover Markers

Parameters	Mean	SD
Sclerostin (pmol/L)	79.84	20.04
Blood sugar fasting (mg/dL)	154.98	43.66
Blood sugar postprandial (mg/dL)	209.7	52.08
HbA1c (%)	8.93	1.73
25(OH)D (pg/mL)	24.66	3.18
BSAP (U/L)	28.8	9.2
BMD (gm/cm^2)	1.239	0.0619
Serum calcium (mg/dL)	8.75	0.348
Serum phosphate (mg/dL)	3.35	0.6

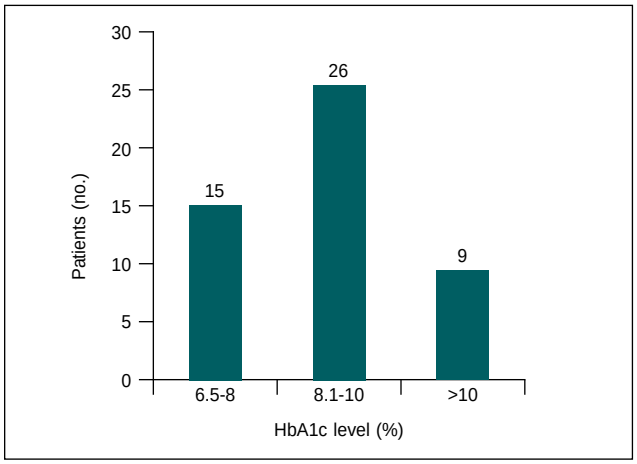

Figure 1. Distribution of HbA1c levels in subjects.

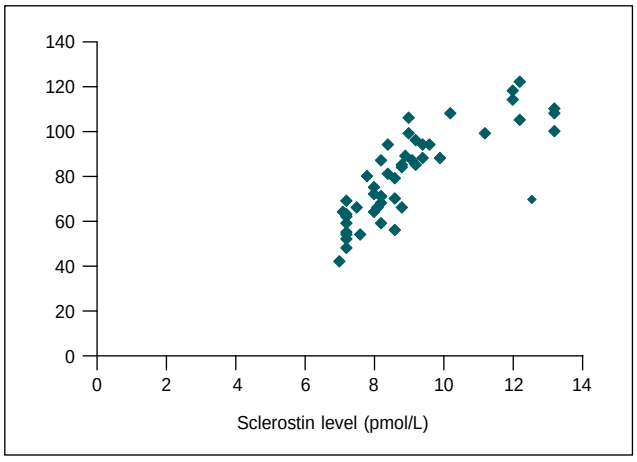

Figure 2. Relationship between HbA1c and serum sclerostin.

was negatively correlated with serum sclerostin levels ($r = –0.638$, $p < 0.001$) (Fig. 5). Table 2 summarizes the correlation between the markers of glycemic control and bone turnover with sclerostin level.

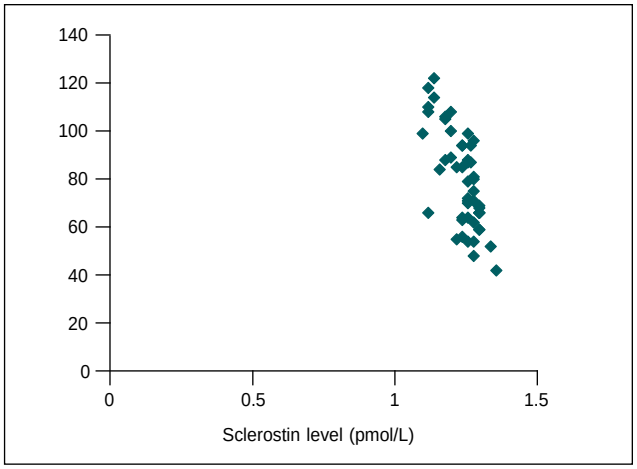

Figure 3. Relationship between serum sclerostin level and bone mineral density.

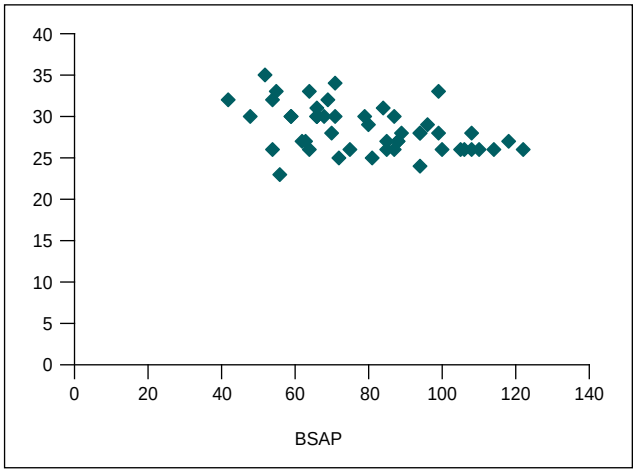

Figure 4. Relationship between serum sclerostin and BSAP.

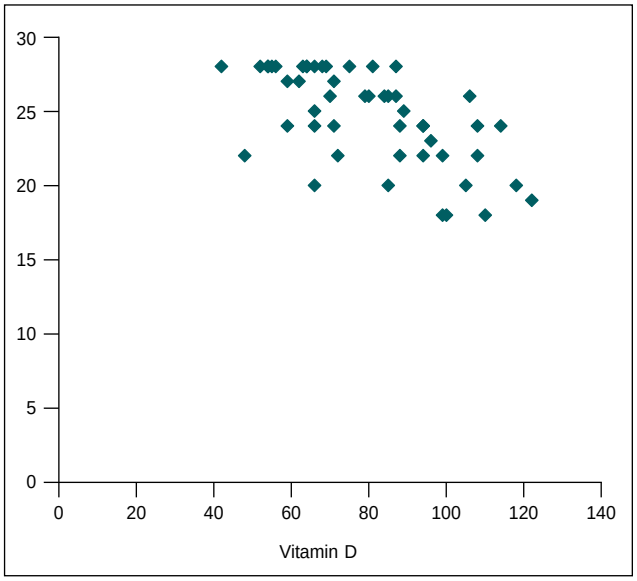

Figure 5. Relationship between serum sclerostin and 25(OH)D level.

Table 2. Correlation Between the Markers of Glycemic Control and Bone Turnover with Sclerostin Level

Variables	BS (F)	BS (PP)	HbA1c	Vit. D	BMD	Calcium	BSAP	Phosphate
Correlation coff ('r' value)	+0.66	0.713	0.846	–0.638	–0.701	–0.459	–0.225	–0.35
P value	<0.001	<0.001	<0.001	<0.001	<0.001	0.01	<0.004	0.685

BS = Blood sugar; F = Fasting; PP = Postprandial.

DISCUSSION

The role of sclerostin has been evaluated and compared with other markers of bone turnover in different subgroups of patients like hemodialysis dependant chronic kidney disease (CKD) patients, immobilized patients, etc. Our study aimed to add to the existing knowledge by evaluating the sclerostin levels in type 2 diabetes patients and correlating its level with glycemic indices and markers of bone metabolism.

The mean level of serum sclerostin in our study was 79.84 ± 20.04 pmol/L, which was more than the normal levels observed in previous studies. In a study conducted by Mödder et al, in a population-based sample, the mean sclerostin level in healthy adult male was 33.3 ± 1.0 pmol/L. The higher levels may be partly be attributed to the age of our patients as serum sclerostin levels are observed to increase with age.

In our study, the serum levels of sclerostin were positively correlated with HbA1c levels ($r = 0.846$, $p < 0.001$). Similar association has been demonstrated by García-Martín et al. They postulated that it may be attributed to direct effect of hyperglycemia on bone cells and indirectly by formation of advanced glycation end products. Also the low physical activity in diabetic patients may lead to elevation in serum sclerostin levels. The result of immobilization on sclerostin has been observed by Gaudio et al.

The BMD in our study population was observed to have negative correlation with serum sclerostin level ($r = –0.701$, $p < 0.001$). This is expected observation since sclerostin inhibits osteoblastic activity. This is in accordance with the data derived from patients of sclerostosis and Van Buchem's disease and in mice over-expressing sclerostin. But is in contrast to the results observed in hemodialysis patients where sclerostin levels correlated positively with BMD. Ardawi et al also found negative correlation between the two parameters in pre- and postmenopausal females.

In the study population, the serum levels were correlated with the levels of BSAP and the correlation coefficient was $r = –0.225$ with a significant p value of <0.004. This is conceptually correct as sclerostin has an inhibitory effect on bone turnover. Our results are consistent with those reported by Mödder et al where bone alkaline phosphatase (B-ALP) and sclerostin levels were inversely associated in elderly females.

Our study also evaluated the relation between the levels of sclerostin and 25(OH)D and observed a negative correlation between the two variables ($r = –0.638$, $p < 0.001$). Ardawi et al also identified an inverse association between serum 25(OH)D and sclerostin levels in healthy postmenopausal women. This negative correlation prompted Dawson-Hughes et al to conduct an interventional study to evaluate the role of supplemental calcium and vitamin D on serum sclerostin levels.

CONCLUSION

In our study, we have demonstrated increased level of serum sclerostin in type 2 diabetes patients and correlated it with HbA1c, BMD, BSAP and 25(OH)D. Sclerostin may be a contributing factor in poor osteogenesis and increased bone fragility in these patients by inhibiting Wnt pathway. But our study is limited by the small size of our sample, selection bias and the cross-sectional nature of our study. Thus, further studies are needed to evaluate the role of sclerostin as a marker of bone biology in all patients, including diabetics and using it as a target for therapeutic intervention.

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