



Association of Sclerostin with Bone Metabolism and Bone Mineral Density in Adults with Type 1 Diabetes Mellitus

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Authors' contributions

This work was carried out in collaboration between all authors. Authors NSA and BUA designed the study and wrote the protocol. Authors NSA and BB wrote the first draft of the manuscript. Author BEB managed the literature searches and author HC managed the experimental process. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Type 1 DM (T1DM) patients are shown having lower bone mineral density (BMD). Wnt signaling pathway is important in bone homeostasis. Sclerostin is a major inhibitor of this pathway. The objectives of our study are to evaluate sclerostin levels of T1DM patients and to analyse its relationships with bone turnover markers.

Place and Duration of Study: Department of Endocrinology, Tekirdağ State Hospital, between January to December 2013.

Methodology: 48 T1DM patients and age, sex and BMI-matched 40 healthy control cases were included in this study. BMD measurements of T1DM patients were done by dual energy x ray absorptiometry. Serum samples were used to measure *albumin, calcium, phosphorus, alkaline*

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phosphatase, intact parathyroid hormone, osteocalcin, pyrilinks-D, 25(OH) Vitamin D and sclerostin levels of all cases.

Results: Sclerostin levels of T1DM patients (803,9±92,01 pg/ml) were significantly higher than control cases (522,9±76,23 pg/ml) ($P=0.000$). Sclerostin has no correlation with age or gender. Sclerostin level was negatively correlated with lumbar vertebrae and femur neck BMD; however, positively correlated with *alkaline phosphatase, intact parathyroid hormone, osteocalcin, pyrilinks-D*. Lumbar vertebrae and femur neck BMD has negative correlation with HbA1c and duration of T1DM.

Conclusions: Sclerostin is increased in T1DM patients and this increment is associated with degradation of lumbar vertebrae and femur neck BMD.

Keywords: Type 1 diabetes mellitus; sclerostin; bone mineral density; bone turnover markers.

ABBREVIATIONS

25OHD : 25(OH)Vitamin D

ALP : Alkaline phosphatase

BMD : Bone mineral density

BMI : Body mass index

Ca : Calcium

DEXA : Dual energy x ray absorptiometri

GFR : Glomerular filtration rate

iPTH : Intact parathyroid hormone

LRP : Low-density lipoprotein receptor related proteins

P : Phosphorus

PTH1R : PTH receptor 1

SOST : Sclerostin gene

T1DM : Type 1 diabetes mellitus

T2DM : Type 2 diabetes mellitus

osteoprogenitor cells and decreases apoptosis of osteoblasts. So these anabolic influence leads to an increase of bone mass. Sclerostin which is secreted by the osteocytes, is one of the main regulators of Wnt signaling. It's an endogenous inhibitor of pathway [8]. Together with Dickkopf1, sclerostin binds to coreceptors low-density lipoprotein receptor related proteins 5 and 6 (LRP5 and 6) and leads to inhibition of differentiation of osteoblasts [9]. Sclerosteosis and Van Buchem disease are two rare genetic disorders caused by genetic defects in sclerostin gene (SOST). Impaired sclerostin production results with markedly high bone mass [10]. Experimental studies with SOST knockout mice results a higher bone formation and BMD [11]. Sclerostin inhibition is a novel therapeutic target in osteoporosis.

1. INTRODUCTION

Diabetes mellitus is associated with increased risk of fracture [1] but diabetic bone disease is an often neglected complication. Type 1 diabetes mellitus (T1DM) patients are shown having lower bone mineral density (BMD). Especially the younger patients may not reach the peak bone mass [2]. But fracture risk of the patients increases beyond that decrease of BMD. So some other factors about bone quality must participate the case. Bone metabolism is associated with disease duration and glycaemic control [3]. Low BMD is reported in the presence of microvascular complications of diabetes [4]. But the exact mechanism influence the bone metabolism is not elucidated yet.

Wnt signaling pathway is an important modulator for many cellular mechanisms such as regulation of cell cycle, cell proliferation, differentiation and adhesion, tumor formation and angiogenesis [5]. Recent studies prove that Wnt signaling pathway is also essential for bone homeostasis [6,7]. Activation of pathway stimulates expansion of

It's known that T1DM is associated with a decrease in bone formation rather than an increase in bone resorption. There are contradictory data on sclerostin with T1DM. Although some studies have shown increased levels of sclerostin in T1DM [12], some studies have found no association [13,14].

The objectives of the present study are to evaluate sclerostin levels of adult T1DM patients and to analyse relationships of sclerostin with bone turnover markers and BMD.

2. METHODOLOGY

A total of 48 (25 female, 23 male) patients with T1DM and 40 (25 female, 15 male) healthy control cases were included in this cross-sectional study. T1DM patients referred to the Endocrinology outpatient clinic between January to December 2013 were recruited. Microalbumin levels were measured by 24-hour urine collection and the estimated glomerular filtration rate (GFR) was calculated by Cockcroft-Gault Equation. T1DM patients with normal renal function were

included in the study. Control group cases were recruited from healthy volunteers. Exclusion criteria included current smoking, alcohol intake, usage of drugs affecting bone (such as calcium and vitamin D supplements or glucocorticoids), systemic inflammatory disorders, malignancy and the pregnancy.

The study protocol was prepared according to the Declaration of Helsinki and approved by the local ethics committee. Informed consent forms were read to and signed by all participants.

Anthropometric measurements (height as cm and weight as kg) were recorded. Body mass index (BMI) was calculated as body weight divided by square of height (kg/m^2).

Fasting blood glucose, *albumin*, *calcium (Ca)*, *phosphorus (P)* and *alkaline phosphatase (ALP)* levels were measured by automated routine procedures (Beckman Coulter LX20, USA). *Intact parathyroid hormone (iPTH)*, *osteocalcin* and *pyrilinks-D 25* were measured by Beckman Coulter DXI600-800, USA. *25(OH)Vitamin D (25OHD)* and HbA1c was measured by the high performance liquid chromatography method (Tosoh G7, USA). Sclerostin levels were measured using ELISA method (R&D Systems, Minneapolis, MN, USA).

BMD measurements at the lumbar spine and femoral neck of T1DM patients were done by dual energy x ray absorptiometry (DEXA) (GE Lunar DPX-IQ, GE Healthcare, UK).

2.1 Statistical Analysis

All statistical analyses were performed using SPSS 20.0 software for Windows. Post-hoc power analysis exhibited 100% power with a sample size of 48 T1DM patients. Chi-square test was used to compare proportions. The normality of the distribution of all variables was assessed by the Kolmogorow-Smirnoff test. Student's t test and ANOVA test were used for normally distributed variables. Mann-Whitney U test and Kruskal-Wallis test were used for non-parametric variables. Pearson's and Spearman rank correlation analyses were done to determine the associations between parameters. Level of statistical significance was set as $P < 0.05$.

3. RESULTS

Clinical characteristics, biochemical and DEXA results of the study population are shown in

Table 1. T1DM and control groups were age, sex and BMI matched.

Sclerostin levels of T1DM patients (803.9 ± 92.01 pg/ml) were significantly higher than control cases (522.9 ± 76.23 pg/ml) ($P=0.000$). Sclerostin has no correlation with age or gender. In T1DM group, HbA1c levels were positively correlated with diabetes duration ($P=0.057$) but there was no significant association between sclerostin level and HbA1c or diabetes duration.

Sclerostin level was negatively correlated with lumbar vertebrae and femur neck BMD; however, positively correlated with ALP, iPTH, osteocalcin, pyrilinks-D (Table 2).

ALP, iPTH, osteocalcin and pyrilinks-D levels were significantly increased in T1DM patients (Table 1). There was no statistically significant difference between 25OHD levels of the groups.

Lumbar vertebrae and femur neck BMD has negative correlation with HbA1c and duration of T1DM (Table 3).

4. DISCUSSION

Our study evaluated the relationship between sclerostin and bone turnover markers in T1DM. Sclerostin is a key inhibitor for Wnt signaling pathway. Sclerostin is critical both for inhibition of bone formation and inducing bone resorption. Recently, many studies have focused on sclerostin and bone metabolism [8,9,11-14]. But there is still few data about sclerostin in T1DM.

We found a marked increase in serum sclerostin levels in T1DM patients. There are studies have found increased sclerostin in Type 2 diabetes mellitus (T2DM) [15] but data in T1DM were contradictory. In an experimental study using rats rendered diabetic by streptozotocin, sclerostin levels found increased and study results suggested decreased osteoblastogenesis in insulin dependent DM [16]. Gennari and colleagues have found no significant difference between sclerostin levels of adult T1DM and control groups [13]. Tsentidis and colleagues have shown a similar result in pediatric age group [14]. However, Neumann and colleagues have found that sclerostin levels were increased in T1DM [12]. Sclerostin and gender relationship has also conflicting results. Gennari and colleagues have found that sclerostin levels were

higher in males in both T1DM and T2DM groups [13]. On the contrary, Catalano and colleagues have shown higher sclerostin levels in females [17]. However, Neumann and colleagues have found no gender difference [12]. Our results also found no correlation of sclerostin either with age or gender.

When we evaluated the bone turnover markers, we found that iPTH, ALP, osteocalcin and pyrilinks-D levels were significantly increased in T1DM patients and sclerostin level was positively correlated with them.

Trio of Ca, iPTH and vitamin D is very important for bone metabolism. Because it's affected by dietary intake, Ca is not a good marker of bone resorption. Serum Ca levels doesn't differ between T1DM and controls in many studies [12,18,19]. iPTH is also difficult to evaluate because it may increase in case of diabetic nephropathy. In the studies excluding patients with renal impairment, serum PTH levels were found increased in T1DM patients [13,20]. In accordance with the literature, we also found no difference between serum Ca levels of T1DM patients and control group but iPTH levels were

Table 1. Characteristics of the study population

	T1DM	Control	P
Age (years)	29,67±9,9	27,22±7,9	0,203
Female/male (n)	25/23	25/15	0,330
BMI (kg/m ²)	23,99±3,81	23,26±3,68	0,364
HbA1c (%)	8,39±1,91		
Diabetes duration (years)	11,35±7,6		
Sclerostin (pg/ml)	803,9 ±92,01	522,9 ±76,23	0,000
cCa (mg/dL)	9,04±0,41	9,14±0,45	0,358
P (mg/dL)	3,35±0,67	2,95±0,75	0,362
ALP (U/L)	78,52±25,74	48,17±16,32	0,000
iPTH (pg/ml)	57,90±41,16	40,11±12,15	0,008
Osteocalcin (ng/ml)	9,10±7,66	5,70±1,74	0,005
Pyrilinks-D (nM/mM creatinin)	11,85±19,32	4,82±1,87	0,017
25OHD (mmol/l)	28,59±20,10	32,56±11,54	0,332
L1-L4 BMD (gr/cm ²)	0,94±0,13		
L1-L4 Z score	-0,81±1,21		
Femur neck BMD (gr/cm ²)	0,75±0,12		
Femur neck Z score	-0,94±1,04		

BMI: Body Mass Index; cCa: Albumin Corrected Calcium; P: Phosphorus; ALP: Alkaline Phosphatase; iPTH: Intact Parathyroid Hormone; 25OHD: 25(OH) Vitamin D; BMD: Bone Mineral Density.

Table 2. Correlations of sclerostin with bone mineral density and bone turnover markers

Sklerostin	r	P
L1-L4 BMD	-0.571	0.000
L1-L4 Z score	-0,489	0,001
Femur neck BMD	-0.367	0.012
Femur neck Z score	-0,411	0,005
ALP	0.405	0.001
iPTH	0.259	0.026
osteocalcin	0.228	0.049
pyrilinks-D	0.430	0.000

BMD: Bone Mineral Density; ALP: Alkaline Phosphatase; iPTH: Intact Parathyroid Hormone

Table 3. Association of bone mineral density with HbA1c and duration of T1DM

	HbA1c		Duration of T1DM	
	r	P	r	P
L1-L4 BMD	-0,375	0,012	-0,212	0,168
L1-L4 Z score	-0,322	0,033	-0,148	0,339
Femur neck BMD	-0,298	0,049	-0,326	0,031
Femur neck Z score	-0,368	0,014	-0,322	0,033

BMD: Bone Mineral Density

significantly increased in T1DM. The results of our study differs from the literature in terms of 25OHD levels. Many studies have found that 25OHD was lower in T1DM than controls [2,13,18,20]. In our study, 25OHD levels of T1DM cohort were $28,59 \pm 20,10$ mmol/l. This level indicates insufficiency. 25OHD levels of healthy controls were $32,56 \pm 11,54$ mmol/l, thus at the lower limit of normal. 25OHD levels of T1DM patients were lower than control group but this difference was not statistically significant. This result may be explained by the already low 25OHD levels in our country. Vitamin D deficiency is a significant health problem in Turkey especially in young female population [21-23]. Additionally, small size of our study group may affect the results.

It's well known that iPTH increases bone resorption; it stimulates osteoclasts by binding to PTH receptor 1 (PTHR1). But interestingly iPTH may also decrease bone resorption by inhibiting the expression of (SOST) [24-26]. If we reflect this data to laboratory, a negative correlation between sclerostin and iPTH is expected. However, results of the studies are contradictory. Garcia-Martin and colleagues found an inverse association between sclerostin and iPTH in T2DM patients [15], whereas studies with T1DM have shown no association [12,13]. We found a positive correlation between sclerostin and iPTH in accordance with bone resorptive effects of them. More detailed studies are needed to understand the mechanism of PTH and PTHR1 on bone metabolism.

ALP is a bone formation marker. Large studies found no difference of ALP levels in T1DM patients in comparison to controls [20]. We found that ALP levels were increased in T1DM patients. Unfortunately in our study bone-ALP measurement couldn't be done. So we couldn't separate different iso-enzymes like the liver-ALP. Hamilton and colleagues have demonstrated that ALP increase in T1DM may be caused by liver involvement [27].

An osteoblast-produced factor, osteocalcin is the major non-collagenous bone matrix protein. Besides it's a bone formation marker, osteocalcin is also involved in the regulation of glucose metabolism. Glycemic control may affect osteoblast function and may cause alterations in osteocalcin levels [28]. Serum levels of osteocalcin have found significantly decreased in T1DM patients [19,29,30]. In our study, osteocalcin levels of T1DM patients were

significantly higher than healthy controls. This finding seems inconsistent with other studies about osteocalcin. Maddaloni and colleagues showed that osteocalcin levels are inversely correlated with HbA1c in T1DM cohort with long disease duration [31]. Our cohort also consist of adult T1DM patients and average disease duration is more than ten 10 years. Longstanding glucose metabolism impairment may cause a fluctuation of osteocalcin level.

Pyrilinks-D (also called Deoxypyridinoline) is a specific marker of bone resorption and osteoclastic activity. It is excreted unmetabolized in urine. Despite there are studies show that the pyrilinks-D level in T1DM has not changed [2], many studies have found the urinary Pyrilinks-D excretion higher in young diabetics [19,32]. Catalano and colleagues found a similar result in adult age T1DM patients [33]. Our results showed increased pyrilinks-D levels and a positive association with sclerostin in T1DM patients and suggested increased bone resorption in T1DM.

In our study, sclerostin level was negatively correlated with lumbar vertebrae and femur neck BMD. This finding is compatible with sclerostin function demonstrated in sclerosteosis and Van Buchem disease [10] or in experimental studies with SOST knockout mice [11]. But it's not exactly compatible with the literature. Whereas there are studies that found significant negative correlation between sclerostin and BMD in older age group [34], positive correlation was determined in pediatric age T1DM and adult age T2DM cohorts [14,15]. Environmental and genetic influences may discuss to explain this difference. Our study cohort consist of adult T1DM patients with long disease duration and low 25OHD levels. Lumbar vertebrae and femur neck BMD has negative correlation with HbA1c and duration of T1DM. So low BMD may be independent of sclerostin.

5. CONCLUSION

In conclusion, our study demonstrated a marked increase in serum sclerostin levels in T1DM patients. Bone turnover markers, iPTH, ALP, osteocalcin and pyrilinks-D levels were also significantly increased. Sclerostin had an inverse association with lumbar vertebrae and femur neck BMD and BMD is associated with glycaemic control and diabetes duration. Increased risk of fracture in T1DM is a

multifactorial process and future researchs are needed to illuminate sclerostin association.

ETHICAL APPROVAL

The study was approved by the Trakya University Faculty of Medicine ethics committee (project number: 2012-547).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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