

## **Exploration of serum levels of SOST and its clinical significance in elderly patients with type 2 diabetes mellitus complicated with osteoporosis.**

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### **Abstract**

**Objective:** To observe the Serum Sclerostin (SOST) level of elderly patients with Type 2 Diabetes Mellitus (T2DM) complicated with Osteoporosis (OP), and to analyse the correlation between T2DM and OP and the clinical significance.

**Methods:** From January 2014 to December 2016, 210 elderly patients with T2DM were divided into two groups: T2DM complicated with OP group (group A) and T2DM group (group B), 105 cases in each group. 105 elderly healthy subjects were recruited as the control group (group C). The levels of serum FPG, 2hPG, FINS, HbA1c and SOST were measured in all subjects. HOMA-IR was calculated and the Bone Mineral Density (BMD) of the radius, femur and lumbar vertebrae of the three groups were measured and compared.

**Results:** The levels of FPG, 2hPG, FINS, HbA1c, HOMA-IR and SOST in groups A and B were significantly higher than those in group C ( $P < 0.05$ ). The FPG, 2 h PG and SOST levels in group A were significantly higher than those in group B ( $P < 0.05$ ). There was no significant difference in FINS, HbA1c and HOMA-IR between groups A and B ( $P > 0.05$ ). The BMDs of the radial, femur and lumbar vertebrae of group A were significantly lower than that of groups B and C ( $P < 0.05$ ), while there was no significant difference between groups B and C ( $P > 0.05$ ). There was a linear positive correlation between SOST and FPG, 2 h PG, radial BMD, femur BMD and lumbar BMD ( $P < 0.05$ ).

**Conclusion:** The expression of serum SOST in elderly patients with T2DM complicated with OP is obviously increased, which may be related to the increase of blood glucose level. The interaction between them is of great significance in the occurrence and development of T2DM complicated with OP.

**Keywords:** Elderly patients, Type 2 diabetes, Osteoporosis, Sclerostin, Clinical significance.

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### **Introduction**

The incidence of Type 2 Diabetes Mellitus (T2DM) is increasing as the current process of population aging and the improvement of living standard [1]. Some studies have shown that [2,3], the incidence of Osteoporosis (OP) in Type 2 Diabetes (T2DM) patients was significantly higher than the population with normal blood glucose level. The clinical manifestations of the initial OP are not very significant, but when the disease progress to a certain extent, fracture often occurred in hip, vertebral or distal radius, which increase patient's pain and cause socio-economic burden [4]. Sclerostin (SOST) is a secretory glycoprotein containing 213 amino acids with a relative molecular mass of 24,000, including one sequence for the secretory signal and two possible N-glycosylation sites [5]. The SOST plays an important role in the process of bone metabolism, which can inhibit the Wnt signaling pathway to regulate bone metabolism, and have significant effect on osteoblastogenesis and bone formation process [6]. It has been reported [7] that SOST monoclonal

antibody can antagonize SOST to negatively regulate bone formation, not only can inhibit bone loss, also promote bone formation, with a significant advantage for osteoporosis treatment. This study was aimed to explore the relationship between serum SOST and T2DM complicated with OP by determining the SOST level of elderly patients with type 2 T2DM complicated with OP to provide basis for early diagnosis and effective treatment of this disease.

### **Materials and Methods**

#### **General information**

210 elderly patients with T2DM from January 2014 to December 2016 were recruited as subjects. T2DM diagnosis is referred to the 1999 WHO diagnostic and typing criteria [8]; OP diagnosis is based on the 2011 WHO diagnostic criteria for osteoporosis [9]. Patients with severe heart and kidney disease, patients with severe endocrine and metabolic disorders, patients with malignant tumors, patients with gastrointestinal

diseases, patients with long-term bed rest, patients who have used glycolipid metabolism drugs for nearly one month, and Pregnant and lactating women were excluded. All patients were divided into T2DM complicated with OP group (group A) and T2DM group (group B), 105 cases in each group. In group A, there were 52 males and 53 females, aged from 60 to 79 y, mean age of ( $65.42 \pm 9.37$  y); In group B, there were 51

males and 54 females, aged from 60 to 78 y, mean age of ( $67.26 \pm 8.58$  y). At the same time, 105 healthy subjects were selected as the control group (group C), including 50 males and 55 females, aged from 60 to 78 y, mean age of ( $66.91 \pm 8.40$  y). There were no significant differences in general information between the three groups ( $P > 0.05$ , Table 1).

**Table 1.** Comparison of general information between the three groups (n/%,  $\bar{x} \pm s$ ).

Groups	Number of cases	Gender (male/female)	Age (y)	BMI (kg/m <sup>2</sup> )	Comorbidity (n (%))
A group	105	52/53	$65.42 \pm 9.37$	$25.45 \pm 3.56$	49 (46.67%)
B group	105	51/54	$67.26 \pm 8.58$	$24.99 \pm 2.73$	51 (48.57%)
C group	105	50/55	$66.91 \pm 8.40$	$24.49 \pm 2.89$	45 (42.86%)
F/ $\chi^2$ value		0.076*	1.296#	2.550#	0.716*
P value		0.963	0.275	0.080	0.699

Note: \*data analysed by  $\chi^2$  test, #data analysed by variance analysis

## Methods

All subjects were asked to collect 5 ml venous blood in fasting early morning. Fasting Plasma Glucose (FPG) was used to determine glucose oxidase method (Hitachi 7600 Automated Biochemical Analyzers, Japan), chemiluminescence method was used for fasting insulin (FINS) level (Roche E170 electrochemistry luminescence immunity analyzers, Switzerland), glycosylated hemoglobin (HbA1c) was detected by VARI-ANT II high pressure liquid chromatography (Bole D-10 glycosylated hemoglobin analyzer, America), SOST level were detected by Enzyme-Linked Immunosorbent Assay (ELISA) for SOST ELISA kit (Beijing craymeibio co., LTD, China). The insulin resistance index of steady-state model was calculated based on the FPG and FINS levels:  $HOMA-IR = FPG \times \text{fasting insulin} / 22.5$ . The levels of 2 h postprandial blood glucose (Oral Glucose Tolerance Test (OGTT)) were compared between the three groups. The Bone Mineral Densities (BMD) of the radius, femur and lumbar vertebrae were measured by dual energy X-ray absorptiometry.

## Statistical methods

The experimental data were analysed by SPSS 19.0 statistical software. The measurement data were present as mean  $\pm$

standard deviation ( $\bar{x} \pm s$ ). Multiple comparisons were performed using variance analysis. The comparisons between two groups were analysed by SNK and Bonferroni t test. The data between the groups were compared by  $\chi^2$  test, and the bivariate correlation analysis was analysed by Pearson correlation analysis.  $P < 0.05$  means the difference was statistically significant.

## Results

### Comparison of biochemical indexes between three groups

The levels of FPG, 2hPG, FINS, HbA1c, HOMA-IR and SOST in groups A and B were significantly higher than those in group C ( $P < 0.05$ ). The FPG, 2 h PG and SOST levels in group A were significantly higher than those in group B ( $P < 0.05$ ). There was no significant difference between FINS, HbA1c and HOMA-IR in groups A and B ( $P > 0.05$ ) (Table 2).

**Table 2.** Comparison of biochemical markers between the three groups ( $\bar{x} \pm s$ ).

Group	Number cases	FPG (mmol/L)	2 h PG (mmol/L)	FINS (mU/L)	HbA1c (%)	HOMA-IR	SOST(ng/ml)
A group	105	$10.56 \pm 2.64^{①②}$	$14.58 \pm 3.26^{①②}$	$16.62 \pm 4.54^{②}$	$8.12 \pm 1.45^{②}$	$2.70 \pm 0.84^{②}$	$16.15 \pm 3.58^{①②}$
B group	105	$9.25 \pm 2.51^{②}$	$13.24 \pm 3.13^{②}$	$16.35 \pm 3.67^{②}$	$7.87 \pm 1.32^{②}$	$2.51 \pm 0.78^{②}$	$9.17 \pm 2.71^{②}$
C group	105	$5.13 \pm 1.49$	$6.69 \pm 2.08$	$7.68 \pm 2.80$	$5.36 \pm 0.85$	$1.62 \pm 0.53$	$5.28 \pm 1.75$
F value		163.28	226.86	194.32	160.70	65.66	411.47
P value		0.000	0.000	0.000	0.000	0.000	0.000

Note: data analysed by variance analysis, <sup>①</sup> $P < 0.05$ , compared with group B; <sup>②</sup> $P < 0.05$ , compared with group C.

**Comparison of BMDs of radius, femur and lumbar vertebrae between the three groups**

The BMDs of the radial, femur and lumbar vertebrae of group A was significantly lower than those of groups B and C

( $P < 0.05$ ). There was no significant difference in BMDs of radius, femur and lumbar vertebrae between groups B and C ( $P > 0.05$ ). There was no significant difference between groups B and C ( $P > 0.05$ ) (Table 3).

**Table 3.** Comparison of BMDs of radius, femur and lumbar vertebrae between the three groups ( $\bar{x} \pm s$ ,  $g/cm^2$ ).

Group	Number of cases	Radius	Femur	Lumbar vertebrae
A group	105	0.52 ± 0.13 <sup>①②</sup>	0.85 ± 0.20 <sup>①②</sup>	0.98 ± 0.17 <sup>①②</sup>
B group	105	0.56 ± 0.10	0.93 ± 0.18	1.10 ± 0.14
C group	105	0.59 ± 0.15	0.92 ± 0.12	1.12 ± 0.12
F value		7.86	6.90	28.71
P value		0.000	0.001	0.000

Note: data analysed by variance analysis, <sup>①</sup> $P < 0.05$ , compared with group B; <sup>②</sup> $P < 0.05$ , compared with group C.

**Bivariate correlation analysis**

Pearson correlation analysis showed that SOST was defined as dependent variable, FPG, 2 h PG, FINS, HbA1c, radial BMD, femoral BMD and lumbar vertebrae BMD as independent variable. SOST was positively correlated with FPG, 2 h PG, radial BMD, femur BMD and lumbar BMD, while there was no significant correlation with the other indexes ( $P > 0.05$ ) (Table 4).

**Table 4.** Bivariate correlation analysis.

Index	R value	P value	Index	R value	P value
FPG	0.384	0.004	HOMA-IR	0.036	0.576
2 h PG	0.470	0.000	Radial BMD	0.412	0.002
FINS	0.045	0.524	Femur BMD	0.475	0.000
HbA1c	0.163	0.214	Lumbar vertebrae BMD	0.526	0.000

Note: data analysed by Pearson correlation analysis.

**Discussion**

OP is a systemic disease more common in elderly patients, due to various causes of bone loss makes bone tissue microstructure easy to damage and bone brittleness significantly increased. The OP patients are prone to fracture, leading to the disability in elderly population. Compared with population with normal blood glucose levels, the incidence of OP in T2DM patients were significantly higher, but the current pathogenesis was not fully elucidated [10]. The causes of T2DM-induced osteoporosis may include the following aspects: 1. elevated blood glucose levels can cause osmotic diuresis, resulting in calcium, phosphorus and magnesium excretion significantly increased, parathyroid hormone secretion also increased, making Bone resorption increased

significantly, leading to bone loss [11]. 2. Long-term increased blood glucose levels can stimulate the final product of glycosylation to stimulate osteoclast bone resorption of cytokines, leading to the ability of osteoblast adhesion to collagen significantly reduced [12]. 3. The insulin receptors exists on osteoblast membrane, insulin can directly stimulate osteoblasts and promote the synthesis of collagen and bone marrow secretion.

SOST is a newly discovered regulatory factor for bone formation and is a member of the cystine structure superfamily. Recent studies have shown that [13] SOST plays an important role in osteoblastogenesis and bone formation, which may be associated with the development of osteoporosis. However, there are few studies on the changes of serum SOST levels in T2DM patients. Early studies suggest that SOST can attenuate the BMP signaling by competitive binding of Bone Morphogenetic Protein (BMP) receptors. However, current studies have confirmed that SOST is not a classical antagonist of BMP pathway. The role of negative regulation of bone formation is mainly related to Wnt/ $\beta$ -catenin signaling pathway [14]. Wnt/ $\beta$ -catenin signaling pathway can regulate the proliferation, differentiation and apoptosis of bone tissue cells to regulate bone mass [15]. SOST has a high affinity to the common receptor LRP5/6 in Wnt signal pathway. Once binding with LRP5/6, SOST can inhibit the activation of Wnt signaling pathway, and thus suppress the formation of bone [16].

The results of this study showed the levels of serum FPG, 2 h PG, FINS, HbA1c, HOMA-IR and SOST in T2DM patients were significantly higher than those in healthy controls ( $P < 0.05$ ), suggesting that blood glucose, insulin and SOST of T2DM patients are abnormal. The levels of FPG, 2 h PG and SOST in T2DM+OP group were significantly higher than those in T2DM group ( $P < 0.05$ ), but there was no statistic difference of FINS, HbA1c and HOMA-IR between T2DM+OP group and T2DM group ( $P > 0.05$ ), indicating compared with

T2DM group, blood glucose and SOST expression of T2DM patients with OP were higher. There is no uniform understanding of bone mineral density in T2DM patients. The results showed that the BMDs of radial, femur and lumbar of T2DM+OP group were significantly lower than those of the T2DM group and healthy control group ( $P<0.05$ ), indicating that bone mineral density of T2DM patients with OP has a downward trend. However, there was no statistically significant difference in BMD between the T2DM group and the healthy control group ( $P>0.05$ ), which may be related to the sample size of the study. The results still need to enlarge the sample size for further observation.

It is demonstrated that SOST was positively correlated with FPG, 2 h PG, radial BMD, femur BMD and lumbar BMD ( $P<0.05$ ). The expression of SOST gene was regulated by various factors, including Parathyroid Hormone (PTH), estrogen, angiotensin system (RAS) and Tumor Necrosis Factor (TNF). The above factors can regulate the expression of SOST gene to regulate bone formation [17,18]. Studies have shown that [19,20], serum PTH and estradiol levels of T2DM patients are obvious abnormalities. Therefore, it may be due to the higher blood glucose levels can promote the expression of SOST, thereby inhibiting bone formation, and long-term action may lead to the occurrence of OP.

In summary, the expression of serum SOST of elderly T2DM patients complicated with OP was significantly increased, which may be related to the high blood glucose level of the patients. The interaction between the two was of great significance in the occurrence and development of T2DM complicated with OP. This study provides the basis for the diagnosis and treatment of T2DM complicated with OP. However, the specific mechanism of SOST gene expression regulated by T2DM still needs further study.

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