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ORIGINAL ARTICLE

Role of Sclerostin in the Bone Loss of Postmenopausal Chinese Women with Type 2 Diabetes

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Key words: sclerostin; type 2 diabetes mellitus; postmenopause; bone markers; bone mineral density

Objective To evaluate the role of sclerostin in bone loss of postmenopausal Chinese women with type 2 diabetes mellitus.

Methods The postmenopausal patients suffering from type 2 diabetes mellitus and age, body mass index, and duration of menopause matched healthy controls were enrolled into this cross-sectional study according to criteria of inclusion and exclusion. The serum sclerostin level and bone mineral density of the anterior-posterior lumbar spine (L1-L4), femoral neck, and total hip were determined by using a quantitative sandwich ELISA kit and dual X-ray absorptiometry, respectively. Meanwhile, the clinical and laboratory indexes of bone mineral metabolism were analyzed. Associations between serum sclerostin level and bone mineral density as well as bone turnover markers were evaluated by linear regression analysis.

Results Finally, 265 postmenopausal women with type 2 diabetes and 225 non-diabetic women were recruited in the diabetic group and control group, respectively. Serum sclerostin level of the diabetic group was significantly higher than that of the control group (48.2 ± 19.4 vs. 37.2 ± 18.6 pmol/L, $P < 0.001$) and was increased with age in both groups (diabetic group, $r = 0.374$, $P < 0.001$; control group, $r = 0.312$, $P < 0.001$). In type 2 diabetes patients, serum sclerostin concentration was positively correlated with hemoglobin A_{1c} level ($r = 0.237$; $P = 0.021$). Biochemical bone turnover markers, intact parathyroid hormone and bone-specific alkaline phosphatase, were negatively associated with serum sclerostin level ($r = -0.138$, $P = 0.078$ and $r = -0.265$, $P < 0.001$). Conversely, the positive correlation between sclerostin and C-terminal cross-linking telopeptide of type I collagen was found in diabetic patients ($r = 0.354$, $P < 0.001$). Serum sclerostin levels of the diabetic group were positively correlated with bone mineral density of the lumbar spine, femoral neck, and total hip ($r = 0.324$, 0.367 , and 0.416 , respectively; all $P < 0.001$).

Conclusions Sclerostin might participate in the pathogenesis of bone loss of type 2 diabetes. The high sclerostin level might serve as a marker of increased osteocyte activity in postmenopausal patients with type 2 diabetes mellitus.

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SCLEROSTIN is produced by osteocytes and acts on osteoblasts as a negative regulator, inhibiting osteoblast-driven bone formation through the Wnt/ β -catenin signaling pathway.^{1,2} Serum sclerostin level has already been found to be increased in patients with renal osteodystrophy, rheumatoid arthritis, and under hemodialysis.³⁻⁵ Sclerostin is implicated in the pathogenesis of osteoporosis. Some studies demonstrated circulating sclerostin level was significantly higher in postmenopausal women with low free estrogen index compared with premenopausal women.^{6, 7} Because of its important role in bone formation and skeletal development in sclerosteosis, sclerostin has also been proposed to play a role in the pathogenesis of postmenopausal osteoporosis.

Diabetes is a great risk factor of osteoporosis. Bone fragility unrelated to the decreased bone density may be the pathological conditions of fracture peculiar to diabetes. Moreover, type 2 diabetes mellitus (T2DM) patients have higher serum sclerostin levels compared with control subjects.^{8, 9} T2DM has a high prevalence in elderly postmenopausal women. At present, there is no report on the levels of serum sclerostin in postmenopausal Chinese (non-Caucasian) women with T2DM and its possible role in the development of bone loss in Chinese diabetic patients. This cross-sectional study therefore measured the serum sclerostin level in postmenopausal Chinese women with T2DM and analyzed the association of serum sclerostin level with relevant clinical characteristics and bone turnover markers, in order to investigate whether sclerostin have a role in the pathogenesis of postmenopausal osteoporosis in Chinese diabetic patients.

PATIENTS AND METHODS

Patients

The postmenopausal inpatient and outpatients with T2DM presenting to the diabetes clinic of our hospital during January 2010 and December 2011 were enrolled as the diabetic group. All the patients were being treated with oral antidiabetic drugs other than thiazolidinediones. The postmenopausal non-diabetic individuals were randomly selected from subjects admitted to our hospital for an annual physical check-up during the same period as the control group. All included controls had normal glucose homeostasis as assessed by 75-g oral glucose tolerance test. Postmenopausal status was defined as more than 1 year of amenorrhea, or age older than 50 years for women who had had a hysterectomy.

Exclusion criteria were as follows: renal dysfunction; inflammatory disorders such as rheumatoid arthritis and

systemic lupus erythematosus with abnormal cytokine values; and endocrine disorders, such as hypothyroidism, hyperthyroidism, primary or secondary hyperparathyroidism, and hypercorticism. Subjects who had a history of using active vitamin D₃, corticosteroids, bisphosphonates, calcitonin injection, estrogens, steroids, thyroid hormone, diuretics, immunosuppressant medications or anticonvulsants that could interfere with normal bone turnover during the last 6 months were excluded. Written informed consent was obtained from all participants, and the study was approved by the Ethics Committee of China Medical University.

Measurement of sclerostin

The serum sclerostin levels were measured by a quantitative sandwich ELISA kit (Biomedica, Vienna, Austria) according to manufacturer's instructions.¹⁰ Briefly, 20 μ L standards or samples were incubated with 50 μ L of detection antibody at room temperature overnight. After wells were washed, 200 μ L horseradish peroxidase-conjugated streptavidin was added into each well and they were incubated for 1 hour at room temperature in the dark. After washing step, 3, 3', 5, 5'-tetramethylbenzidine used as substrate was added, followed by addition of 50 μ L stop solution. Absorbance was measured at 450 nm. The intra- and inter-assay coefficients of variation of the kit were 5.8% and 6.4%, respectively.

Bone mineral density (BMD) measurement

BMD of the anterior-posterior lumbar spine (L1-L4), femoral neck, and total hip was determined by dual-energy X-ray absorptiometry (DXA) technique, using the LUNAR DPX densitometer (GE-Lunar, Madison, WI, USA).

Biochemical and hormonal assays

Fasting plasma glucose (FPG), 2-hour plasma glucose (2hPG), and hemoglobin A_{1c} (HbA_{1c}) were measured using standard automated laboratory techniques. Serum estradiol was measured by chemiluminescent immunoassay (Siemens, Gwynedd, UK). Kidney function was determined by calculation of the estimated glomerular filtration rate [eGFR; mL/(min $\cdot$ 1.73 m²)] according to the modification of diet in renal disease formula. As indexes of bone formation and resorption, serum calcium, phosphate, 25(OH) vitamin D, parathyroid hormone (PTH), calcitonin, osteocalcin, bone-specific alkaline phosphatase (bALP), and C-terminal telopeptide of type I collagen (CTX) were measured. Serum calcitonin was measured by an immunochemiluminescent assay (Immulite, Diagnostic Products Corporation, Los Angeles, CA, USA). bALP was measured by enzyme immunoassay (Immunodiagnostic Systems, Boldon, UK). Intact PTH, osteocalcin,

and CTX were measured by immunoradiometric assay (CIS Bio. International, ORIS Group, France). Inter- and intra-assay coefficients of variation for all parameters were less than 10%.

Statistical analysis

Data were analyzed using SPSS 11.5 statistical package (SPSS Inc., Chicago, IL, USA). Results were expressed as mean±standard deviation (SD) values, unless stated otherwise. Comparisons of continuous variables between groups were carried out using a Student's *t* test. Comparisons of categorical variables between groups were performed using the χ^2 test. Association of serum sclerostin levels with anthropometric, clinical, and biochemical parameters, BMD, and hormone levels were determined by linear regression analysis after logarithmic transformation of the data for normalization of the distribution. A *P*-value of < 0.05 was considered as statistically significant.

RESULTS

This cross-sectional study included 265 patients with T2DM and 225 control subjects. Baseline characteristics of T2DM patients and matched control subjects are shown in Table 1. Age, duration of menopause, and body mass index (BMI) had no significant difference between the two groups (all *P*>0.05). There was no significant difference in serum levels of estradiol, calcium, phosphate, PTH, and calcitonin (all *P*>0.05). The levels of FPG and 2hPG were significantly

higher in T2DM women than those in controls (all *P*<0.05). Bone resorption markers in diabetic patients were also higher than those in the controls (all *P*<0.05). Diabetic patients and BMI matched control subjects had similar BMDs at the total hip, femoral neck, and lumbar spine (all *P*>0.05).

Postmenopausal women with T2DM had elevated serum levels of sclerostin compared to control subjects without diabetes (*P*<0.001, Table 1). Linear regression analysis revealed a strong positive association between serum sclerostin levels and age in postmenopausal women with (*r*=0.374, *P*<0.001) and without (*r*=0.312, *P*<0.001) diabetes. Serum sclerostin level was positively correlated with BMI in diabetic patients (*r*=0.221, *P*=0.004). By contrast, serum sclerostin level had no significant association with BMI in the controls (*r*=0.157, *P*>0.05). It is of interest to notice that serum sclerostin level was positively correlated with time since diagnosis (year) independently of age (data not shown) in the T2DM patients. Changes of sclerostin level in serum did not significantly correlate with FPG or 2hPG in the diabetic patients (*r*=0.035 or 0.027; all *P*>0.05) as well as in the controls (*r*=0.002 or 0.013; all *P*>0.05). Conversely, a trend for a positive correlation between serum sclerostin and HbA_{1c} levels was observed in diabetic patients (*r*=0.237, *P*=0.021), but we did not found the trend in the controls (*r*=0.024, *P*>0.05). There was slight but not significant association between sclerostin levels and estradiol in the overall cohort of subjects (control

Table 1. Baseline characteristics of the study participants[§]

Groups	<i>n</i>	Age (yr)	Menopause Duration (yr)	BMI (kg/m ²)	Smoker (%)	Alcohol use (%)	FPG (mmol/L)	Sclerostin (pmol/L)	2hPG (mmol/L)	HbA _{1c} (%)	Total estradiol (nmol/L)	eGFR [mL/(min · 1.73 m ²)]
Control group	225	57.7±7.8	7.4±2.3	24.5±5.8	9.3	8.8	4.6±0.9	37.2±18.6	6.2±1.4	5.1±0.8	0.38±0.07	84.5±16.8
Diabetic group	265	58.4±5.7	8.2±2.1	25.7±3.8	9.6	7.4	9.5±1.4	48.2±19.4	12.3±3.5	8.1±1.2	0.37±0.08	82.9±15.4
<i>P</i> value		NS	NS	NS	NS	NS	<0.001	<0.001	<0.001	<0.001	NS	NS

Groups	bALP (IU/L)	Calcium (mmol/L)	Phosphate (mmol/L)	25(OH)D (nmol/L)	Intact PTH (pg/mL)	Osteocalcin (ng/mL)	Calcitonin (ng/L)	CTX (ng/mL)	BMD (g/cm ²)		
									Total hip	Femoral neck	Lumbar spine
Control group	84.7±14.8	2.47±0.21	1.42±0.11	46.3±18.3	28.38±9.34	16.9±0.1	93.18±15.65	0.32±0.12	0.773±0.127	0.685±0.143	0.868±0.153
Diabetic group	76.3±16.8	2.45±0.25	1.48±0.28	43.1±15.5	29.47±8.83	12.5±0.8	94.17±13.52	0.48±0.11	0.746±0.151	0.654±0.127	0.827±0.162
<i>P</i> value	<0.001	NS	NS	NS	NS	<0.001	NS	<0.001	NS	NS	NS

§: Plus-minus values are means±SD.

BMI: body mass index; FPG: fasting plasma glucose; 2hPG: 2-hour plasma glucose; HbA_{1c}: hemoglobin A_{1c}; eGFR: estimated glomerular filtration rate; bALP: bone-specific alkaline phosphatase; 25(OH)D: 25(OH) vitamin D; PTH: parathyroid hormone; CTX: C-terminal cross-linking telopeptide of type I collagen; BMD: bone mineral density; NS: not significant.

group, $r=-0.125$, $P>0.05$; diabetic group, $r=-0.132$, $P>0.05$). Sclerostin levels in serum were negatively correlated with eGFR in the controls and in the diabetic group ($r=-0.237$ and -0.285 ; all $P<0.001$).

Linear regression analysis showed there were no significant correlations between sclerostin levels and serum calcium ($r=-0.014$, $P>0.05$) or phosphate levels ($r=0.037$, $P>0.05$) in the total postmenopausal women. Bone-relevant biochemical markers, namely, 25(OH) D ($r=0.063$, $P>0.05$), calcitonin ($r=0.016$, $P>0.05$), and osteocalcin ($r=0.027$, $P>0.05$), were also not significantly correlated with serum sclerostin level, except that intact PTH was slightly negatively associated with serum sclerostin level in all subjects (control group, $r=-0.145$, $P=0.059$; diabetic group, $r=-0.138$, $P=0.078$). In T2DM patients, serum sclerostin level was negatively associated with bALP ($r=-0.265$, $P<0.001$). A significantly positive correlation was observed between sclerostin and CTX in the diabetic group ($r=0.354$, $P<0.001$). For the controls, both bALP and CTX had no significant correlations with serum sclerostin level ($r=-0.028$ and 0.136 ; all $P>0.05$). Serum sclerostin was positively associated with BMD values at the lumbar spine L1-L4, femoral neck, and total hip in postmenopausal women with diabetes ($r=0.324$, 0.367 , and 0.416 , respectively; all $P<0.001$), however, the relationship was not found in the control group ($r=0.301$, 0.094 , and 0.154 , respectively; all $P>0.05$).

DISCUSSION

This report reveals a correlation of the serum sclerostin levels with BMD and bone turnover markers in Chinese postmenopausal women with T2DM. Patients with T2DM have an increased risk of certain types of osteoporotic fractures because of the poorer quality of the bone.¹¹ In this clinical study, we found that a marked increase in circulating sclerostin levels in Chinese postmenopausal women with T2DM compare with age-matched control subjects without diabetes, demonstrating sclerostin might participate in the pathogenesis of bone loss of T2DM.

Our data confirm the positive relationship between age and serum sclerostin concentrations in postmenopausal women with and without diabetes. FPG and 2hPG were not significantly associated with sclerostin in the diabetic patients as well as in the controls. Although we did not find a consistent relationship between sclerostin and current blood glucose control in patients with T2DM, our finding suggested that HbA_{1c} was positively associated with sclerostin. Glycaemic levels measured at the time when

sclerostin measurements were performed reflected only short-term blood glucose control. HbA_{1c} level may adequately reflect long-term skeletal glycaemic exposure. We found that serum sclerostin was slightly but not significantly correlated with total estradiol levels. This absence of significant correlation contrasts with previous studies that reported significant negative associations.^{7, 12} These discrepancies may be related to differences in racial characteristics.

In our sample, we found that sclerostin levels were negatively associated with bone regulating hormone intact PTH, both in the T2DM patients and in the controls. PTH has an inhibitory role in sclerostin production in humans. On the contrary, a recent report showed that a trend for a positive association between sclerostin and PTH in diabetic patients.⁹ No significant correlation was found between sclerostin and bone-relevant biochemical markers like serum calcium, phosphate, 25(OH)D, calcitonin, and osteocalcin. These results indicated PTH is probably one of the most important regulators of sclerostin secretion in postmenopausal women. In our T2DM cohort, we showed that the serum sclerostin were positively associated with bone resorption marker CTX and negatively associated with bone formation marker bALP. Some previous studies reported a negative correlation between sclerostin and bone formation markers in postmenopausal women,^{8, 10} other studies showed no correlation^{13, 14} or even a positive association.¹⁵ Our findings may suggest that an increase in sclerostin levels leads to a reduction in bone formation and an increase in bone resorption in diabetes.

In our study, diabetic patients and control subjects had similar BMDs at various skeletal regions. We first analyzed the relationship between the serum sclerostin levels and the BMDs in the Chinese postmenopausal women. We found that serum sclerostin was positively associated with lumbar spine and total hip BMDs in controls, being in consistent with the results of previous studies.^{6, 8} In addition, similar results were obtained for diabetic patients in whom circulating sclerostin positively correlated with BMDs in all studied sites. High sclerostin levels in our diabetic women may be associated with decreased bone formation or low bone turnover. Therefore, low bone turnover could slow bone loss and explain the positive relationship between serum sclerostin and BMDs in the postmenopausal women.

This study has some limitations. First, as a cross-sectional study, our data show associations only and cannot prove causation. Therefore, the conclusions of this study are weakened by its design. Second, our study is the relatively small size. The patients might not be representative of

Chinese diabetic patients. In addition, T2DM is associated with an increased risk of fractures. We did not evaluate fracture risks in T2DM patients and analyze their relationship with sclerostin.

In summary, the results of this study show that circulating sclerostin is elevated in Chinese postmenopausal women with T2DM. Moreover, increased serum sclerostin is associated with HbA_{1c}, bone turnover markers, and BMDs in T2DM patients. These findings suggest that high sclerostin may serve as a marker of increased osteocyte activity in diabetic patients.

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