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Original Article

Association between sclerostin, serum bone turnover markers and bone density in postmenopausal women with fragility fracture

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Abstract: In this study we sought to determine the relationship between circulating sclerostin levels, serum bone turnover markers and bone mass in postmenopausal women with fragility fracture. 80 premenopausal women and 80 postmenopausal women with femoral neck fracture were evaluated in a cross-sectional observational study. There were significant differences between the two groups in bone turnover makers. Serum sclerostin levels were higher in postmenopausal women. There was a significant negative correlation between serum sclerostin level and mean femoral neck BMD, mean trochanter BMD and mean total hip BMD in postmenopausal women. No significant correlations were seen between serum sclerostin levels and lumbar spine BMD or serum bone turnover markers in either group. There was significant positive correlation between serum sclerostin level and age in combined pre- and postmenopausal women. But there was no correlation in either group separately. In the postmenopausal women, there were no significant correlations between serum sclerostin levels and any of the measured bone turnover markers. We conclude that measurement of serum sclerostin levels may represent a novel approach to predict the potential risks of fraglity fracture in postmenopausal women.

Keywords: Osteoporosis, sclerostin, postmenopausal

Introduction

Osteoporosis, which is a common bone disorder, especially prevalent in postmenopausal women, is characterized by reduced bone density, alterations of the bone microarchitecture and an increased risk of fragility fracture. The primary complication of osteoporosis is bone fractures, which can occur at almost any site, but most common at the hip, vertebral spine and wrist [1, 2]. Osteoporosis and related fragility fractures are prevalent and major causes of morbidity and mortality in older men and postmenopausal women. Although the pathogenesis of bone loss and skeletal fragility in this disease is not well understood, the imbalance between bone formation and bone resorption plays a important role in its development and will lead to bone pain, skeletal deformities and fracture.

Recently, the Wnt/β-catenin signaling pathway was found to be involved in the control of bone mass in many experimental humans' and animals' researches [3-5]. Activation of this pathway results in increased proliferation and differentiation of osteoblast cells as well as reduced apoptosis of mature osteoblasts. These changes will deposit new bone and increase bone density. Sclerostin, a glycoprotein secreted by osteocytes partially in response to mechanical loading, is a strong negative regulator of osteoblast differentiation and bone formation through antagonizing effects on the Wnt/β-catenin signaling pathway [6].

Sclerostin regulates bone mass by binding to LRP5 and LRP6 and inhibiting canonical Wnt/β-catenin signaling [7, 8]. In vitro studies have shown that sclerostin promotes osteoblast apoptosis, inhibits osteoblast proliferation and suppresses mineralization of osteoblastic cells [9]. Conditions associated with defective sclerostin production such as sclerosteosis and Van Buchem's disease are also associated with high bone mass [10, 11]. Similarly, animal study

has also showed sclerostin-deficient mice increase bone mass and bone strength [12]. Bone biopsy specimens from these patients show more activated osteoblasts compared with normal controls [13].

Though serum levels of sclerostin increase with aging, high serum sclerostin predicts high osteoporotic fractures with negative corrections between sclerostin and BMD have been reported [3, 8, 14-16], other reports showed patients with higher sclerostin serum levels had a higher BMD [15, 17]. However, there are no reports in the literature that assess the relationship between sclerostin, and bone density in fragility fracture. In this study we sought to determine the relationship between circulating sclerostin levels, serum bone turnover markers and bone mass in postmenopausal women with fragility fracture.

Methods

Subjects

80 healthy premenopausal women and 80 postmenopausal women with femoral neck fracture were evaluated in a cross-sectional observational study. This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Shanghai Jiaotong University. Written informed consent was obtained from all participants. The groups were evaluated for serum levels of sclerostin, bone density, and bone turnover markers.

Premenopausal women recruitment for the study was done by open advertisement in the hospital's broadcast system. For inclusion in the study, healthy premenopausal women had to be 20-40 year of age with a body mass index (BMI) of 28 kg/m^2 or less. Postmenopausal women were enrolled from the patients with acute femoral neck fractures, which were caused by low energy such as falling down without collusion.

All subjects had a brief baseline history obtained at the time of initial studying. Smokers were excluded from participation in the study. Subjects were excluded if they had a history of chronic heart, kidney, gastrointestinal, or liver diseases; prior cancer diagnosis; Paget's disease of bone; primary hyperparathyroidism; multiple myeloma.

They were also excluded if they received treatment with medications that interfere with bone metabolism within the last 6 months, such as chronic corticosteroids, seizure medications, selective estrogen receptor modulators, calcitonin, or any prior treatment with bisphosphonates (alendronate, ibandronate, zoledronic acid).

Serum samples were collected in the morning and stored at -50°C until they were assayed for sclerostin (Genetimes Technology, Inc. Shanghai, China, collagen type 1 cross-linked C-telopeptide (CTX1) (R&D systems, Inc, Minneapolis, MN), collagen type 2 cross-linked C-telopeptide (CTX2) (R&D systems, Inc, Minneapolis, MN), bone-specific alkaline phosphatase (bAP) (QUIDEL CORPORATION, San Diego, CA), and procollagen type 1 N-terminal propeptide (P1NP) (QUIDEL CORPORATION, San Diego, CA), Receptor Activator of Nuclear Factor kappa-B (RANK) (R&D systems, Inc, Minneapolis, MN), Receptor activator of nuclear factor kappa-B ligand (RANKL) (R&D systems, Inc, Minneapolis, MN) in our laboratory.

Bone density measurements

Bone mineral density (BMD) was measured by dual-energy x-ray absorptiometry (Lunar Prodigy, Madison, WI). The CV of BMD measurement, based on reproducibility scans, were 1.5% for femoral neck, 1% for total hip, 1% for trochanter and 2% for L1-L4 spine.

Statistical analysis

The study was approved by the Shanghai sixth people's hospital. All subjects provided written informed consent for their participation trial and for all additional measurements presented here. Results are presented as means ± SD, and categorical variables are expressed as frequencies. Data were analyzed using SPSS v.15.0 software (SPSS Inc., Chicago, IL, USA). Student's t test was used to compare the groups for differences in baseline characteristics, sclerostin, osteocalcin, CTX1, CTX2, bAP, P1NP, RANK, RANKL and BMD. Pearson product moment correlation coefficients were calculated between the sclerostin level and bone turnover markers, as well as between sclerostin level and BMD. The association of sclerostin levels with BMD was assessed using multivariable regression adjusted for age. Any p value < 0.05 was considered statistically significant.

Table 1. Subject characteristics, circulating levels of bone proteins and markers of bone turnover

	Premenopausal women	Post-menopausal women with femoral neck fracture	*P* value
Age (years)	29.81 ± 5.48	72.99 ± 10.46	*P* < 0.0001
BMI (kg/m^2)	20.71 ± 2.97	22.96 ± 3.61	*P* < 0.0001
Sclerostion (pg/ml)			
Unadjusted	412.13 ± 15.57	488.51 ± 15.67	0.006
Adjusted by age	338.56 ± 98.33	458.10 ± 42.64	0.054*
P1NP (μg/ml)			
Unadjusted	156.35 ± 3.22	38.72 ± 3.20	< 0.001
Adjusted by age	146.97 ± 8.74	15.78 ± 20.153	< 0.001
Osteocalcin (pg/ml)			
Unadjusted	1442.61 ± 38.76	1151.65 ± 38.51	< 0.0001
Adjusted by age	1420.55 ± 105.67	1098.48 ± 243.66	0.036**
bAP (μg/L)			
Unadjusted	25.03 ± 2.86	82.59 ± 2.84	< 0.0001
Adjusted by age	22.26 ± 7.80	75.51 ± 17.99	< 0.001
CTX 1 (μg/L)			
Unadjusted	749.19 ± 10.31	851.41 ± 10.25	< 0.0001
Adjusted by age	741.38 ± 28.12	831.31 ± 64.84	0.028**
CTX 2 (μg/L)			
Unadjusted by age	713.78 ± 20.41	913.67 ± 20.28	< 0.0001
Adjusted by age	685.77 ± 55.59	844.62 ± 128.18	0.050**
RANK (pg/ml)			
Unadjusted	0.49 ± 0.01	0.39 ± 0.01	< 0.0001
Adjusted by age	0.484 ± 0.04	0.374 ± 0.08	0.036**
RANKL (pg/ml)			
Unadjusted	491.34 ± 20.41	769.23 ± 20.29	< 0.0001
Adjusted by age	464.35 ± 55.62	703.19 ± 128.24	0.003

*P > 0.05, **P < 0.05. BMI=body mass index; bAP=bone-specific alkaline phosphatase; PINP=N-terminal propeptide of type I procollagen; CTX 1=C-terminal telopeptide of type I collagen; CTX 2=C-terminal telopeptide of type II collagen; RANK=Receptor Activator of Nuclear Factor kappa-B; RANKL=Receptor activator of nuclear factor kappa-B ligand.

Table 2. BMD of two groups

	Premenopausal women	Post-menopausal women with femoral neck fracture	*P* value
L1-L4 BMD (g/cm^2)	1.182 ± 0.093	1.086 ± 0.143	< 0.01
Mean femoral neck BMD (g/cm^2)	1.030 ± 0.118	0.877 ± 0.086	< 0.01
Mean trochanter BMD (g/cm^2)	1.045 ± 0.135	0.833 ± 0.126	< 0.01
Mean total hip BMD (g/cm^2)	1.035± 0.116	0.846 ± 0.097	< 0.01

BMD=bone mineral density.

Results

Healthy premenopausal women were 29.8 ± 5.5 year old, and postmenopausal women with femoral neck fracture were 72.9 ± 10.1 year old. BMI was 20.7 ± 2.9 kg/m^2 for premenopausal and 22.9 ± 3.6 kg/m^2 for postmenopausal women (P < 0.0001). There were significant differences between the two groups in sclerostin, osteocalcin, CTX1, CTX2, bAP, P1NP, RANK and RANKL (**Table 1**). The bone formation marker serum bAP, the bone resorption markers serum CTX1 and CTX2 were significantly higher in postmenopausal women (**Table 1**). The bone formation marker serum osteocalcin was significantly lower in postmenopausal women compared to premenopausal women (**Table 1**). After adjusting for age, there were still differences between the two groups in CTX1, CTX2, bAP, P1NP, RANK and RANKL. But there was no difference in serum sclerostin level (P= 0.054). In postmenopausal women group, BMD was also significantly lower at the lumbar spine, total hip, greater trochanter and femoral neck (**Table 2**).

Serum sclerostin levels were higher in postmenopausal women (488.51 ± 15.67 pg/ml) compared with premenopausal women (412.13 ± 15.57 pg/ml) (P=0.006) (**Table 1**). There was a significant negative correlation between serum sclerostin level and mean femoral neck BMD (r=-0.269; P= 0.016), mean trochanter BMD (r=-0.235; P=0.036) and mean total hip BMD (r=-0.376; P=0.0006) in postmenopausal women (**Table 3**; **Figure 2**). No significant correlations were seen between serum sclerostin levels and lumbar spine BMD

Table 3. The correction between sclerostin and BMD

		Neck BMD		Trochanter BMD		Total hip BMD		L1-L4 BMD	
		r value	*p* value	r value	*p* value	r value	*p* value	r value	*p* value
Sclerostion	Unadjusted	-0.269	0.016	-0.235	0.036	-0.376	< 0.001	-0.120	0.289
	Adjusted by age	-0.279	0.013	-0.252	0.025	-0.375	< 0.001	-0.132	0.245

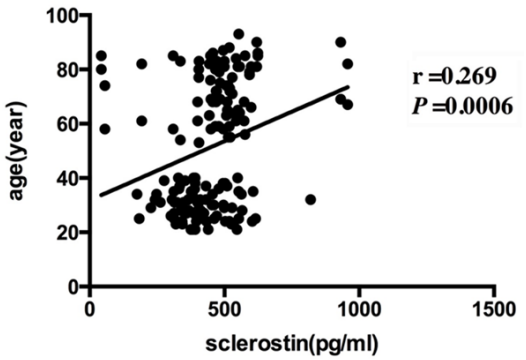

Figure 1. Relationship between age and sclerostin in this study. Sclerostin levels increase significantly with age (r=0.269, *P*=0.0006) in both groups.

(r=-0.120; *P*=0.289). After adjusting for age, the results were same. There was significant positive correlation between serum sclerostin level and age in combined pre- and postmenopausal women (r=0.269; *P*=0.0006) (**Figure 1**). But there was no correlation in either group separately (r=-0.068, *P*=0.55; r=0.102, *P*= 0.36). In the postmenopausal women, there were no significant correlations between serum sclerostin levels and any of the measured bone turnover markers.

Discussion

In this study we examined the relationship between serum sclerostin levels and bone density in postmenopausal women with femoral neck fracture. We found that serum sclerostin levels were significantly higher in postmenopausal women compared with healthy premenopausal women. The present study also shows a strong association between increased sclerostin levels and fragility fracture risk. The fracture risk increased in high sclerostin level. Moreover, bone turnover markers (osteocalcin, CTX1, CTX2, bAP, P1NP, RANK, RANKL) were significantly associated with fragility fracture in postmenopausal women. There was a significant negative correlation between serum sclerostin level and mean femoral neck BMD, mean trochanter BMD and mean total hip BMD in postmenopausal women. It is not clear why there was no correlation between serum sclerostin and lumber BMD.

Age influences sclerostin levels in women. Osteoblastic expression of Wnt-related proteins is modulated by aging [18]. We observed there were high serum sclerostin levels in elder women, with negative association between sclerostin levels and age. Ardawi found a positive association between sclerostin levels and age [3]. Other studies reported increased sclerostin levels with age [3, 15, 19]. It appeared that osteocytic sclerostin production increases with aging; however, diminished sclerostin protein clearance and age-related changes in the hormonal milieu may also contribute to sclerostin increases [15, 19]. In this study, we also calculated the corrections between serum sclerostin and age in postmenopausal women and premenopausal women respectively. However, there was no positive or negative association in either group. The reason was not clear. Maybe there were few subjects in each of these two groups, the findings should be confirmed in a larger study. After we adjusted the age of two groups, we found the serum sclerostin level was no difference between two groups. The result proved that there was correction between serum sclerosin and age. However, it is still believed there were high serum sclerostin levels in elder women. Modder et al. [15] observed that at any age, serum sclerostin levels were higher in men than in women. They thought the larger skeleton in men simply may produce and release more sclerostin into the circulation.

For postmenopausal women with femoral neck fracture, sclerostin levels were significantly negative correlation with BMD at the femoral neck, trochanter and total hip. These results are in agreement with a recent report that serum sclerostin levels are negatively associated with bone density in postmenopausal women [3]. Winkler et al. also reported the results on low BMD and bone volume in mice overexpressing sclerostin [20]. By contrast, the results are different to the findings of Modder

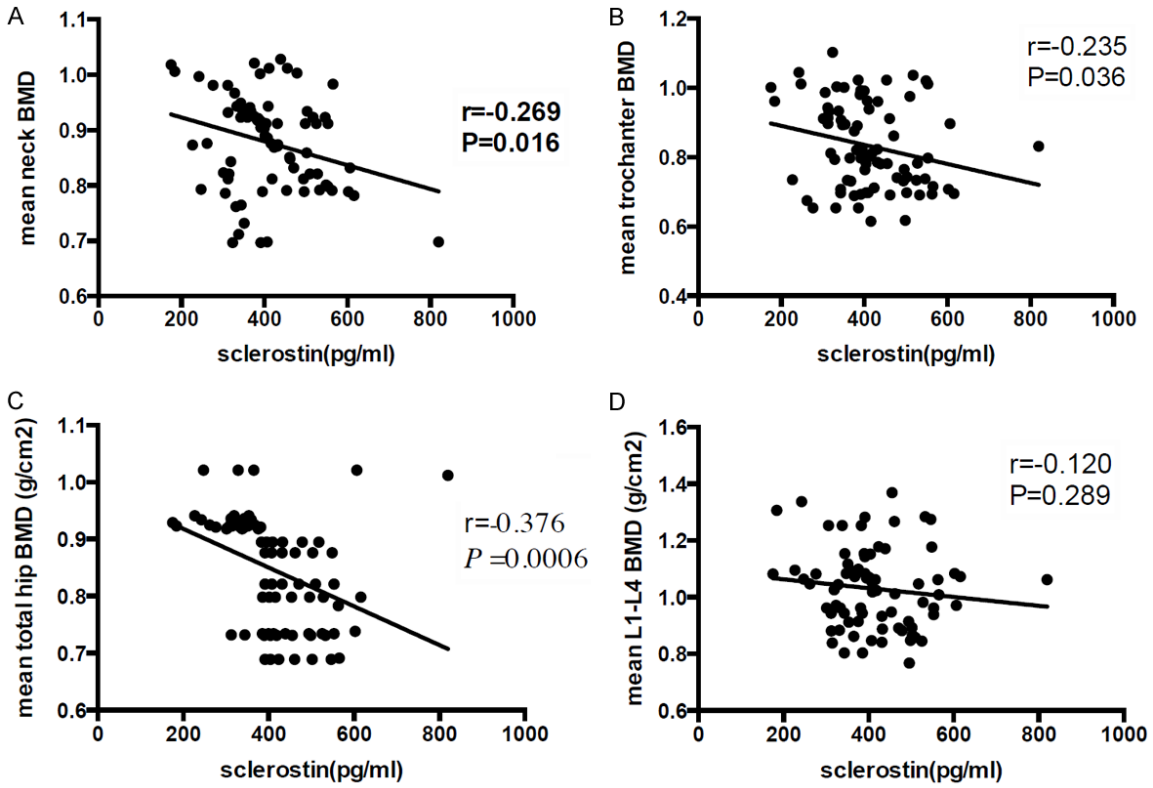

Figure 2. Association between sclerostin and bone density in postmenopausal women with femoral neck fracture. Higher sclerostin levels are significantly associated with lower bone mineral density in subjects with femoral neck fracture at femoral neck (r=-0.269; *P*=0.016) (A), trochanter (r=-0.235; *P*=0.036) (B) and total hip (r=-0.376; *P*=0.0006) (C), but not lumbar spine (r=-0.120; *P*=0.289) (D). These relationships did not change when the age was adjusted.

et al. [15] Modder demonstrated that all the bone density and microstructural parameters including total-body bone mineral content (TBBMC) and total-body bone mineral density (TBBMD) in elder man and women showed positive associations with sclerostin levels. Cejka et al. [17] also found patients with higher sclerostin serum levels had a higher BMD measured by DXA. Furthermore, trabecular bone volume and trabecular BMD were positively associated with sclerostin based on HRp-QCT measurements. The reason for this positive association between sclerostin and BMD and bone structure is unknown. A possible explanation could be that serum levels of sclerostin, which is produced by osteocytes, reflect osteocyte number. A higher bone mass would result in more osteocytes and therefore higher sclerostin levels. Since sclerostin is a potent inhibitor of bone formation, high lever sclerostin will reduce bone formation and decrease Bone density. In the study of association between sclerostin and bone density in chronic spinal cord injury, Morse et al. [4] found lower total limb bone mineral content was significantly associated with lower circulating sclerostin levels. Sclerostin levels were reduced, not elevated. They explained, fewer osteoytes exist to produce sclerostin, and sclerostin levels fall to levels lower than those found under normal conditions in severe osteoporosis. These findings suggest that, in chronic SCI, circulating sclerostin is a biomarker of osteoporosis severity, not a mediator of ongoing bone loss.

In this study, there were also significant differences between the two groups in osteocalin, bone ALP, P1NP, CTX1, CTX2, RANK and RANKL. At present, osteocalin, bone ALP and P1NP, which reflect the rate of synthesis of the main constituent of bone tissue, are the most specific and sensitive markers of bone formation [21]. We found P1NP level in premenopausal women was about 4-fold higher than that in postmenopausal women. Osteocalcin in premenopausal women was also higher than that in postmenopausal women. Bone ALP in postmenopausal women was about 3-fold higher

than that in postmenopausal women. These results proved that the rate of bone synthesis was lower in postmenopausal women than that in premenopausal women. At the same manner, CTX1, CTX2, RANK and RANKL are also valuable biochemical markers reflecting the rate of bone resorption [21]. In our study, serum CTX1, CTX2 and RANKL levels in postmenopausal women were all higher than those in premenopausal women. These results also proved there was higher bone resorption rate in postmenopausal women. Mirza et al. [8] also reported the same results of higher serum CTX level in postmenopausal women. We also observed that at any age, serum sclerostin level did not show statistically positive or negative correlation with bone turnover markers (osteocalcin, CTX1, CTX2, bone ALP, P1NP, RANK, RANKL). These findings also suggest that circulating sclerostin is a biomarker of osteoporosis severity, not a indicator of bone formation or bone loss.

Bone is a dynamic tissue with continuous cycles of bone resorption and bone formation, and which are regulated by osteocytes [22]. Sclerostin is an osteocyte-derived protein that signals to osteoblasts to reduce bone formation [23]. Based on an analysis of its amino acid sequence, sclerostin was initially recognized as a secreted cystine knot protein most closely related to the dan/cerberus family of proteins [24] which regulate bone morphogenic proteins (BMPs). Sclerostin was shown to bind to BMPs and inhibit their signaling [20, 25]. In the meantime, further investigation into the function of sclerostin suggested that it did not behave in the same way as classical BMP antagonists such as noggin, but rather may actually antagonize a BMP-inducible factor [13]. Li et al. [7] subsequently showed that sclerostin inhibited Wnt signaling by binding to LRP5/6 and that LRP5 containing the high bone mass mutation bound less well to sclerostin than wild type LRP5. As evidence showed that sclerostin was a Wnt antagonist it was even suggested that sclerostin did not directly inhibit BMP activity [26]. Krause et al. [27] confirmed that sclerostin could both inhibit Wnt signaling and bind intracellularly to BMPs to prevent secretion of active BMP protein.

There are limitations to the current study that must be considered. This is not a big study limited to. Larger, longitudinal studies that include postmenopausal women without fragility fracture are needed to confirm these findings and to establish the utility of sclerostin in fracture risk. Also, limited fragility fracture cases only exist in the femoral neck fracture women, a larger subjects with fragility fractures such as femoral neck fracture, trochanter fracture and lumber fracture are need to be enroll to the study. With a larger study, the subjects can be divided into different subgroup according to age. The dates will be more correct.

In conclusion, our study demonstrates that serum sclerostin levels are significantly higher in postmenopausal women with femoral neck fracture compared with premenopausal women. There are statistical differences between postmenopausal women and premenopausal women in bone turnover markers such as CTX1, CTX2, bAP, P1NP, RANK and RANKL. More importantly, we found significant positive correlations between serum sclerostin levels and age, as well as negative corrections between serum sclerostin levels and BMD in postmenopausal women. Our results imply that sclerostin is an additional serum marker of fragility fracture in elder. Additionally, the relationships between serum sclerostin levels, bone turnover markers and bone density, and the predictive value of serum sclerostin levels in determining postmenopausal fragility fracture need to be explored further in larger prospective studies. We conclude that measurement of serum sclerostin levels may represent a novel approach to predict the potential risks of fragility fracture in postmenopausal women.

Disclosure of conflict of interest

None.

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