Decreased trabecular bone deterioration of proximal tibiae and lumbar vertebrae in postmenopausal women with osteoarthritis

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Abstract. Osteoporosis and osteoarthritis (OA) are two common age-related skeletal disorders, which are associated with substantial morbidity and disability, particularly among elderly women. The present study was performed to investigate the trabecular bone deterioration of proximal tibiae and lumbar vertebrae in postmenopausal women with OA. The results demonstrated that the histomorphometric section of trabecular bone below the growth plate was markedly different between the healthy control and OA group. However, the loss of trabecular bone underneath the growth plate in the healthy control group was significantly worse than that of the OA group. Hematoxylin and eosin staining demonstrated the increased disconnection and separation of the trabecular bone network as well as the reduction of trabecular bone mass of primary and secondary spongiosa throughout the proximal metaphysis of tibia in the healthy control compared to the OA group; similar results were found when the same experiment was repeated on the lumbar vertebrae of healthy control subjects and OA patients from postmenopausal women. The biological properties of trabecular bone in the proximal tibia and lumbar vertebrae were measured in postmenopausal women with OA. Spearman's rank correlation analysis revealed that the bone volume fraction was both positively correlated with radiographic severity and Western Ontario McMaster University Osteoarthritis Index scores in in the proximal tibia and lumbar vertebrae from postmenopausal women with OA. In conclusion, the structural properties of the proximal tibia and lumbar vertebrae supported that an inverse correlation existed between postmenopausal women with OA and healthy controls. Moreover, there is an important protective mechanism of OA on trabecular microstructure in proximal tibiae and lumbar vertebrae from postmenopausal women.

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Introduction

Osteoporosis (OP) and osteoarthritis (OA) are two common age-related skeletal disorders, which are associated with substantial morbidity and disability, particularly among elderly women (1). Low bone mineral density (BMD) is reported in patients with early ankylosing spondylitis and rheumatoid arthritis (2,3). However, reports of increased risk of bone deterioration in patients with OA remain controversial (4,5). The correlation between OA and bone fragility has been subject to a considerable number of studies (6,7). Epidemiological surveys suggest that OA and OP are rarely present together in the same patient (1,8). In addition, cross-sectional studies have indicated that OA is associated with an increase in BMD (1,9), leading to a common assumption that an increased BMD is protective against OA progression. This has been demonstrated in postmenopausal women with OA undergoing total hip arthroplasty (THA), also revealing that the trabecular microarchitecture of the femoral neck in postmenopausal osteoarthritic subjects is enhanced compared with age-matched healthy controls (10,11). Contrastingly, the presence of OP within the femoral head in OA patients has been confirmed, and OA does not appear to protect a patient from generalized primary OP (12,13).

Postmenopausal osteoporosis (PMO) is characterized by excessive bone resorption, which also occurs at the early stages in the development of OA (14). However, increased bone formation, subchondral sclerosis and osteophyte formation take place in both the early and late stages of OA development (15). A previous study demonstrated significant differences in the trabecular bone, collagen fibers, lacunae and osteoblasts between PMO and OA in the femoral head (16). These observations support the hypothesis that there is an inverse correlation between OP and OA (11,14,17). Moreover, an inverse correlation between vertebral fractures and spine OA in postmenopausal women with OP is reported (18). To the best of our knowledge, there has been no study of patients with knee OA who may be at risk of OP due to trabecular bone deterioration of the proximal tibiae and lumbar vertebrae. Therefore, the aim of the present study was to investigate trabecular bone deterioration of proximal tibiae and lumbar vertebrae in postmenopausal women with OA by two-dimensional analysis, and to provide further proof to support the idea that there is an inverse correlation between knee OA and OP in postmenopausal women.

Materials and methods

Patients and specimens. The present study included a total of 20 subjects (10 used as healthy controls and 10 patients with OA). Subjects were recruited from September, 2012 to December, 2014 from The First People's Hospital in Xianyang, China, and osteoarthritic bone specimens were taken from postmenopausal women suffering from primary hip arthrosis undergoing THA. The diagnosis of primary OA referred to the criteria of the American College of Rheumatology (19). In both groups, patients with illnesses that may affect bone metabolism, including hyperparathyroidism, hyperthyroidism, osteomalacia, renal dysfunction and diabetes mellitus or drug supplementation were excluded. The detailed data of all subjects are shown in Table I. Clinical experiments were performed with written informed consent from all patients, and the present study was approved by the Ethics Committee of the First People's Hospital (Xianyang, China).

Bone histomorphometry. The proximal tibiae and lumbar vertebrae were decalcified in 0.5 M EDTA (pH=8.0) and then embedded in paraffin by standard histological procedures. Sections of 5 μm in size were cut and stained with hematoxylin and eosin (H&E), and visualized under a microscope (Olympus Corporation, Tokyo, Japan). In addition, two or three visual fields were selected for bone histomorphometry, and all the images were obtained at a magnification of x40 using a digital camera (Olympus Corporation) that was linked to a desktop computer. A commercial image analysis software, Image Pro Plus 5.1 (Media Cybernetics Inc., Rockville, MD, USA) was used to measure three semi-automatically measured values, namely, bone area, trabecular tissue area and trabecular perimeter. Next, the bone volume fraction (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N) and trabecular space (Tb.Sp) were calculated with the following formulas according to Parfitt's theory (20,21): BV/TV = 100 x B.Ar/T.Ar; Tb.Th = (B.Ar/B.Pm)(π/2); Tb.N = (B.Pm/T.Ar) x 10; Tb.Sp = (1,000 x T.Ar-B.Ar)/B.Pm.

Radiographic assessment and symptomatic severity in OA. OA severity was determined using weight-bearing anteroposterior radiographs of the affected knee. Radiographic severity was evaluated according to the Kellgren and Lawrence (KL) grading system (22). In addition, the symptomatic severity of the disease was evaluated according to the Western Ontario McMaster University Osteoarthritis Index (WOMAC), which consists of 3 subscales: Pain, stiffness and physical function (23).

Statistical analysis. The data from these experiments are reported as the mean ± standard deviation for each group. All statistical analyses were performed using PRISM version 5.0 (GraphPad Software, Inc., La Jolla, CA, USA). The Kolmogorov-Smirnov test was performed to analyse data normality, while an unpaired t-test was used to compare each structural parameter (BV/TV, Tb.N, Tb.Th and Tb.Sp) between the knees of OA patients and healthy controls. The correlation between BV/TV and disease severity classified according to the KL grading system and the correlation between BV/TV and WOMAC scores were determined using Spearman's correlation coefficient. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Histological analysis. Histological analysis on trabecular bone in the proximal tibial head and lumbar vertebrae were performed by H&E staining (Figs. 1 and 2). The histomorphometric section of trabecular bone below the growth plate was markedly different between the healthy control and OA groups. However, the loss of trabecular bone underneath the growth plate in the healthy control group was significantly worse than that of the OA group (Fig. 1). Importantly, H&E staining demonstrated the increased disconnections and separation of the trabecular bone network as well as the reduction of trabecular bone mass of primary and secondary spongiosa throughout the proximal metaphysis of tibia in the healthy control as compared to the OA group (Fig. 1). It is of note that similar results were observed when the same experiment was repeated on the lumbar vertebrae of healthy control subjects and OA patients from postmenopausal women (Fig. 2).

Bone histomorphometry. The biological properties of trabecular bone were determined at the proximal tibia and lumbar vertebrae. As shown in Table II, patients with OA had higher BV/TV, Tb.Th, Tb.N values at the proximal tibia compared to that of subjects in the healthy control group (BV/TV, 32.68±3.54% vs. 24.75±2.47%, P=0.002; Tb.N, 2.54±0.78/mm vs. 1.28±0.46/mm, P=0.001; Tb.Th, 124.83±14.83 μm vs. 97.23±8.25 μm, P=0.001). Meanwhile, Tb.Sp was significantly lower in the OA group than that of subjects in the healthy control group (Tb.Sp, 253.68±22.27 μm vs. 347.39±30.65 μm, P=0.014). As shown in Table III, patients with OA had higher BV/TV, Tb.Th, Tb.N values at the lumbar vertebrae than that of subjects in the healthy control group (BV/TV, 26.37±2.69% vs. 20.71±2.05%, P=0.005; Tb.N, 2.25±0.64/mm vs. 1.62±0.51/mm, P=0.025; Tb.Th, 121.49±12.52 μm vs. 80.31±9.26 μm, P=0.001). Meanwhile, Tb.Sp was significantly lower in the OA group than that of subjects in the healthy control group (Tb.Sp, 312.43±30.56 μm vs. 562.74±43.67 μm, P<0.001).

Scoring. OA was divided into 3 subgroups according to the KL grading system. The association of BV/TV at the proximal tibia with radiographic severity and WOMAC scores was illustrated. As shown in Fig. 3A and B, Spearman's rank correlation analysis demonstrated that BV/TV was both positively correlated with radiographic severity and WOMAC scores in the proximal tibia of OA patients ($r=0.737$, $P<0.001$ and $r=0.478$, $P=0.033$, respectively). Similar results were observed when the same experiment was repeated on the lumbar vertebrae. Spearman's rank correlation analysis demonstrated that BV/TV at the lumbar vertebrae was both positively correlated with radiographic severity and WOMAC scores in OA patients ($r=0.842$, $P<0.001$ and $r=0.765$, $P=0.033$, respectively, Fig. 3C and D).

Discussion

The aim of the present study was to determine whether there were altered structural properties of trabecular bone in

Table I. Detailed data of donors with osteoarthritis or healthy control from postmenopausal women.

Subject	Group	Age (years)	Height (cm)	BW (kg)	BMD (g/cm^2)	T-score
1	OA	54	165	65	1.09	-0.39
2	OA	50	163	72	0.96	-0.57
3	OA	58	158	68	0.87	-0.97
4	OA	49	155	60	0.93	-1.22
5	OA	56	168	57	0.78	-1.55
6	OA	57	156	62	0.92	-0.84
7	OA	53	164	69	1.04	-0.45
8	OA	60	158	73	1.13	-0.34
9	OA	55	167	60	0.91	-0.95
10	OA	54	163	76	1.25	-0.27
11	HC	52	165	63	0.86	-1.45
12	HC	56	160	70	0.79	-1.78
13	HC	55	162	65	0.91	-0.86
14	HC	50	158	72	0.88	-1.43
15	HC	59	163	62	0.79	-1.74
16	HC	55	164	68	0.72	-2.45
17	HC	56	157	58	0.65	-2.82
18	HC	54	162	63	0.81	-1.69
19	HC	53	160	70	0.74	-2.07
20	HC	54	166	65	0.82	-1.79

OA, osteoarthritis; HC, healthy control; BW, body weight; BMD, bone mineral density.

Table II. Comparison of bone parameters of proximal tibia between osteoarthritis and healthy controls from postmenopausal women.

Bone parameters	OA (n=10)	HC (n=10)	P-value
BV/TV (%)	32.68±3.54	24.75±2.47	0.002[a]
Tb.N/mm	2.54±0.78	1.28±0.46	0.001[a]
Tb.Th (μm)	124.83±14.83	97.23±8.25	0.001[a]
Tb.Sp (μm)	253.68±22.27	347.39±30.65	0.014[b]

OA, osteoarthritis; HC, healthy control; BV/TV, bone volume over total volume; Tb. N, trabecular number; Tb. Th, trabecular thickness; Tb. Sp, trabecular separation. [a]P<0.001 and [b]P<0.05.

Table III. Comparison of bone parameters of lumbar vertebrae between osteoarthritis and healthy control from postmenopausal women.

Bone parameters	OA (n=10)	HC (n=10)	P-value
BV/TV (%)	26.37±2.69	20.71±2.05	0.005[a]
Tb.N/mm	2.25±0.64	1.62±0.51	0.025[b]
Tb.Th (μm)	121.49±12.52	80.31±9.26	0.001[a]
Tb.Sp (μm)	312.43±30.56	562.74±43.67	<0.001[a]

OA, osteoarthritis; HC, healthy control; BV/TV, bone volume over total volume; Tb. N, trabecular number; Tb. Th, trabecular thickness; Tb. Sp, trabecular separation. [a]P<0.001 and [b]P<0.05.

proximal tibia and lumbar vertebrae when comparing healthy control subjects and patients with OA in postmenopausal female groups. Compared with the healthy control group, patients with OA had higher BV/TV, Tb.Th and Tb.N values and lower Tb.Sp values in the proximal tibia and lumbar vertebrae. These results demonstrated that a different bone mineralization status in the proximal tibia and lumbar vertebrae existed between healthy control subjects and OA patients from postmenopausal women, and that OA may be associated with an increased BMD.

Previous large epidemiological surveys confirm that radiographic knee OA is associated with high femoral neck BMD (14,24). However, to the best of our knowledge, there are no studies that have simultaneously investigated the association of OA with the BMD at the proximal tibia and lumbar vertebrae. The present study identified that the correlation of BV/TV with radiographic severity and WOMAC scores at lumbar vertebrae was more closely at proximal tibia. In addition, the study demonstrated marked differences in the histomorphometric sections of some microarchitectural parameters at the proximal tibia and lumbar vertebrae between OA patients and healthy control subjects in postmenopausal women. Moreover, previous studies have demonstrated clear trends of trabecular microarchitectural deterioration during aging in women (11,25). In postmenopausal women, lumbar OA leads to an increase in lumbar spine areal BMD (aBMD), and lumbar spine aBMD increases with the KL grade (26).

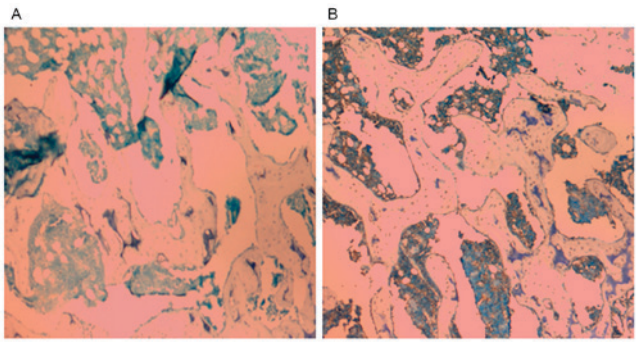

Figure 1. Histomorphometric section of the proximal tibia of (A) a healthy control and (B) osteoarthritis from postmenopausal women.

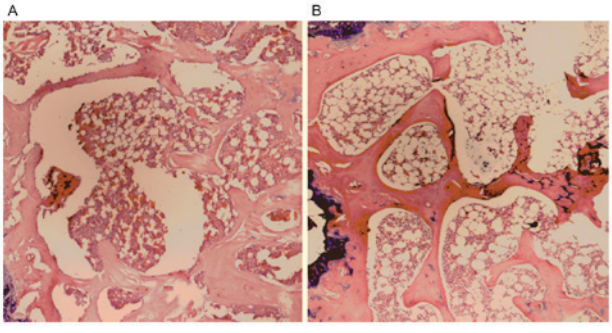

Figure 2. Histomorphometric section of the lumbar vertebrae of (A) a healthy control and (B) osteoarthritis from postmenopausal women.

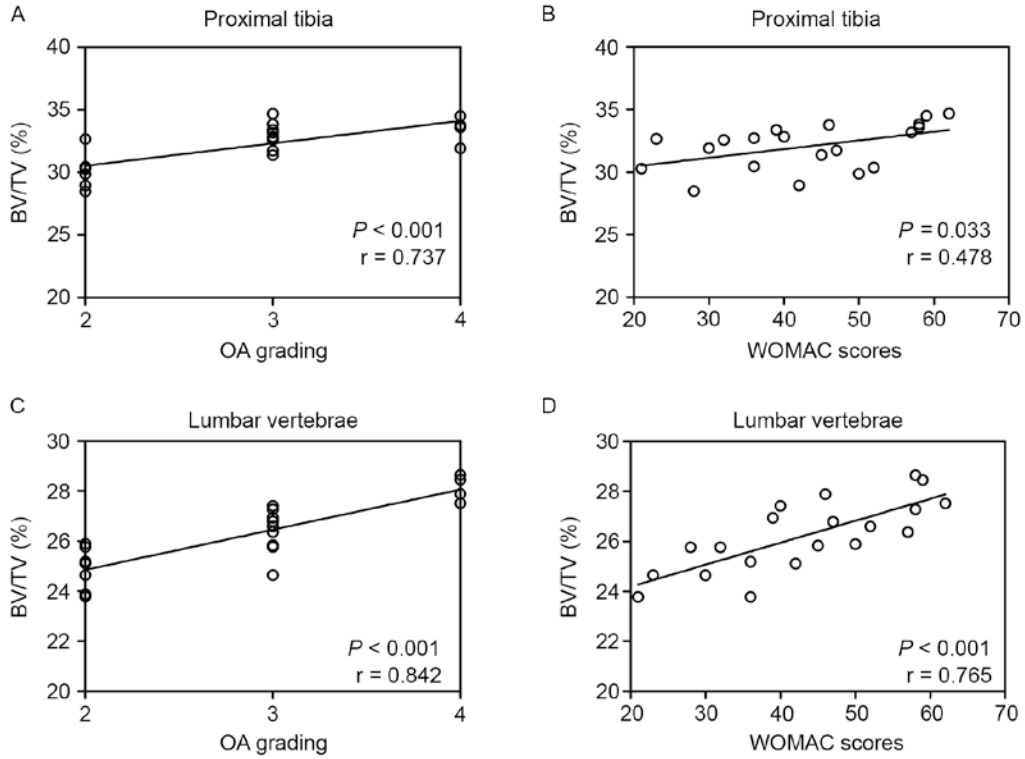

Figure 3. (A) Correlation between BV/TV of proximal tibia in OA patients from postmenopausal women and disease severity classified according to the KL grading system (r=0.737, P<0.001). (B) Correlation between BV/TV of proximal tibia in OA patients from postmenopausal women and WOMAC scores (r=0.478, P=0.033). (C) Correlation between BV/TV of lumbar vertebrae in OA patients from postmenopausal women and disease severity classified according to the KL grading system (r=0.842, P<0.001). (D) Correlation between BV/TV of proximal tibia in OA patients from postmenopausal women and WOMAC scores (r=0.765, P<0.001). BV/TV, bone volume over total volume; KL, Kellgren and Lawrence; WOMAC, Western Ontario McMaster University Osteoarthritis Index.

Consistent with previous reports, the present study demonstrated an increased BMD and BV/TV in OA patients. This is in accordance with what was previously identified as knee OA and was associated with increased hip and even spine aBMD (27,28). Lumbar vertebrae and proximal tibia BV/TV also increase with the KL grade and WOMAC scores in postmenopausal women with OA.

A previous study demonstrated that there was a high prevalence of OA presenting with osteophytes (90% of patients) and disc space narrowing (65% of patients) in postmenopausal women aged 74 years on average (18). When OA is defined by the presence of osteophytes, the risk of vertebral fracture has already been shown to be decreased (29,30). Although the risk of vertebral fracture was not performed, the data of the present study demonstrated that there was a significant difference in aBMD between patients with OA and the healthy control. In concordance with the results of the majority of studies concerning BMD measurement, an increased BMD in OA patients was observed. Moreover, an apparent density divided by the total volume was higher in OA because of increased Tb.Th and BV/TV.

Previous comparative research that investigated the association between OA and OP merely emphasized the importance of fracture risk or mechanical properties (31). To the best of our knowledge, this is the first time to evaluate trabecular bone deterioration of the proximal tibia and lumbar vertebrae in postmenopausal women with OA. In addition, the present study revealed differential trabecular structure in proximal tibia and lumbar vertebrae between postmenopausal women with OA and OP, which may be a potential influence on the mechanism of OA and OP. Evidence from the present study supported that there was an important protective mechanism of OA on trabecular microstructure in proximal tibia and lumbar vertebrae from postmenopausal women.

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