Osteoarthritis and Cartilage 21 (2013) 1716–1723

Osteoarthritis and Cartilage

Bone loss at subchondral plate in knee osteoarthritis patients with hypertension and type 2 diabetes mellitus

C.Y. Wen †[a], Y. Chen †[a], H.L. Tang ‡, C.H. Yan †, W.W. Lu †, K.Y. Chiu †*

† Department of Orthopaedics and Traumatology, LKS Faculty of Medicine, Hong Kong
‡ Medical Engineering Program, Faculty of Engineering, The University of Hong Kong, Hong Kong

ARTICLE INFO

Article history:
Received 5 January 2013
Accepted 27 June 2013

Keywords:
Osteoarthritis
Subchondral plate
Microstructure
Hypertension
Type 2 diabetes mellitus

SUMMARY

Objectives: This study aimed to characterize subchondral bone damages of knee osteoarthritis (OA) patients in presence of the comorbidities, i.e., hypertension and type 2 diabetes mellitus (T2DM).
Methods: A total of 43 patients with advanced stage of primary knee OA were recruited, and tibial plateau specimens were collected during surgery with informed consent. The specimens were processed for micro-CT and histological examination to assess the severity of subchondral bone damages. The presence of the comorbid disease, e.g., hypertension and T2DM, and the data on covariates, such as the age, gender and body mass index (BMI), were taken into account in a multi-variable linear regression model to explore the potential effect of the comorbidities on subchondral bone damages in knee OA after adjusting the covariates.
Results: As compared to 15 subjects without the comorbidities, significant bone loss was observed at subchondral plate in 28 knee OA patients with hypertension and T2DM, in terms of the lower bone mineral density (BMD) ($P = 0.034$) and higher porosity ($P = 0.032$) on the medial portion of tibial plateau. After adjusting the age, gender and BMI, the presence of hypertension or T2DM was included in a regression model to explain in part the decreased BMD ($r^2 = 0.551$, $P = 0.004$) and increased porosity ($r^2 = 0.545$, $P = 0.003$) at subchondral plate in knee OA.
Conclusion: Our findings suggest the biological link between bone loss at subchondral bone plate in knee OA and the comorbid diseases, i.e., hypertension and T2DM, which prompt the needs for a large-scale cohort study to confirm the causality.

Introduction

Knee osteoarthritis (OA) is the most common form of joint disorders and a leading cause of walking disability among the older adults with a prediction of 67 million OA patients in the United States by 2030[1]. Knee OA is not only a problem of articular cartilage, but also a whole joint disease, involving the other joint tissues, such as subchondral bone[2]. For example, subchondral bone provides mechanical support and nutrition supply to overlying articular cartilage; subchondral bone and articular cartilage act as a unit to maintain the structural and functional integrity of joint[3,4]. In addition, there is mount evidence showing that subchondral osteoblasts in OA altered the cellular behavior of articular chondrocytes[5–10]. All these findings support the notion that subchondral bone disturbance plays an important role in pathomechanism of OA and contributes to the progression of disease[11].

The etiology of OA is idiopathic and multifactorial. Aging, menopause, obesity and genetic variations have been identified as the major risk factors in the pathogenesis of knee OA[12–15]. Metabolic syndrome, including hypertension and Type 2 diabetes mellitus (T2DM), are frequently encountered comorbidities in the elderly patients with knee OA. It was reported that there was around 55% of knee OA patients of over 65 years old having hypertension and 13% of them showing T2DM[16]. Hence, there is growing research interest recently on the role of metabolic syndrome in the pathomechanism of knee OA among the elderly persons[17]. The intimate relationship between knee OA and metabolic syndrome significantly contributes to the mortality of patients compared to generation population[18]. The walking disabilities of OA knees have been identified as a major risk factor for the death of elderly patients with T2DM and cardiovascular diseases[18]. Although atheromatous vascular disease and diabetes were once speculated in the pathogenesis of OA[19–21], the exact impact of these comorbidities, such as hypertension and T2DM, on the severity of

* Address correspondence and reprint requests to: K.Y. Chiu, Li Shu Fan Medical Foundation, The University of Hong Kong, Room 216, New Clinical Building, Queen Mary Hospital, Pokfulam, Hong Kong. Tel: 852-22554259; Fax: 852-28174392.
E-mail address: pkychiu@hku.hk (K.Y. Chiu).
[a] Equal contribution to this manuscript.

1063-4584/$ – see front matter
http://dx.doi.org/10.1016/j.joca.2013.06.027

knee OA and the progression of disease remains to be elucidated[22]. As previously reported, high blood pressure was associated with the low bone mass and high risk of fractures in elderly persons[23–25]. In T2DM patient, bone quality was also reduced with high risk of fracture[26], yet bone mineral density (BMD) increased in lumbar spine, hip and radius in patients[27]. We hypothesized that hypertension and T2DM as the comorbidities could interfere with subchondral bone remodeling, and aggravate the severity of OA[17].

Because of no prior knowledge on the relationship of subchondral bone damages in knee OA with its comorbidities, we initially proposed a cost-effective cross-sectional study to evaluate the subchondral bone damages in knee OA in presence or absence of hypertension and T2DM. Then we further investigated the possible effect of these comorbidities on subchondral bone mass and microstructure in knee OA after adjusting covariates, i.e., the age, gender and BMI of patients. The findings generated from this study would build up a solid scientific foundation for a large-scale cohort study to test our hypothesis.

Patients and methods

Study design

The ethic committee of authors' institute proved all the experimental procedures in this study (Ref Nr: UW-09368). An experienced orthopaedic specialist (CHY) recruited a total of 43 patients of primary knee OA, and collected tibial plateau specimens during total knee replacement surgery with informed consent from February to October 2012 in authors' institute (10 male, 33 females, 63–85 years old). The subjects were screened before recruitment; the patients with the early-onset of knee OA prior to 40 years old, history of knee joint trauma, infection and other rheumatic diseases, such as gout *etc.*, were not included in the current study. Two subjects with multiple myeloma or osteoporosis on the treatment of alendronate were excluded after initial screening. The knee OA patients were then divided into different groups for comparisons according to the presence or absence of hypertension and T2DM.

Clinical assessments

The severity of knee OA was assessed by the experienced orthopaedic surgeons (CHY, KYC) in our outpatient clinic prior to surgery, The radiographic severity of OA knee was graded using Kellgren–Lawrence (K–L) system on plain X-radiograph, and the functional disability of OA knee was routinely examined using knee society knee score (KSKS) and functional scale (KSFS)[28].

The diagnosis of essential hypertension and T2DM was taken from clinical record. The diagnosis is made in our institute according to "the Seventh Report of the Joint National Committee on prevention, detection, evaluation, and treatment of high blood pressure" and "WHO report on the diagnosis and classification of diabetes mellitus". In the current study, essential hypertension was defined as blood pressure over 140/90 mmHg or previous history of essential hypertension on the medication; T2DM was defined as a single raised glucose reading with symptoms or history of T2DM on medication or with the documented complications.

The data on covariates, such as the patients' age, gender and BMI, were taken from clinical records in authors' institute. Results of physical examination, e.g., systolic and blood pressure monitoring, and the clinical biochemistry tests of fast glucose test, calcium/phosphate metabolism, liver and kidney function, which were performed within one week prior to the surgery, were also collected.

Micro-CT evaluation

The serial scans of tibial specimens was performed under a μCT scanner (SkyScan 1,076, Kontich, Belgium) with the following scanning parameters: 18 μm isotropic voxel size, 55 kV voltage, 109 μA current, 200 ms integration time and 4,000 projections. The microstructure of subchondral plate and trabecular bone was clearly visible in the 2D reconstructed images and separately for analyses. Generally, they were converted into discrete binary objects by the global thresholding and binarisation procedures combined with image filtering such as despeckle to remove noise, and then processed for 3D measurement in SkyScan CTAn software. The surface rendered 3D models were created for visualization via the SkyScan CTVol software.

Subchondral bone in the central one third of medial or lateral tibial plateau was chosen as the region of interest for analyses. The parts of subchondral plate and trabecular bone underneath were analyzed respectively. The irregular boundary of subchondral plate was drawn with the aid of the edge detection program in MATLAB®R2010a. Briefly, the boundary of binary objects was detected in the micro-CT images after segmentation. The edges of subchondral

Table I
Demographic and clinical assessments of knee OA patients*

Parameters		Comorbidities of knee OA patients			
		No hypertension no T2DM	Hypertension, No T2DM	Hypertension and T2DM	*P* value
Number of patients		15	15	12	/
Gender (M: male; F: female)		3 (M), 12 (F)	5 (M), 10 (F)	3 (M), 9 (F)	0.037*
Age		69 ± 9 (58–82)	72 ± 10 (52–85)	71 ± 9 (52–86)	0.662#
Body Mass Index (BMI, kg/m^2)		27 ± 4 (23-36)	28 ± 4 (23–39)	25 ± 3 (18–27)	0.257#
Standing X-ray	Kellgren–Lawrance (K–L) grade	8 (Grade 3), 7 (Grade 4)	6 (Grade 3), 9 (Grade 4)	5 (Grade 3), 7 (Grade 4)	0.535*
Knee Society Knee Score	Pain (0–50)	23 ± 8 (10 ~ 30)	22 ± 9 (10 ~ 30)	26 ± 11 (10 ~ 45)	0.652^
	Passive range of motion (0–25)	21 ± 3 (15 ~ 24)	18 ± 4 (12 ~ 25)	18 ± 4 (10 ~ 24)	0.238^
	Stability (0–25)	23 ± 4 (15 ~ 25)	21 ± 7 (0 ~ 25)	24 ± 4 (15 ~ 25)	0.645^
	Fixed flexion contracture (−15 to 0)	−1 ± 1 (−2 ~ 0)	−4 ± 4 (−15 ~ 0)	−3 ± 5 (−15 ~ 0)	0.152^
	Extension lag (−15 to 0)	0 ± 0 (0 ~ 0)	0 ± 1 (−5 ~ 0)	−1±2 (−5 ~ 0)	0.417^
	Alignment (−20 to 0)	−16 ± 7 (−20 ~ 0)	−19 ± 3 (−20 ~ −9)	−13 ± 10 (−20 ~ 0)	0.102^
	Sum	44 ± 19 (0–59)	36 ± 17 (0 ~ 61)	51 ± 21 (20 ~ 87)	0.163^
Knee Society Functional Assessment	Walking (0–50)	27 ± 8 (20 ~ 40)	22 ± 4 (20 ~ 30)	20 ± 5 (10 ~ 30)	0.051^
	Stairs (0–50)	30 ± 0 (30 ~ 30)	26 ± 9 (0 ~ 30)	24 ± 15 (0 ~ 40)	0.487^
	Aids (−20 to 0)	−5 ± 0 (−5 ~ −5)	−4 ± 2 (−5 ~ 0)	−7±5 (−20 ~ −5)	0.175^
	Sum	52 ± 8 (45–65)	44 ± 10 (15 ~ 60)	37 ± 23 (−10 ~ 65)	0.145^

All data were expressed as mean ± standard deviation and range in the bracket. The comparisons of the demographic data and clinical severity of OA knee were performed among the groups of patients in presence or absence of hypertension and T2DM using one-way ANOVA (#), Kruskal–Wallis H test (^) or Chi-square test (*) when appropriate. T2DM: type 2 diabetes mellitus.

* The data of 1 patients having T2DM only were listed in supplementary materials but not included for comparison. All data were expressed as mean ± standard deviation.

Table II
Physical and laboratory examinations of knee OA patients taken from clinical record*

Parameters/Reference values		Comorbidities in knee OA patients			
		No hypertension, no T2DM	Hypertension, no T2DM	Hypertension and T2DM	*P* value
Systolic blood pressure (mmHg)	95 ~ 140	119 ± 15 (110 ~ 138)	135 ± 16 (112 ~ 174)	132 ± 15 (108 ~ 149)	0.076
Diastolic blood pressure (mmHg)	60 ~ 90	70 ± 8 (60 ~ 80)	81 ± 13 (60 ~ 103)	75 ± 11 (57 ~ 89)	0.129
Calcium (mmol/L)	2.24–2.63	2.33 ± 0.12 (2.16 ~ 2.51)	2.30 ± 0.12 (2.13 ~ 2.50)	2.30 ± 0.06 (2.23 ~ 2.38)	0.812
Phosphate (mmol/L)	0.88–1.45	1.05 ± 0.16 (0.85 ~ 1.25)	1.16 ± 0.21 (0.73 ~ 1.41)	1.05 ± 0.07 (0.99 ~ 1.16)	0.398
Glucose (mmol/L)	<7.8	6.7 ± 1.5 (5.2 ~ 8.7)	6.3 ± 1.3 (3.7 ~ 8.7)	8.6 ± 3.6 (4.5 ~ 14.6)	0.085
Total Protein (g/L)	67–87	74 ± 5 (69 ~ 86)	76 ± 5 (67 ~ 85)	75 ± 5 (69 ~ 84)	0.778
Albumin (g/L)	39–50	43 ± 2 (39 ~ 46)	42 ± 4 (33 ~ 46)	41 ± 3 (34 ~ 44)	0.502
Globulin (g/L)	26–40	32 ± 4 (25 ~ 40)	34 ± 4 (28 ~ 43)	34 ± 8 (26 ~ 50)	0.421
ALP (U/L)	47–124	68 ± 12 (47 ~ 86)	75 ± 21 (42 ~ 115)	76 ± 24 (50 ~ 119)	0.565
ALT (U/L)	8 ~ 45	24 ± 13 (11 ~ 53)	21 ± 9 (8 ~ 36)	25 ± 17 (7 ~ 66)	0.748
AST (U/L)	15–37	27 ± 9 (17 ~ 47)	25 ± 6 (17 ~ 35)	26 ± 10 (15 ~ 49)	0.949
Urea (mmol/L)	2.9–8.0	6.0 ± 1.8 (3.7 ~ 9.6)	7.2 ± 2.9 (2.5 ~ 14.2)	6.3 ± 2.0 (4.6 ~ 11.0)	0.384
Creatinine (umol/L)	49–82	68 ± 13 (51 ~ 93)	86 ± 36 (39 ~ 161)	81 ± 16 (59 ~ 102)	0.260

All data were expressed as mean ± standard deviation and range in the bracket. The comparisons of physical and laboratory data were performed among the groups of knee OA patients in presence or absence of hypertension and T2DM using one-way ANOVA.

* The data of 1 patients having T2DM only were listed in supplementary materials but not included for comparison. All data were expressed as mean ± standard deviation.

plate were saved as the ROI in the binary bitmap images and unwanted edges were removed according to their coordinate in the segmented images. After smoothing procedure, the selected ROI was then input into SkyScan CT analyser for analyses. The following parameters of subchondral plate: bone volume fraction (BV/TV), bone mineral density (BMD), total (open plus close) porosity and pore size, were calculated accordingly. For trabecular bone, a cubic ROI of $10 \times 10 \times 2$ mm^3 was chosen and the following parameters were taken, including BV/TV, BMD, trabecular thickness/number/separation (Tb.Th, Tb.N, Tb.Sp), structure model index, degree of anisotropy and connectivity density.

Histological evaluation

After micro-CT scans, the osteochondral plugs were harvested from the central one third of medial and lateral tibial plateau respectively, and then processed for histological evaluation. Briefly, the specimens were decalcified and embedded in wax. The sections were cut into 5 μm thick and stained with Hematoxylin–Eosin (H&E).

Statistics analysis

All data were expressed as mean ± standard deviation. The comparisons of the clinical, laboratory and radiological data were performed among the groups of knee OA patients using one-way analysis of variance (ANOVA), Kruskal–Wallis *H* test or Chi-square test when appropriate. In addition to the covariates such as the age, gender and BMI of patients, the comorbidities of knee OA, hypertension and T2DM, were examined as an independent categorical variable in a multi-variable linear regression model to investigate their effect on the microstructural damages of subchondral bone.

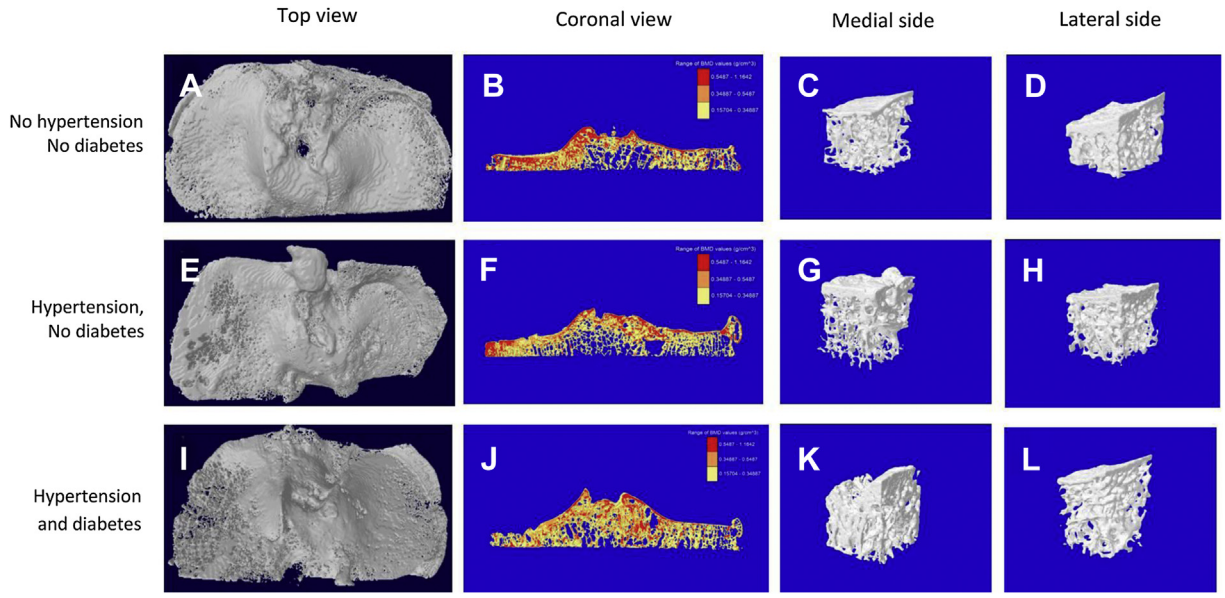

Fig. 1. Micro-CT images of subchondral bone damages in different groups of knee OA patients (A ~ D: patient without hypertension or T2DM; E ~ H: patient with hypertension but no T2DM; I ~ L: patient with both hypertension and T2DM). As compared to the knee OA patients without the comorbidities (A ~ D), subchondral bone plate was severely damaged at medial tibial plateau in the patients with hypertension and T2DM (I ~ L). BMD, which was coded by different colors, also significantly decreased on both medial and lateral sides in these patients (J). Moreover, the thickness of subchondral plate on lateral tibial plateau turned to be thinner in the subjects with the comorbidities (H, L). (A, E, I: top views of tibial plateau of OA knee; B, F, J: BMD distribution at coronal section view of tibial plateau of OA knee, which are coded in color bar on right corner: red – high BMD 0.55 ~ 1.16 g/mm^3; pink – medium BMD 0.35 ~ 0.55 g/mm^3 and yellow – low BMD 0.16 ~ 0.35 g/mm^3; C, G, K: cubic regions of interest (ROI) from medial tibial plateau, D, H, L: cubic regions of interest from lateral tibial plateau).

SPSS 20.0 (Chicago, IL, USA) was used for analyses with the statistical significance set at 0.05.

Results

Among all knee OA patients recruited in this study, 79% of them were overweight or obese. There were 63% (27 out of 43) of knee OA patients having primary hypertension and 30% (13 out of 43) of them with T2DM. It was noted that 12 out of 13 OA patients with T2DM co-existed with essential hypertension. OA knee patients were then divided into three groups for comparisons: Group 1: subjects without hypertension nor T2DM, Group 2: subjects with hypertension but no T2DM and Group 3: with both hypertension and T2DM (Table I). There was only one OA patient having T2DM but no hypertension, which was listed for the completeness and transparency of our data but not included for statistical analyses subsequently (Supplementary Tables I–III).

There were no significant differences in the patients' age, gender and BMI among three groups. Patients with hypertension and T2DM had the worse walking function than the subjects without the comorbidities ($P = 0.051$) although they showed the similar severity of radiological knee OA in terms of K–L grade (Table I). There existed marginal differences in systolic blood pressure ($P = 0.076$) and fast glucose test ($P = 0.085$) among the groups. Apart from that, patients with hypertension and diabetes did not differ from the subjects without these comorbidities regarding the data from the tests of the electrolytes, liver and renal function (Table II).

As shown in micro-CT images, significant loss of subchondral bone plate structural integrity on medial tibial plateau was noted in knee OA patients with hypertension and T2DM as compared to those without these comorbidities (Fig. 1). It was accompanied by full-thickness articular cartilage worn away in these patients (Fig. 2). Loss of subchondral bone plate integrity was characterized by the decrease of BMD and an increase of structural porosity (Fig. 3, Supplementary Table IV). The similar trend was also found in subchondral plate at lateral tibial plateau, and underneath trabecular bone on medial tibial plateau showing relatively lower BV/TV

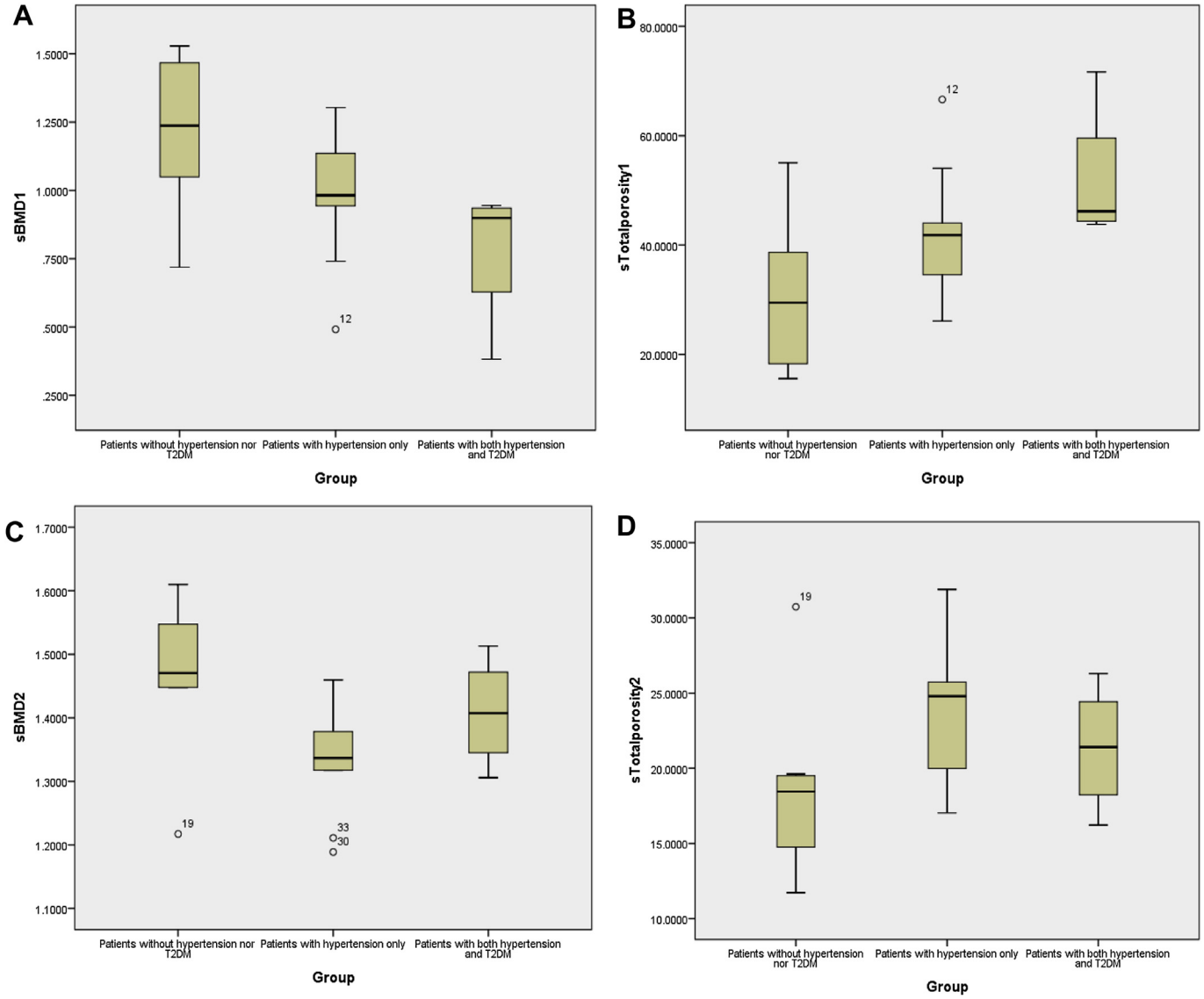

Fig. 2. Comparisons of BMD and porosity of medial subchondral bone plate between knee OA patients in presence or absence of hypertension and T2DM. In comparison with the subjects without the comorbidities, knee OA patients with hypertension and T2DM showed significantly lower BMD (A, $P = 0.034$) and higher porosity (B, $P = 0.032$) at subchondral plate on medial tibial plateau. Similar trend was also found in BMD (C, $P = 0.042$) and total porosity (D, $P = 0.088$) of subchondral plate at lateral tibial plateau. The comparisons among the groups were performed using one-way ANOVA and the significance level was set at $P < 0.05$.

and marginally thinner trabeculae but not all of them were statistically significant (Supplementary Table IV).

The comorbidities in knee OA were categorized as follows: "1" no hypertension, no T2DM; "2" hypertension, no T2DM; "3" hypertension and T2DM. With the change in the categories of the comorbidities from 1 to 3, BMD of subchondral plate decreased by 0.312 g/cm^3 and the porosity increased by15.347% after fully adjustment of the covariant (Table III). As shown in Supplementary Fig. 1, BMI correlated with BMD of subchondral plate at medial tibial plateau (A, $P = 0.025$). Meanwhile, it was also associated with the porosity of subchondral plate on medial side of tibial plateau in OA knees (C, $P = 0.031$). So BMI could be a confounding factor in the estimated effect of comorbid diseases on subchondral bone damages in a regression model. As shown in Table III, the standardized β weight of the comorbid diseases contributing to BMD of subchondral plate obviously increased from unadjusted −0.577 to minor adjusted −0.701 by the age, gender and BMI of knee OA patients. After adjusting the age, gender and BMI, the presence of hypertension and T2DM was included in a regression model to contribute in part to the decreased BMD ($r^2 = 0.551$, $P = 0.004$) and increased porosity ($r^2 = 0.545$, $P = 0.003$).

Discussion

The scientific merit of this study was to put the statistically significant relationship of knee OA with the coexisting hypertension and diabetes in epidemiological studies under microscopic examination. Our findings pave a road to further explore the underlying mechanism of the mutual relationship between knee OA and its comorbidities. In the present study, we observed significant bone loss at subchondral plate at medial tibial plateau, in terms of reduced BMD and elevated porosity, in knee OA patients with hypertension and/or T2DM as compared to the subjects without these comorbidities. Our findings suggest a potential biological link between bone loss at subchondral plate in knee OA and its comorbid diseases. As shown in a linear regression model, the presence of hypertension and diabetes could explain in part (around 50%) the decreased BMD and increased porosity of subchondral plate in OA. This finding was generated after considering the covariate, such as the age, gender, and BMI of knee OA patients etc. Among the covariate, it was not surprised to find the overweight (BMI >24 and <30) or obesity (BMI ≥30) in southern Chinese subjects as a confounding factor to correlate with BMD and porosity of subchondral

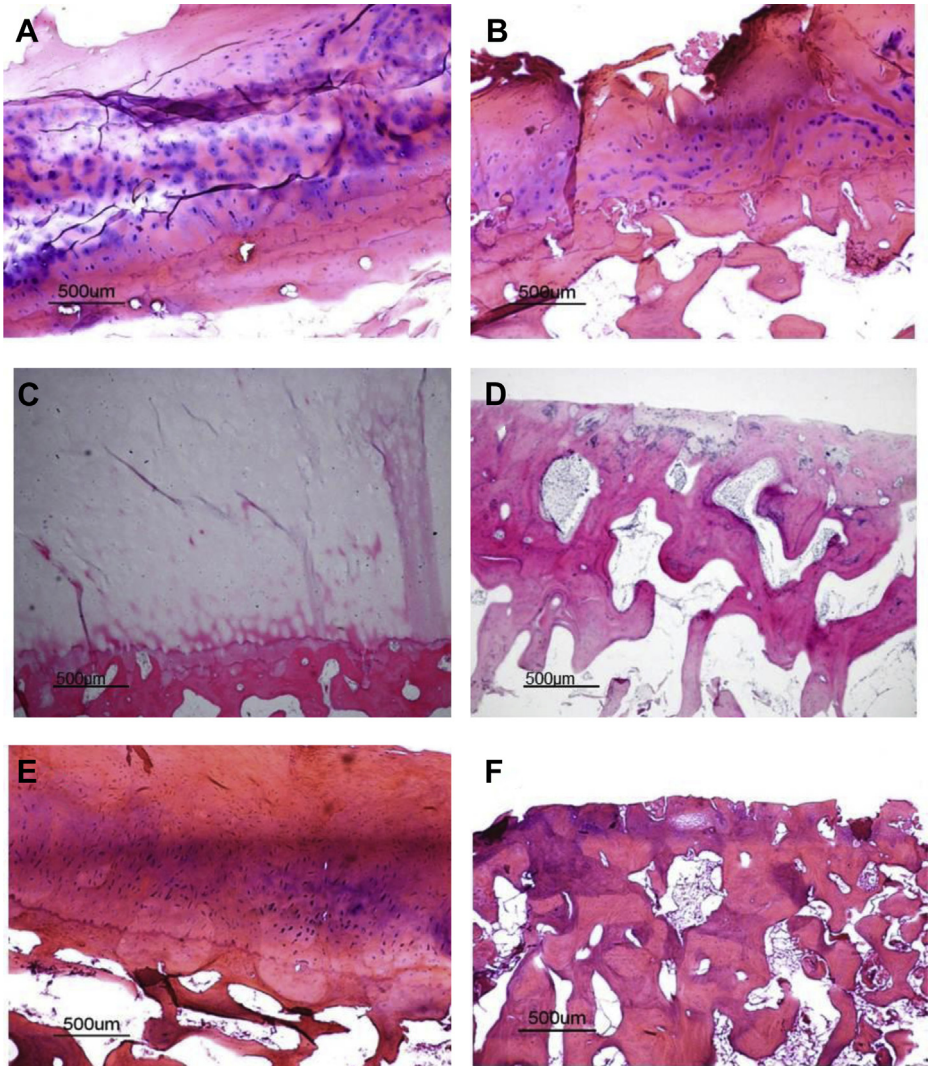

Fig. 3. Histological images of osteochondral junction at lateral (A, C, E) and medial (B, D, F) tibial plateau in knee osteoarthritis (OA) patients (A ~ B: patients without hypertension and T2DM; C ~ D: patients with hypertension only; E ~ F: patients with both hypertension and T2DM). As compared to the subjects without these comorbid diseases (B), subchondral bone was severely damaged on medial tibial plateau in patients with hypertension and diabetes, which was accompanied by wearing away the full-thickness of overlying articular cartilage (D, F). Furthermore, the thickness of subchondral bone plate on lateral tibial plateau was also obviously thinner in these patients with the comorbidities together with the elevated porosity (C, E) (Hematoxylin–Eosin staining).

Table III
Unadjusted, minor and fully adjusted multivariate linear regression analysis of the comorbidities in knee OA on subchondral bone damages of medial tibia

Variables	Unadjusted				Adjusted by age, gender and BMI				Fully adjusted*			
	Unstandardized β	Standardized β	95% CI	*P*	Unstandardized β	Standardized β	95% CI	*P*	Unstandardized β	Standardized β	95% CI	*P*
BMD (g/cm^3)	−0.214	−0.577	(−0.387 ~ −0.040)	0.019	−0.260	−0.701	(−0.409 ~ −0.110)	0.002	−0.312	−0.742	(−0.498 ~ −0.125)	0.004
Total porosity (%)	10.503	0.572	(2.224 ~ 18.783)	0.016	12.788	0.697	(5.731 ~ 19.846)	0.002	15.347	0.738	(6.526–24.168)	0.003

Notes: The comorbidities in knee OA were categorized as follows: "1" no hypertension, no T2DM; "2" hypertension, no T2DM; "3" hypertension and T2DM. With the change of the categories of the comorbidities from 1 to 3, BMD of subchondral plate decreases by 0.312 g/cm^3 and the porosity increases by15.347% after fully adjustment.

* Fully adjusted by age, gender, BMI, knee society knee scores and functional assessments.

plate. After adjusting these covariates, we can clearly notice the add-on effect of the comorbidities on the subchondral bone damages on the top of the major risk factors in the pathomechanism of knee OA, for example the obesity. To our best knowledge, this is the first time to establish such numerical relationship between subchondral bone microstructural damages in knee OA and its comorbidities.

The emerging view of knee OA is that part of joint pain and disorder might be related to altered vascular function[19,20,29]. The scenario of the development of OA with hypertension was proposed as follows: narrow or constricted vessels restrict blood flow to subchondral bone, and impair the circulation and nutrition supply to overlying articular cartilage, and ultimately contribute to the deterioration of cartilage degeneration in OA[30].

This hypothetic model for vascular pathology in pathomechanism of OA was supported by a recent epidemiological study showing a novel relationship between arterial stiffness with development of hand OA[29]. Our findings enriched this hypothetic model that high blood pressure might contribute to the decreased BMD at subchondral plate in knee OA. As a consequence, subchondral plate with low bone mass might be susceptible for microcrack or microfracture at osteochondral junction under repetitive mechanical loading during joint movement, which in turn triggers or aggregates the development of OA[31]. It was compatible with previous findings on the association of high blood pressure with low bone mass and high risk of fracture in general population[23–25].

T2DM is another disease having a strong relationship with knee OA[16,21,32,33]. In a recent study, 927 men and women at the age of 40–80 years old were studied by a 20-year follow-up[33]. It was found that type 2 diabetes emerged as an independent risk predictor for arthroplasty after adjustment for age, BMI, and other risk factors for OA[33]. In addition, the subjects with diabetes developed more severe clinical symptoms of OA (Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) score) and structural joint damages in terms of joint effusion and synovitis under ultrasonography[33]. Researchers concluded that type 2 diabetes predicted the development of severe OA independent of age and BMI[33]. However, they did not provide any information regarding articular cartilage degeneration and subchondral bone abnormality, which are the primary concern in knee OA, in presence of type 2 diabetes. Our findings contributed to fill out this research gap. We observed more severe subchondral plate and articular cartilage damages in knee OA patients with type 2 diabetes and hypertension as compared to the subjects without these comorbidities. These findings further strengthen the concept of a strong metabolic component in the pathogenesis of OA.

There were two possible pathways for T2DM involved in the pathogenesis of OA. Firstly, the insulin resistance in T2DM might trigger the cascade inflammatory reactions[21,34–36], which is known to play an important role in the development of OA[37]. On the other hand, inflammation produces C-reactive protein (CRP), which, unfortunately, can damage the arteries by helping to form plaque while attempting to tackle a long-term condition like high blood pressure – often an accompanying T2DM[38]. In the case series of this study, we found that the majority of knee OA patients with T2DM (12 out of 13) also had essential hypertension. This likely means that paying attention to insulin resistance, and high blood pressure could be important in preventing the progression of OA.

Clinical management of knee OA, hypertension and T2DM in the elderly patients is a dilemma for clinicians because of the complex interaction among them. It was known that NSAIDs, widely used for pain management in knee OA, induce high blood pressure in OA patients[39]. In addition, some anti-hypertensive medicines were known to aggregate bone loss[40]. The findings generated from this study raise the public awareness on the phenomenon of bone loss

at subchondral plate of OA knee in presence of hypertension and type 2 diabetes. Understanding of the interrelationship between subchondral bone loss in OA and its comorbidities would bring the hope to break down the vicious cycle in management of the elderly persons with these multiple organ disorders.

The pores in subchondral plate at osteochondral junction are necessary for nutrition supply, and exchanges of metabolites between subchondral bone and overlying articular cartilage[3]. Previous study has identified the increase of the pore size in subchondral plate and the decrease of bone mass together with the development of OA-like changes induced by collagenase in a mice model[41]. The increased porosity of subchondral plate at osteochondral junction might facilitate the invasion of the heterozygous clustered bone marrow cells, including multi-nucleated cells, and blood vessel (Supplementary Figs. 2 and 3). As a consequence, the ingrowth of blood vessels and bone marrow cells might trigger the endochondral ossification in the hyaline cartilage, lead to the stiffening of cartilage collagen fibrils[42], ultimately the failure of articular cartilage.

There are several major limitations in the current study. First, the cross-sectional observation in a case series study does not allow us to answer any question regarding the causative effect of the comorbidities on knee OA, or *vice versa*. Second, the sample number was relative small and we only analyzed two most frequently encountered comorbidities in knee OA: hypertension and T2DM, but did not cover all the other comorbidities, for example hyperlipidemia, chronic ischemic heart disease, atherosclerosis and stroke *etc.* Third, the clinical and laboratory data were taken from clinical record, and the non-routine examinations, such as the PWV/ABI data for arterial stiffening, were not available. Some record on the duration of diseases and all treatments for the comorbidities were incomplete although the latest medications, previous cardiovascular events or diabetes complications were well documented. Last but not least, there are many more potential confounders in the observed associations and the potential of residual confounding factors might bring the bias due to incomplete adjustment.

We appreciated that knee OA is a multifactorial disorder and only 43 human subjects were analyzed in the present study. Here, we mainly reported a phenomenon that bone loss occurred at subchondral plate of OA knee in presence of hypertension and T2DM. Our further analyses imply the biological link between subchondral bone loss in knee OA and its comorbid diseases. By doing that, we hope to raise the public awareness of their complex interactions in elderly persons, and prompt the needs of a large-scale cohort study to confirm the causality between them.

Authors' contribution

The authors met all the following conditions: (1) substantial contribution to the conception and design (Wen CY, and Chiu KY), (2) acquisition of data (Wen CY, Chen Y and Tang HL), analysis and interpretation of data, and drafting the article or revising it critically for important intellectual content (Wen CY, Yan CH, Chiu KY, Lu WW); also (3) final approval of the version to be published (Wen CY, Yan CH, Chiu KY, Lu WW).

Competing interests

The authors declare that they have no competing interests.

Acknowledgement

The authors acknowledge the support from Li Shu Fan Medical Foundation, National Science Foundation of China (NSFC 81270967#) and ShenZhen Peacock Project.

Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.joca.2013.06.027.

References

1. Zhang Y, Jordan JM. Epidemiology of osteoarthritis. Rheum Dis Clin North America 2008;34:515–29.
2. Mahjoub M, Berenbaum F, Houard X. Why subchondral bone in osteoarthritis? The importance of the cartilage bone interface in osteoarthritis. Osteoporosis international: a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA 2013;23(Suppl 8):S841–6.
3. Suri S, Walsh DA. Osteochondral alterations in osteoarthritis. Bone 2012;51:204–11.
4. Lories RJ, Luyten FP. The bone-cartilage unit in osteoarthritis. Nature reviews. Rheumatology 2011;7:43–9.
5. Prasadam I, van Gennip S, Friis T, Shi W, Crawford R, Xiao Y. ERK-1/2 and p38 in the regulation of hypertrophic changes of normal articular cartilage chondrocytes induced by osteoarthritic subchondral osteoblasts. Arthritis Rheum 2010;62:1349–60.
6. Couchourel D, Aubry I, Delalandre A, Lavigne M, Martel-Pelletier J, Pelletier JP, *et al*. Altered mineralization of human osteoarthritic osteoblasts is attributable to abnormal type I collagen production. Arthritis Rheum 2009;60:1438–50.
7. Sanchez C, Deberg MA, Bellahcene A, Castronovo V, Msika P, Delcour JP, *et al*. Phenotypic characterization of osteoblasts from the sclerotic zones of osteoarthritic subchondral bone. Arthritis Rheum 2008;58:442–55.
8. Sanchez C, Deberg MA, Piccardi N, Msika P, Reginster JY, Henrotin YE. Subchondral bone osteoblasts induce phenotypic changes in human osteoarthritic chondrocytes. Osteoarthritis and cartilage/OARS. Osteoarthritis Res Soc 2005;13:988–97.
9. McErlain DD, Ulici V, Darling M, Msika P, Reginster JY, Henrotin YE. An in vivo investigation of the initiation and progression of subchondral cysts in a rodent model of secondary osteoarthritis. Arthritis Res Ther 2012;14:R26.
10. Hilal G, Massicotte F, Martel-Pelletier J, Fernandes JC, Pelletier JP, Lajeunesse D. Endogenous prostaglandin E2 and insulin-like growth factor 1 can modulate the levels of parathyroid hormone receptor in human osteoarthritic osteoblasts. J Bone Miner Res: Official J Am Soc Bone Miner Res 2001;16:713–21.
11. Lajeunesse D. The role of bone in the treatment of osteoarthritis. Osteoarthritis and cartilage/OARS. Osteoarthritis Res Soc 2004;12(Suppl A):S34–8.
12. Hochberg MC, Lethbridge-Cejku M, Tobin JD. Bone mineral density and osteoarthritis: data from the Baltimore Longitudinal Study of Aging. Osteoarthritis and cartilage/OARS. Osteoarthritis Res Soc 2004;12(Suppl A):S45–8.
13. Sridhar MS, Jarrett CD, Xerogeanes JW, Labib SA. Obesity and symptomatic osteoarthritis of the knee. J Bone Joint Surg Br 2012;94:433–40.
14. Kerkhof HJ, Lories RJ, Meulenbelt I, Jonsdottir I, Valdes AM, Arp P, *et al*. A genome-wide association study identifies an osteoarthritis susceptibility locus on chromosome 7q22. Arthritis Rheum 2010;62:499–510.
15. Valdes AM, Spector TD, Tamm A, Kisand K, Doherty SA, Dennison EM, *et al*. Genetic variation in the SMAD3 gene is associated with hip and knee osteoarthritis. Arthritis Rheum 2010;62:2347–52.

16. Singh G, Miller JD, Lee FH, Pettitt D, Russell MW. Prevalence of cardiovascular disease risk factors among US adults with self-reported osteoarthritis: data from the Third National Health and Nutrition Examination Survey. Am J Manag Care 2002;8: S383–91.
17. Zhuo Q, Yang W, Chen J, Wang Y. Metabolic syndrome meets osteoarthritis. Nature reviews. Rheumatology 2012;8:729–37.
18. Nuesch E, Dieppe P, Reichenbach S, Williams S, Iff S, Juni P. All cause and disease specific mortality in patients with knee or hip osteoarthritis: population based cohort study. BMJ 2011;342:d1165.
19. Findlay DM. Vascular pathology and osteoarthritis. Rheumatology (Oxford) 2007;46:1763–8.
20. Conaghan PG, Vanharanta H, Dieppe PA. Is progressive osteoarthritis an atheromatous vascular disease? Ann Rheum Dis 2005;64:1539–41.
21. Berenbaum F. Diabetes-induced osteoarthritis: from a new paradigm to a new phenotype. Postgrad Med J 2012;88:240–2.
22. Yoshimura N, Muraki S, Oka H, Tanaka S, Kawaguchi H, Nakamura K, *et al*. Accumulation of metabolic risk factors such as overweight, hypertension, dyslipidaemia, and impaired glucose tolerance raises the risk of occurrence and progression of knee osteoarthritis: a 3-year follow-up of the ROAD study. Osteoarthritis and cartilage/OARS. Osteoarthritis Res Soc 2012;20:1217–26.
23. Metz JA, Morris CD, Roberts LA, McClung MR, McCarron DA. Blood pressure and calcium intake are related to bone density in adult males. Br J Nutr 1999;81:383–8.
24. Lethbridge-Cejku M, Tobin JD, Scott Jr WW, Cauley JA. Axial and hip bone mineral density and radiographic changes of osteoarthritis of the knee: data from the Baltimore Longitudinal Study of Aging. J Rheumatol 1996;23:1943–7.
25. Vestergaard P, Rejnmark L, Mosekilde L. Hypertension is a risk factor for fractures. Calcified Tissue Int 2009;84:103–11.
26. Schwartz AV, Vittinghoff E, Bauer DC, Hillier TA, Strotmeyer ES, Ensrud KE, *et al*. Association of BMD and FRAX score with risk of fracture in older adults with type 2 diabetes. JAMA 2011;305:2184–92.
27. Hofbauer LC, Brueck CC, Singh SK, Dobnig H. Osteoporosis in patients with diabetes mellitus. J Bone Mineral Res 2007;22: 1317–28.
28. Liow RY, Walker K, Wajid MA, Bedi G, Lennox CM. The reliability of the American knee society score. Acta Orthopaedica Scand 2000;71:603–8.
29. Saleh AS, Najjar SS, Muller DC, Shetty V, Ferrucci L, Gelber AC, *et al*. Arterial stiffness and hand osteoarthritis: a novel relationship? Osteoarthritis and cartilage/OARS. Osteoarthritis Res Soc 2007;15:357–61.
30. van den Berg WB. Osteoarthritis year 2010 in review: pathomechanisms. Osteoarthritis and cartilage/OARS. Osteoarthritis Res Soc 2011;19:338–41.
31. Burr DB, Radin EL. Microfractures and microcracks in subchondral bone: are they relevant to osteoarthrosis? Rheum Dis Clin North America 2003;29:675–85.
32. Leaverton PE, Peregoy J, Fahlman L, Sangeorzan E, Barrett Jr JP. Does diabetes hide osteoarthritis pain? Med Hypotheses 2012;78:471–4.
33. Schett G, Kleyer A, Perricone C, Sahinbegovic E, Iagnocco A, Zwerina J, *et al*. Diabetes is an independent predictor for severe osteoarthritis: results from a longitudinal cohort study. Diabetes Care 2013;36:403–9.
34. Waine H, Nevinny D, Rosenthal J, Joffe IB. Association of osteoarthritis and diabetes mellitus. Tufts Folia Med 1961;7: 13–9.
35. Sturmer T, Brenner H, Brenner RE, Gunther KP. Non-insulin dependent diabetes mellitus (NIDDM) and patterns of osteoarthritis. The Ulm osteoarthritis study. Scand J Rheumatol 2001;30:169–71.
36. Caterson B, Baker JR, Christner JE, Pollok BA, Rostand KS. Diabetes and osteoarthritis. Ala J Med Sci 1980;17:292–9.
37. Wang Q, Rozelle AL, Lepus CM, Scanzello CR, Song JJ, Larsen DM, *et al*. Identification of a central role for complement in osteoarthritis. Nat Med 2011;17:1674–9.
38. Nielen MM, van Sijl AM, Peters MJ, Verheij RA, Schellevis FG, Nurmohamed MT. Cardiovascular disease prevalence in patients with inflammatory arthritis, diabetes mellitus and osteoarthritis: a cross-sectional study in primary care. BMC Musculoskelet Disord 2012;13:150.
39. Sowers JR, White WB, Pitt B, Whelton A, Simon LS, Winer N, *et al*. The effects of cyclooxygenase-2 inhibitors and nonsteroidal anti-inflammatory therapy on 24-hour blood pressure in patients with hypertension, osteoarthritis, and type 2 diabetes mellitus. Arch Intern Med 2005;165:161–8.
40. Ilic K, Obradovic N, Vujasinovic-Stupar N. The relationship among hypertension, antihypertensive medications, and osteoporosis: a narrative review. Calcif Tissue Int 2013;92: 217–27.
41. Botter SM, van Osch GJ, Clockaerts S, Waarsing JH, Weinans H, van Leeuwen JP. Osteoarthritis induction leads to early and temporal subchondral plate porosity in the tibial plateau of mice: an in vivo microfocal computed tomography study. Arthritis Rheum 2011;63:2690–9.
42. Wen CY, Wu CB, Tang B, Wang T, Yan CH, Lu WW, *et al*. Collagen fibril stiffening in osteoarthritic cartilage of human beings revealed by atomic force microscopy. Osteoarthritis and cartilage/OARS. Osteoarthritis Res Soc 2012;20:916–22.