Is Retinal Microvascular Abnormalities an Independent Risk Factor of Vertebral Fractures? A Prospective Study From a Chinese Population

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ABSTRACT

Low bone mineral density (BMD) and microvascular diseases (MVD) share various common risk factors; however, whether MVD is an independent risk factor of vertebral fractures is incompletely understood. The aim of this study is to clarify whether MVD is an independent risk factor of vertebral fractures. In this prospective study, calcaneal BMD and retinal microvascular abnormalities were assessed at baseline from June 2011 to January 2012. A total of 2176 premenopausal women, 2633 postmenopausal women, 2998 men aged <65 years, and 737 men aged ≥65 were included. Then with/without retinal microvascular abnormalities cohorts were followed for an average of 2.93 years to find out the relationship between MVD and vertebral fractures. At the baseline, after full adjustment, retinal microvascular abnormalities were related to risk of low BMD only in men aged ≥65 years (odds ratio [OR] = 2.506; 95% confidence interval [CI] 1.454–4.321; p = 0.001). After follow-up of 2.93 years, retinal microvascular abnormalities were related to risk of vertebral fractures in men aged ≥65 years (OR = 2.475; 95% CI 1.085–5.646; p = 0.031) when adjustment for confounding factors. However, no associations were found between MVD and vertebral fractures in men aged <65 years, premenopausal women, and postmenopausal women. When stratified by diabetes, in the without-diabetes group, the men with retinal microvascular abnormalities had higher risk for vertebral fractures than without retinopathy (OR = 2.194; 95% CI 1.097–4.389; p = 0.026); however, the difference was not found in women. In the diabetes group, there were no significant differences of risk for vertebral fractures between those with retinal microvascular abnormalities and those without both in men and women. Stratified by hypertension, the men with retinopathy had higher risk for vertebral fractures than those without among the hypertension group (OR = 2.034; 95% CI 1.163–3.559; p = 0.013), but a difference was not found among women. In the without-hypertension group, no relation was found between MVD and fracture both in men and women. In conclusion, MVD is an independent risk factor of vertebral fractures in old men. © 2017 The Authors. JBMR Plus is published by Wiley Periodicals, Inc. on behalf of the American Society for Bone and Mineral Research.

KEY WORDS: OSTEOPOROSIS; GENERAL POPULATION STUDIES; BONE MINERAL DENSITY (BMD); RETINAL MICROVASCULAR ABNORMALITIES; VERTEBRAL FRACTURE

Introduction

Bone loss and vascular abnormalities both occur insidiously and are initially asymptomatic processes that increase markedly with advancing age. As the number of elderly increases, so will the magnitude of the problem. Multiple factors including proteins, parathyroid hormone (PTH), phosphate, oxidized lipids, and vitamins D and K are implicated in both bone and vascular metabolism, illustrating the interaction of these two seemingly unrelated conditions. Some study has shown that diabetic microvascular complications contribute to risk of fracture. For people who do not suffer from diabetes or hypertension with microvascular disease (MVD), the risk for fracture remains elusive. In addition, the association between MVD and fracture in large prospective study is limited. Evidence linking fragile fracture with microvascular complications in bone remains incompletely understood.
It is a well-established principle that blood supply to the bone is a vital basis of bone growth and remodeling. Peripheral vascular resistance and the perfusion pressure gradient are two main factors controlling the rate of flow through the microvascular bed. Reduced blood flow has been linked low bone mass disorders. Two studies have found that a special capillary subtype (CD31hi/Emcnhi vessels) and Notch signaling pathway are involved in the murine bone growth. The decline of CD31hi/Emcnhi vessels and the concomitant reduction of osteoprogenitor cells could potentially offer a compelling explanation for bone loss during aging. For technical difficulties, the precise overall structure of the skeletal vasculature in human has remained poorly understood. The human eye offers an excellent opportunity to visualize the microcirculation. Direct ophthalmoscopic examination provides a noninvasive means by which MVD can be assessed in vitro, and because the anatomy and physiology of retinal arterioles are similar to those of cerebral and coronary arterioles, retinal microcirculation abnormalities may reflect generalized microcirculatory pathology. The spatial distribution of arteriole-capillary connections determines regional differences in oxygenation and metabolic activity in bone. Calcaneal quantitative ultrasound (QUS) is a quick, simple, and inexpensive method free of ionizing radiation that appears to be effective in detecting bone loss. A great deal of literature has shown that severe height loss (at least 2 cm over 3 to 7 years) is often a consequence of osteoporotic vertebral fractures, so we use height loss of at least 2 cm as a surrogate of vertebral fragile fractures. Estrogen and age have a significant impact on bone mineral density (BMD) and vascularization. Therefore, we evaluate the different associations of microvascular abnormalities with bone loss/fatigue, among premenopausal women, postmenopausal women, men aged <65 years, and men aged ≥65 years. Because diabetes and hypertension can cause microangiopathy, we further categorized diabetic, hypertensive, and sex to explore the relations between the microvascular and vertebral fragile fractures.

Materials and Methods

See the Supplemental Methods for more detail.

Study cohort

This was a prospective observational cohorts study design is shown in reference 24 and supplemental material. A total of 10,906 Chinese respondents to the survey who were not pregnant and had no cognitive dysfunction. Among them, 1876 subjects who refused to undergo retinal examination or were temporarily outside, 165 with history of thyroid disorders, postmenopausal women, men aged <65 years, and men aged ≥65 years. Because diabetes and hypertension can cause microangiopathy, we further categorized diabetic, hypertensive, and sex to explore the relations between the microvascular and vertebral fragile fractures.

Baseline assessment

BMD was assessed by scanning the left calcaneus with Sahara (Hologic, Inc., Waltham, MA, USA). All the parameters were measured twice by the same experienced operator, and the mean value was used for analysis. Results for calcaneal BMD were transformed to T-scores (calculated as the difference between the actual measurement and the mean value of healthy sex-matched adult controls, divided by their standard deviation), from the data provided by the densitometer manufacturer. According to the World Health Organization (WHO) criteria, bone status was categorized into three groups: normal BMD (T-score ≥−0.9), osteopenia (−2.4 ≤ T-score ≤ −1.0), or osteoporosis (T-score ≤−2.5). Low BMD refers to T-score ≤−1.0.

Direct ophthalmoscopic examination from both eyes after 5 minutes of dark adaptation was conducted by two qualified retinal ophthalmologists independently. A research ophthalmologist undertook a quality assurance check of 10% of the diagnosis and no instances of discordance were identified. Microvascular abnormalities were defined as present if any of the following lesions were observed in any of the four quadrants: microaneurysms, retinal hemorrhages (blot or flame shaped), soft exudates (cotton-wool spots), hard exudates, macular edema, intraretinal microvascular abnormalities, venous beading, new vessels at the disc or elsewhere, vitreous hemorrhage, disc swelling, laser photocoagulation scars, arteriovenous nicking, or focal arteriolar narrowing.

Standing height was measured in light clothing without shoes with the use of an electronic stadiometer. Subjects placed both heels, buttoks, and their back against the stadiometer backboard, with the head positioned in the Frankfort horizontal plane. The participants were instructed to stretch to a fully erect position while keeping the feet flat on the floor. Excessive stretching was avoided. Besides the subjects’ heads being maintained in the Frankfort plane, the heads did not necessarily touch the backboard. The horizontal plate of the stadiometer was pressed firmly onto the heads, flattening the hair. Height was measured by certified nurses to the nearest 0.1 cm during normal respiration.

A questionnaire including information on demographic characteristics, medical history, and lifestyle factors was administered. Anthropometric measurements were conducted. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. Standard laboratory tests determined blood glucose, HbA1c, serum insulin, creatinine, and lipids. The index of homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as: fasting glucose (mmol/L) × fasting insulin (μU/mL)/22.5. The CKD-EPI equation was used to estimate glomerular filtration rate (eGFR). Age is divided into four groups: ≤45, 46 to 55, 56 to 65, and ≥65 years.

Diabetes mellitus was defined as having fasting blood glucose (FBG) ≥7.0 mmol/L, porphobilinogen (PBG) ≥11.1 mmol/L, or...
history of treatment for diabetes. Insulin resistance (IR) was defined as HOMA-IR higher than 2.50. (28) Hypertension was defined as having systolic blood pressure (SBP) ≥ 140 mmHg, diastolic blood pressure (DBP) ≥ 90 mmHg, or history of treatment for hypertension. Dyslipidemia was defined as current treatment with cholesterol-lowering medication or having one or more of the following: TC > 6.22 mmol/L, TG > 2.26 mmol/L, LDL > 4.4 mmol/L, and HDL < 1.04 mmol/L. (29) Furthermore, general obesity was defined as BMI ≥ 27.5 kg/m² and abdominal obesity as waist circumference (WC) ≥ 90 cm for men and ≥ 80 cm for women. (30,31) We defined abnormal eGFR as < 60 mL/min/1.73 m². (32)

Collection of follow-up data

Three-year follow-up visits measured height. Height loss of at least 2 cm over 3 years was an indication of vertebral fractures. Subjects self-reported any history of trauma fracture(s), details of the way, site, and date. Methods of followed data collection were similar to the baseline. However, direct ophthalmoscopic examination and fasting insulin were not collected in the follow-up visit. In addition, Calcaneal quantitative ultrasound was followed only in Wuyishan city.

Statistical analysis

EpiData software (The EpiData Association, Odense, Denmark) was used to establish the database. All data were double entered in a database and then compared and corrected for errors. Continuous variables were shown by medians with interquartile ranges (IQR; the range between the 25th and 75th percentile) because of the non-normal distribution, and categorical variables were expressed by counts and percentages. The differences among subjects in different groups were detected using the Kruskal-Wallis test for continuous variables and chi-square test for categorical variables. Constructing multiple logistic regression models determined the relationship between low BMD and microvascular abnormalities on baseline. First, the crude associations were evaluated (unadjusted) and then included adjustment for age (model 1). Subsequent models were built with inclusions of education, physical activity, smoking status, alcohol, coffee, milk, and bean products consumption (model 2), then further adjusted for diabetes, IR, hypertension, dyslipidemia, eGFR < 60 mL/min/1.73 m², generalized and abdominal obesity, gastrointestinal disorders, respiratory diseases, and urological diseases (model 3). Additionally, binary logistic regression was used to analyze the association between vertebral fractures and microvascular abnormalities. The detailed process of adjusted confounding factors is given in Table 3. To further control the effects of diabetes mellitus and hypertension, data were split into diabetes mellitus and hypertension, in men and women, and the risk of vertebral fragile fractures with microvascular abnormalities were compared to without microvascular abnormalities by binary logistic regressions.

All data analyses were performed with SPSS 19.0 statistical software package (SPSS, Chicago, IL, USA). All p values were based on two-sided tests, with statistical significance defined as p < 0.05.

Results

At baseline, participants were randomly selected, with 56.3% being women and 43.7% being men, giving a sex ratio of 1.29:1, with a total median age of 53 years (IQR, 46 to 61 years). Prevalence of retinal microvascular abnormalities was 1.3%, 2.9%, 6.9%, and 9.6% in premenopausal women, men aged < 65 years, postmenopausal women, and men aged ≥ 65 years, respectively, and low BMD prevalence was 10.7%, 28.3%, 40.6%, and 43.8% (Supplemental Table S1). The baseline measures, follow-up measures, and percentage change in subjects with and without microvascular abnormalities are shown in Table 1. In the microvascular abnormalities group, the prevalence of vertebral fractures was 22.2%, 24.6%, 22.8%, and 39.5% among premenopausal women, men aged < 65 years, postmenopausal...
| Baseline       | Men                          | p Value | Women                         | p Value |
|---------------|------------------------------|---------|-------------------------------|---------|
| Number        | 3577                         | 0.001   | 4598                         | 0.001   |
| Age (years)   | 53 (45 to 62)                | 0.161   | 51 (46 to 59)                 | 0.001   |
| High school or more | 1285 (42.9) | 0.001   | 921 (42.3)                    | 0.001   |
| Smokers       | 1709 (49.8)                  | 0.001   | 28 (6.0)                      | 0.244   |
| Alcohol drinkers | 1189 (33.2) | 0.086   | 1205 (26.2)                   | 0.001   |
| Coffee drinkers | 204 (5.7)       | 0.379   | 309 (6.7)                     | 0.022   |
| Milk drinkers  | 1831 (51.2)                 | 0.684   | 2057 (44.7)                   | 0.103   |
| Bean products  | 2799 (78.2)                 | 0.281   | 3681 (80.1)                   | 0.023   |
| Physical activity | 0.001           |         | 0.001                        |         |
| Low           | 2399 (67.1)                 | 0.001   | 3744 (81.4)                   | 0.202   |
| Moderate      | 324 (9.1)                   | 0.161   | 328 (7.1)                     | 0.3 (4.4)|
| High          | 854 (23.9)                  | 0.161   | 526 (11.4)                    | 0.6 (2.6)|
| FBG (mmol/L)  | 5.5 (5.1 to 6.0)            | 0.001   | 5.4 (5.0 to 5.8)              | 0.001   |
| PBG (mmol/L)  | 6.4 (5.1 to 8.2)            | 0.001   | 6.8 (5.7 to 8.3)              | 0.001   |
| HbA1c (%)     | 5.7 (5.4 to 6.0)            | 0.001   | 5.7 (5.4 to 6.0)              | 0.001   |
| FIN (mU/mL)   | 5.2 (3.4 to 7.7)            | 0.001   | 6.4 (4.6 to 8.8)              | 0.001   |
| HOMA-IR       | 1.3 (0.8 to 2.0)            | 0.001   | 1.5 (1.1 to 2.2)              | 0.001   |
| SBP (mmHg)    | 134 (123 to 147)            | 0.001   | 127 (117 to 144)              | 0.001   |
| DBP (mmHg)    | 78 (72 to 86)               | 0.001   | 76 (69 to 83)                 | 0.001   |
| HDL (mmol/L)  | 1.3 (1.1 to 1.5)            | 0.045   | 1.4 (1.2 to 1.6)              | 0.237   |
| LDL (mmol/L)  | 2.9 (2.4 to 3.4)            | 0.029   | 3.0 (2.5 to 3.6)              | 0.018   |
| TC (mmol/L)   | 5.0 (4.4 to 5.7)            | 0.810   | 5.2 (4.5 to 5.9)              | 0.001   |
| TG (mmol/L)   | 1.4 (1.0 to 2.1)            | 0.006   | 1.3 (0.9 to 1.8)              | 0.001   |
| BMI (kg/m²)   | 24.0 (21.9 to 26.2)         | 0.001   | 23.7 (21.8 to 25.9)           | 0.001   |
| eGFR (mL/min/1.73 m²) | 96.3 (86.1 to 104.0) | 0.001   | 96.9 (88.3 to 104.5)           | 0.001   |
| Diabetes      | 53 (15.0)                   | 0.001   | 592 (12.9)                    | 0.001   |
| IR            | 506 (14.1)                  | 0.001   | 830 (18.1)                    | 0.001   |
| Hypertension  | 1558 (43.6)                 | 0.001   | 1717 (37.3)                   | 0.001   |
| Dyslipidemia  | 1466 (41.0)                 | 0.001   | 1561 (33.9)                   | 0.001   |
| General obesity | 498 (13.9)   | 0.001   | 641 (13.9)                    | 0.001   |
| Abdominal obesity | 760 (21.2) | 0.001   | 81 (1.8)                      | 0.001   |
| eGFR <60 mL/min/1.73 m² | 89 (2.5)     | 0.001   | 75 (1.6)                      | 0.001   |
| Gastrointestinal disorders | 452 (12.6) | 0.001   | 538 (15.0)                    | 0.001   |
| Respiratory diseases | 112 (3.1)    | 0.001   | 83 (1.8)                      | 0.001   |
| Urological diseases | 233 (6.5)   | 0.001   | 201 (4.4)                     | 0.001   |
| T-score       | –0.3 (–1.2 to 0.8)          | 0.001   | 0.1 (–1.0 to 1.4)             | 0.001   |
| BMD status    | 2475 (69.2)                 | 0.001   | 3378 (73.5)                   | 0.001   |
| Number        | 2156                         | 0.001   | 2676                         | 0.001   |
| Age (years)   | 54 (49 to 62)                | 0.001   | 53 (48 to 60)                 | 0.001   |
| Height loss at least 2 cm | 345 (16.0)   | 0.001   | 519 (19.4)                    | 0.001   |
| Smokers       | 1165 (34.0)                 | 0.001   | 10 (0.4)                      | 0.001   |
| Alcohol drinkers | 768 (35.6)    | 0.086   | 81 (3.0)                      | 0.001   |
| Coffee drinkers | 204 (5.7)      | 0.379   | 309 (6.7)                     | 0.022   |
| Milk drinkers  | 1831 (51.2)                | 0.684   | 2057 (44.7)                   | 0.103   |
| Physical activity | 0.022           |         | 0.001                        |         |
| Low           | 1428 (66.2)                 | 0.001   | 2234 (83.5)                   | 0.126   |
| Moderate      | 216 (10.0)                 | 0.161   | 201 (7.5)                     | 0.3 (3.3)|
| High          | 512 (23.7)                 | 0.001   | 241 (9.0)                     | 0.017   |
| FBG (mmol/L)  | 5.5 (5.2 to 6.0)            | 0.001   | 5.4 (5.1 to 5.9)              | 0.001   |
| PBG (mmol/L)  | 7.0 (5.1 to 9.1)            | 0.001   | 7.1 (6.0 to 9.0)              | 0.001   |
In men aged \( \geq 65 \) years, compared with participants without retinal microvascular abnormalities, those with retinal microvascular abnormalities had significantly higher risk of low BMD even after adjustment for different confounders; the fully adjusted odds ratio (OR) was 2.506 (95% confidence interval [CI] 1.454–4.321; \( p = 0.001 \)). However, no significant associations were found between retinal microvascular abnormalities and low BMD risk in men aged \( < 65 \) years and premenopausal or postmenopausal women (all \( p > 0.05 \)) (Table 2).

Retinal microvascular abnormalities and vertebral fractures risk

As shown in Table 3, retinal microvascular abnormalities were associated with the risk of vertebral fractures in men aged \( \geq 65 \) years, OR was 2.475 (95% CI 1.085–5.646; 0.031). However, no significant associations were found between retinal microvascular abnormalities and vertebral fractures risk.
Table 2. Retinopathy and Low BMD Risk (OR [95% CI])

| Retinopathy | None | Present | p Value |
|-------------|------|---------|---------|
| **Men**     |      |         |         |
| <65 years (n = 2998) | 2911 | 87      | 0.077   |
| No. of cases | 1.00 | 1.491 (0.957, 2.323) | 0.0203  |
| Unadjusted | Model 1 | 1.00 | 1.336 (0.855, 2.089) | 0.295   |
| Model 2 | 1.00 | 1.272 (0.811, 1.995) | 0.141   |
| Model 3 | 1.00 | 1.421 (0.890, 2.268) |         |
| ≥65 years (n = 737) | 666  | 71      |         |
| No. of cases | 1.00 | 1.539 (0.942, 2.515) | 0.085   |
| Unadjusted | Model 1 | 1.00 | 1.620 (0.987, 2.660) | 0.056   |
| Model 2 | 1.00 | 1.899 (1.142, 3.158) | 0.013   |
| Model 3 | 1.00 | 2.506 (1.454, 4.321) | 0.001   |
| **Women**   |      |         |         |
| Premenopausal (n = 2176) | 2147 | 29      | 0.258   |
| No. of cases | 1.00 | 1.753 (0.663, 4.641) |         |
| Unadjusted | Model 1 | 1.00 | 0.916 (0.325, 2.581) | 0.868   |
| Model 2 | 1.00 | 0.918 (0.322, 2.621) | 0.873   |
| Model 3 | 1.00 | 1.275 (0.413, 3.939) | 0.673   |
| Postmenopausal (n = 2633) | 2451 | 182     |         |
| No. of cases | 1.00 | 1.079 (0.795, 1.463) | 0.627   |
| Unadjusted | Model 1 | 1.00 | 0.764 (0.556, 1.051) | 0.098   |
| Model 2 | 1.00 | 0.764 (0.555, 1.053) | 0.100   |
| Model 3 | 1.00 | 0.861 (0.618, 1.199) | 0.376   |

Data are ORs (95% CI). Bold indicates statistically significant (p < 0.05). Each model included the following covariates for adjustment: model 1: age; model 2: model 1 + education, physical activity, smoking status, alcohol, coffee, milk, and bean products consumption; model 3: model 2 + diabetes, insulin resistance, hypertension, dyslipidemia, eGFR <60 mL/min/1.73 m², generalized and abdominal obesity, gastrointestinal disorders, respiratory diseases, and urological diseases.

Table 3. Retinopathy and Vertebral Risk (ORs [95%CI])

| Retinopathy | None | Present | p Value |
|-------------|------|---------|---------|
| **Men**     |      |         |         |
| <65 years (n = 1945) | 1888 | 57      |         |
| No. of cases | 1.00 | 1.690 (0.899, 3.177) | 0.104   |
| Unadjusted | Model 1 | 1.00 | 1.407 (0.743, 2.663) | 0.294   |
| Model 2 | 1.00 | 1.499 (0.786, 2.861) | 0.219   |
| Model 3 | 1.00 | 1.470 (0.752, 2.874) | 0.260   |
| Model 4 | 1.00 | 1.403 (0.713, 2.760) | 0.327   |
| ≥65 years (n = 306) | 268  | 38      |         |
| No. of cases | 1.00 | 2.079 (1.024, 4.222) | 0.043   |
| Unadjusted | Model 1 | 1.00 | 1.981 (0.966, 4.063) | 0.062   |
| Model 2 | 1.00 | 2.277 (1.650, 4.867) | 0.034   |
| Model 3 | 1.00 | 2.514 (1.117, 5.656) | 0.026   |
| Model 4 | 1.00 | 2.475 (1.085, 5.646) | 0.031   |
| **Women**   |      |         |         |
| Premenopausal (n = 1276) | 1267 | 9       |         |
| No. of cases | 1.00 | 1.552 (0.320, 7.525) | 0.585   |
| Unadjusted | Model 1 | 1.00 | 1.630 (0.335, 7.921) | 0.545   |
| Model 2 | 1.00 | 1.358 (0.271, 6.798) | 0.709   |
| Model 3 | 1.00 | 1.338 (0.248, 7.231) | 0.735   |
| Model 4 | 1.00 | 1.387 (0.249, 7.728) | 0.709   |
| Postmenopausal (n = 1531) | 1409 | 122     |         |
| No. of cases | 1.00 | 1.101 (0.716, 1.693) | 0.662   |
| Unadjusted | Model 1 | 1.00 | 1.008 (0.650, 1.563) | 0.972   |
| Model 2 | 1.00 | 1.017 (0.654, 1.582) | 0.940   |
| Model 3 | 1.00 | 0.995 (0.631, 1.571) | 0.983   |
| Model 4 | 1.00 | 0.972 (0.613, 1.541) | 0.904   |

Data are ORs (95% CI). Bold indicates statistically significant (p < 0.05). Each model included the following covariates for adjustment: model 1: grouped age, baseline BMD; model 2: model 1 + education, physical activity, smoking status, alcohol, coffee, milk, and bean products consumption; model 3: model 2 + diabetes, insulin resistance, hypertension, dyslipidemia, eGFR <60 mL/min/1.73 m², generalized and abdominal obesity; model 4: model 3 + the new occurrence of smoking status, alcohol, diabetes, hypertension, dyslipidemia, and the change of generalized and abdominal obesity, eGFR <60 mL/min/1.73 m².

Discussion

To our knowledge, the present study is the first attempt to explore the association between the presence of retinal microvascular abnormalities and BMD/vertebral fractures stratified by age, sex, menopausal status, diabetes, and hypertension in a large prospective study. In the baseline study, our team found that participants with retinal microvascular abnormalities have a higher risk of low BMD compared with participants without microvascular abnormalities after adjustment for confounders among men aged ≥65 years. After participants’ height were followed for an average of 2.93 years, results showed that risk for vertebral fractures (height loss of at least 2 cm) was 2.475 times higher compared with the participants without retinal microvascular abnormalities among men aged ≥65 years. In addition, when stratified by diabetes, in no diabetic group, men with microvascular abnormalities had higher risk for vertebral fracture compared with participants without retinopathy. Stratified by hypertension, hypertensive men with microvascular abnormalities had a higher risk for vertebral fracture compared with participants without hypertension.

The current result showed that retinal microvascular abnormalities were associated with an increased risk of low BMD and vertebral fragile fracture(s). These findings are consistent with microvascular abnormalities and vertebral fractures in premenopausal and postmenopausal women and men aged <65 years (all p < 0.05). When stratified by diabetes, in the without-diabetes group, men with retinal microvascular abnormalities had higher risk for vertebral fractures than without retinopathy (OR = 2.194; 95% CI = 1.097–4.389; p = 0.026) after adjusted confounding factor. However, the difference was not found in women. In the diabetes group, both in men and women, the risk for vertebral fractures between those with retinal microvascular abnormalities and those without were not significantly different. Stratified by hypertension, hypertensive men with retinopathy had higher risk for vertebral fractures than those without (OR = 2.034; 95% CI 1.163–3.559; p = 0.013), but the difference was not found among women. In the without-hypertension group, no associations were found between MVD and fracture both in men and women (Table 4).
The difference was not found in subjects without hypertension. The notion that there is an association between vascular disease and bone. In this prospective study, not only baseline results showed that men aged ≥65 years with MVD had higher risk of low BMD compared with participants without microvascular abnormalities, but also after 3-year follow-up visits, the men aged ≥65 years with MVD had a higher risk of fracture after adjustment for age, BMD, and other confounding factors. This result indicated that MVD can lead to low BMD and fracture(s), although various common risk factors between BMD and MVD may lead to interaction of these two. MVD is an independent risk factor of vertebral fractures in old men. Shanbhogue and colleagues, which reported that the reduction of CD31hi/Emcnhi vessels (a special subtype of capillaries that was almost unique to bone) and the concurrent decline of osteoprogenitor cells could potentially offer a compelling explanation for bone loss in mice during aging. Researchers have shown that improved angiogenesis in bone benefits bone formation. For example, platelet-derived growth factor-BB, exogenous or released by preosteoclasts, induces formation of the CD31hi/Emcnhi vessel subtype to promote the coupling of angiogenesis with bone formation; exogenous factors DJ-1 induce osteogenesis and angiogenesis, both of which have properties that are essential for bone regeneration. Our findings suggest that retinal microangiopathy may, as an index of the disturbed bone vascularity (declined CD31hi/Emcnhi vessels and impaired Notch signaling), be a mechanism leading to bone loss by diminished bone formation and, perhaps, excessive bone resorption, which affect BMD and lead to an increased risk for fractures in men aged ≥65 years. Moreover, administration of antiangiogenic drugs are associated with adverse effects in vascular homeostasis and endocrine organs. If our findings are confirmed in other samples, regular ophthalmoscopy and calcaneus quantitative ultrasound are needed among patients with antiangiogenic therapies.

Current research confirms the study by Kusumbe and colleagues, which reported that the reduction of CD31hi/Emcnhi vessels (a special subtype of capillaries that was almost unique to bone) and the concurrent decline of osteoprogenitor cells could potentially offer a compelling explanation for bone loss in mice during aging. Researchers have shown that improved angiogenesis in bone benefits bone formation. For example, platelet-derived growth factor-BB, exogenous or released by preosteoclasts, induces formation of the CD31hi/Emcnhi vessel subtype to promote the coupling of angiogenesis with bone formation; exogenous factors DJ-1 induce osteogenesis and angiogenesis, both of which have properties that are essential for bone regeneration. Our findings suggest that retinal microangiopathy may, as an index of the disturbed bone vascularity (declined CD31hi/Emcnhi vessels and impaired Notch signaling), be a mechanism leading to bone loss by diminished bone formation and, perhaps, excessive bone resorption, which affect BMD and lead to an increased risk for fractures in men aged ≥65 years. Moreover, administration of antiangiogenic drugs are associated with adverse effects in vascular homeostasis and endocrine organs. If our findings are confirmed in other samples, regular ophthalmoscopy and calcaneus quantitative ultrasound are needed among patients with antiangiogenic therapies.

However, the association between retinal microvascular abnormalities and low BMD risk and vertebral fractures has not been found in men aged <65 years and premenopausal and postmenopausal women. It is well known that women after menopause have accelerated bone loss, suggesting that estrogen deficiency plays a major role in this loss, which may

| Table 4. Prevalence and ORs of Vertebral Fracture Among Different Sex Retinopathy Stratified by Diabetes and Hypertension |
|-----------------------------------------------|
| Men retinopathy | Women retinopathy |
| Without | With | Without | With |
| No. without diabetes | 1750 | 43 | 2225 | 59 |
| Prevalence % | 15.5 | 32.6 | 19.1 | 25.4 |
| OR (95% CI)a | 1.0 (ref) 2.194 (1.097, 4.389) | 1.0 (ref) 1.010 (0.545, 1.874) | 0.026 | 0.974 |
| p | | | | |
| No. with diabetes | 406 | 52 | 451 | 72 |
| Prevalence % | 18.0 | 26.9 | 21.1 | 23.6 |
| OR (95% CI)a | 1.0 (ref) 1.358 (0.651, 2.832) | 1.0 (ref) 0.978 (0.501, 1.908) | 0.414 | 0.978 |
| p | | | | |
| No. without hypertension | 1150 | 17 | 1536 | 15 |
| Prevalence % | 15.3 | 23.5 | 18.2 | 33.3 |
| OR (95% CI)a | 1.0 (ref) 1.349 (0.400, 4.551) | 1.0 (ref) 1.625 (0.509, 5.189) | 0.630 | 0.412 |
| p | | | | |
| No. with hypertension | 1006 | 78 | 1140 | 116 |
| Prevalence % | 16.8 | 30.8 | 21.0 | 23.3 |
| OR (95% CI)a | 1.0 (ref) 2.034 (1.163, 3.559) | 1.0 (ref) 0.964 (0.593, 1.568) | 1.358 | 1.625 |
| p | | | | |

*aAll ORs use the without-retinopathy cohort as the reference cohort (ref).

ORs were adjusted for age, baseline BMD, education, physical activity, smoking status, alcohol, coffee, milk, and bean products consumption, dyslipidemia, eGFR <60 mL/min/1.73 m², generalized and abdominal obesity, the new occurrence of smoking status, alcohol, diabetes, hypertension, dyslipidemia, and the change of generalized and abdominal obesity, eGFR <60 mL/min/1.73 m². In addition, adjustment for diabetes when stratified by hypertension and adjustment for hypertension when stratified by diabetes. Bold indicates statistically significant (p < 0.05).
cover the effect of microvascular abnormalities on low BMD. This may partly explain why we fail to detect the association in postmenopausal women.

A strength of this work is that it was a prospective cohorts study, which included a large sample size of both men and women drawn from the general population rather than a specialized sample. The standardized identification of retinal microvascular abnormalities, detailed information collected on a range of risk factors, and potential confounders were collected. Our study examined the different associations of calcaneal BMD and fracture risk with microvascular abnormalities stratified by sex, age, menstrual status, diabetes, and hypertension. Limitations should also be stated. It was indirect to use height loss as a surrogate of vertebral fractures instead of imaging examination, since height loss may be due to weakening of the muscle groups, postural changes, disc degeneration, joint space narrowing, and spinal deformities. Also, the current study used calcaneal ultrasound T-scores, not by conventional dual-energy X-ray absorptiometry. Calcaneal quantitative ultrasound at commonly used cutoff thresholds do not definitively exclude or confirm DXA-determined osteoporosis. Additionally, the inclusion of only persons who are Hans threatens the study's generalizability because patterns of bone density are known to vary by ethnicity. Furthermore, many other variables potentially related to the risk of having low BMD, such as level of vitamin D, sex hormones, PTH, and other unknown or less clearly understood risk factors (for instance, genetic and inflammatory markers), may have played a role or modified the associations that we did not collect or assess because of the financial constraints. In addition, BMD was measured from a single bone site. However, the measurement of different sites of BMD in a multicenter epidemiologic study like this has not proven to be easy or practical, especially in remote mountainous regions.

In summary, our results provide evidence that retinal microvascular abnormalities are an independent risk factor of osteoporosis fracture in elderly men.

Disclosures

All authors state that they have no conflicts of interest.

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Authors’ roles: GC, PZ, JW, KT, FZ, WL, and HR conceived this article. KT and FZ wrote the first draft of the article, with further contributions from GC, PZ, JW, WL, HR, HH, JY, LC, HC, ML, JL, LLI, XG, SP, LLin, JL, YB, WW, and GN. GC (principal investigator), with PZ, JW, and WL, applicants for the study, wrote the study protocol, obtained funding, designed the study, and interpreted the results. KT, LC, HC, ML, XG, and SP were responsible for data collection and delivery. KT, LC, HC, and ML managed data. TK and FZ did the statistical analysis, with support from GC, PZ, JW, and WL. All authors interpreted data, reviewed successive drafts, and approved the final version of the article.

References

1. Lampropoulos CE, Papaioannou I, D’Cruz DP. Osteoporosis—a risk factor for cardiovascular disease? Nat Rev Rheumatol. 2012;8(10):587–98.
2. Fajardo RJ. Is diabetic skeletal fragility associated with microvascular complications in bone? Curr Osteoporos Rep. 2017;15(1):1–8.
3. Ivers RQ, Cumming RG, Mitchell P, Peduto AJ. Diabetes and risk of fracture: the Blue Mountains Eye Study. Diabetes Care. 2001;24(7):1198–203.
4. Trias A, Fery A. Cortical circulation of long bones. J Bone Joint Surg Am. 1979;61(7):1052–9.
5. Kusumbe AP, Ramasamy SK, Adams RH. Coupling of angiogenesis and osteogenesis by a specific vessel subtype in bone. Nature. 2014;507(7492):323–8.
6. Ramasamy SK, Kusumbe AP, Wang L, Adams RH. Endothelial Notch activity promotes angiogenesis and osteogenesis in bone. Nature. 2014;507(7492):376–80.
7. Wong TY, Klein R, Klein BE, Tielsch JM, Hubbard L, Nieto FJ. Retinal microvascular abnormalities and their relationship with hypertension, cardiovascular disease, and mortality. Surv Ophthalmol. 2001;46(1):59–80.
8. Wong TY, Klein R, Couper DJ, et al. Retinal microvascular abnormalities and incident stroke: the Atherosclerosis Risk in Communities Study. Lancet. 2001;358(9288):1134–40.
9. Nayak S, Roberts MS, Greenspan SL. Cost-effectiveness of different screening strategies for osteoporosis in postmenopausal women. Ann Intern Med. 2011;155(11):751–61.
10. Costman F, de Beur SJ, LeBoff MS, et al. Erratum to: Clinician’s guide to prevention and treatment of osteoporosis. Osteoporos Int. 2015;26(7):2045–7.
11. Masunari N, Fujiwara S, Kasagi F, Takahashi I, Yamada M, Nakamura T. Height loss starting in middle age predicts increased mortality in the elderly. J Bone Miner Res. 2012;27(1):138–45.
12. Xu W, Perera S, Medich D, et al. Height loss, vertebral fractures, and the misclassification of osteoporosis. Bone. 2011;48(2):307–11.
13. Moayyeri A, Luben RN, Bingham SA, Welch AA, Wareham NJ, Khaw KT. Measured height loss predicts fractures in middle-aged and older men and women: the EPIC-Norfolk prospective population study. J Bone Miner Res. 2008;23(3):425–32.
14. Briot K, Legrand E, Pouchain D, Monnier S, Roux C. Accuracy of patient-reported height loss and risk factors for height loss among postmenopausal women. CMAJ. 2010;182(6):558–62.
15. Yoshimura N, Kinoshita H, Takijiri T, et al. Association between height loss and bone loss, cumulative incidence of vertebral fractures and future quality of life: the Miyama study. Osteoporos Int. 2008;19(1):21–8.
16. Siminoski K, Warshawski RS, Jen H, Lee K. The accuracy of historical height loss for the detection of vertebral fractures in postmenopausal women. Osteoporos Int. 2006;17(2):290–6.
17. Tobias JH, Hutchison AP, Hunt LP, et al. Use of clinical risk factors to identify postmenopausal women with vertebral fractures. Osteoporos Int. 2007;18(1):35–43.
18. Krego JH, Siminoski K, Adachi JD, Misurski DA, Chen P. A simple method for determining the probability a new vertebral fracture is present in postmenopausal women with osteoporosis. Osteoporos Int. 2006;17(3):379–86.
19. Siminoski K, Jiang G, Adachi JD, et al. Accuracy of height loss during prospective monitoring for detection of incident vertebral fractures. Osteoporos Int. 2005;16(4):403–10.
20. Ismail AA, Cooper C, Felsenberg D, et al. Number and type of vertebral deformities: epidemiological characteristics and relation to back pain and height loss. European Vertebral Osteoporosis Study Group. Osteoporos Int. 1999;9(3):206–13.

21. Huang C, Ross PD, Lydick E, Davis JW, Wasnich RD. Contributions of vertebral fractures to stature loss among elderly Japanese-American women in Hawaii. J Bone Miner Res. 1996;11(3):408–11.

22. Heaney RP. Pathophysiology of osteoporosis. Endocrinol Metab Clin N Am. 1998;27(2):255–65.

23. Losordo DW, Isner JM. Estrogen and angiogenesis: a review. Arterioscler Thromb Vasc Biol. 2001;21(1):6–12.

24. Chen G, Chen L, Wen J, et al. Associations between sleep duration, daytime nap duration, and osteoporosis vary by sex, menopause, and sleep quality. J Clin Endocrinol Metab. 2014;99(8):2869–77.

25. Kanis JA, Melton LJ 3rd, Christiansen C, Johnston CC, Khaltaev N. The diagnosis of osteoporosis. J Bone Miner Res. 1994;9(8):1137–41.

26. Levy JC, Matthews DR, Hermans MP. Correct homeostasis model assessment (HOMA) evaluation uses the computer program. Diabetes Care. 1998;21(12):2191–2.

27. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med. 2009;150(9):604–12.

28. Wang T, Li M, Chen B, et al. Urinary bisphenol A (BPA) concentration associates with obesity and insulin resistance. J Clin Endocrinol Metab. 2012;97(2):E223–7.

29. Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III). JAMA. 2001;285(19):2486–97.

30. WHO Expert Consultation. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. Lancet. 2004;363(9403):157–63.

31. Alberti KG, Zimmet P, Shaw J. The metabolic syndrome—a new worldwide definition. Lancet (London, England). Sep 24–30 2005;366(9491):1059–62. Epub 2005/09/27.

32. Levey AS, Coresh J, Balk E, et al. National Kidney Foundation practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Ann Intern Med. 2003;139(2):137–47.

33. Shanbhogue VV, Hansen S, Frost M, et al. Bone geometry, volumetric density, microarchitecture, and estimated bone strength assessed by HR-pQCT in adult patients with type 1 diabetes mellitus. J Bone Miner Res. 2015;30(12):2188–99.

34. Lecka-Czernik B. Diabetes, bone and glucose-lowering agents: basic biology. Diabetologia. 2017;60(7):1163–9.

35. Xie H, Cui Z, Wang L, et al. PDGF-BB secreted by preosteoclasts induces angiogenesis during coupling with osteogenesis. Nat Med. 2014;20(11):1270–8.

36. Kim JM, Shin HI, Cha SS, et al. DJ-1 promotes angiogenesis and osteogenesis by activating FGF receptor-1 signaling. Nat Commun. 2012;3:1296.

37. Cao Y. VEGF-targeted cancer therapeutics-paradoxical effects in endocrine organs. Nat Rev Endocrinol. 2014;10(9):530–9.

38. Nayak S, Olkin I, Liu H, et al. Meta-analysis: accuracy of quantitative ultrasound for identifying patients with osteoporosis. Ann Intern Med. 2006;144(11):832–41.

39. Ettinger B, Sidney S, Cummings SR, et al. Racial differences in bone density between young adult black and white subjects persist after adjustment for anthropometric, lifestyle, and biochemical differences. J Clin Endocrinol Metab. 1997;82(2):429–34.