Table 1. Background data in control and GC-treated groups

	Control group	GC group	*P*
No. of subjects	132	87	
No. of vertebral fractures	11(8.3%)	30(34.5%)	< 0.001†
Age (years)	53.4 ± 18.4	52.1 ± 16.9	
BMI (kg/m^2)	22.9 ± 3.7	22.4 ± 2.8	
L-BMD (g/cm^2)	0.913 ± 0.158	0.941 ± 0.172	0.216
Z score	-0.41 ± 1.09	-0.31 ± 1.28	0.531
T score	-1.11 ± 1.32	-0.89 ± 1.45	0.257
Current dose of PSL (mg/day)		11.1 ± 9.8	
Maximum dose of PSL (mg/day)		41.5 ± 21.0	
Duration of GC treatment (months)		75 ± 83	

† $P < 0.05$, Chi square test

GC, glucocorticoids; BMI, body mass index; L, lumbar; PSL, prednisolone

254x190mm (96 x 96 DPI)

Table 2. Comparison of various parameters between men with and without vertebral fractures in GC-treated group

GC group	Vertebral fractures		
	No	Yes	*P*
No. of subjects	57	30	
Age (years)	48.7 ± 17.8	58.4 ± 13.5	0.029*
BMI (kg/m^2)	22.4 ± 2.9	22.4 ± 2.6	0.978
L-BMD (g/cm^2)	0.949 ± 0.148	0.927 ± 0.211	0.553
Z score	-0.315 ± 1.149	-0.287 ± 1.513	0.837
Current dose of PSL (mg/day)	9.9 ± 6.9	13.4 ± 13.6	0.420
Maximum dose of PSL (mg/day)	41.3 ± 21.2	41.0 ± 20.7	0.989
Duration of GC treatment (months)	61.8 ± 67.4	96.9 ± 104.8	0.177

* $P < 0.05$, Mann Whitney U test

GC, glucocorticoids; BMI, body mass index; L, lumbar; PSL, prednisolone

254x190mm (96 x 96 DPI)

Table 3. Cut-off values of L-BMD for vertebral fractures

Independent variables	Cut-off value BMD (g/cm^2)	T score (%)	Sensitivity (%)	Specificity (%)
Control group				
L-BMD	0.825	-1.85 (79)	74.0	74.0
GC group				
L- BMD	0.936	-1.03 (89)	53.5	53.5

Cut-off values as well as sensitivity and specificity were calculated by ROC analysis.
GC, glucocorticoids; BMI, body mass index; L, lumbar; PSL, prednisolone

254x190mm (96 x 96 DPI)

Table 4. Association between the presence of vertebral fractures and L-BMD in male control and GC group.

	Presence of vertebral fractures		
Independent variables	OR	(95% CI)	*P*
Control group			
L-BMD	0.26	(0.09-0.74)	0.012
GC group			
L-BMD	0.77	(0.47-1.26)	0.298

Multivariate logistic regression analysis adjusted for age and BMI.
unit of change, per SD increase.
GC, glucocorticoids; OR, odds ratio; CI, confidential intervals; L, lumbar

254x190mm (96 x 96 DPI)

Diabetic patients have an increased risk of vertebral fractures independent of bone mineral density or diabetic complications

Masahiro Yamamoto[1], Toru Yamaguchi[1], Mika Yamauchi[1], Hiroshi Kaji[2], Toshitsugu Sugimoto[1]

[1]Internal Medicine 1, Shimane University Faculty of Medicine, Japan

[2]Division of Endocrinology/Metabolism, Neurology, and Hematology/Oncology, Department of Clinical Molecular Medicine, Kobe University Graduate School of Medicine, Japan

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E-mail address: Masahiro Yamamoto; masa-ya@med.shimane-u.ac.jp

Toru Yamaguchi; yamaguch@med.shimane-u.ac.jp

Mika yamauchi; yamauchi@med.shimane-u.ac.jp

Hiroshi Kaji; hiroshik@med.kobe-u.ac.jp

Toshitsugu Sugimoto; sugimoto@med.shimane-u.ac.jp

Corresponding author:

Masahiro Yamamoto, M.D, Ph.D

Internal Medicine 1, Shimane University Faculty of Medicine,

89-1, Enya-cho, Izumo, Shimane, 693-8501, Japan

Phone: +81-853-20-2183, FAX: +81-853-23-8650

E-mail: masa-ya@med.shimae-u.ac.jp

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Abstract

Introduction:

Although patients with type 2 diabetes (T2DM) have an increased risk of hip fracture, risk of vertebral fracture (VF) and its association with bone mineral density (BMD) are still unclear.

Materials and Methods:

We examined Japanese T2DM patients (161 men older than 50 years and 137 postmenopausal women) and non-DM controls (76 and 622, respectively) by lateral spine radiography as well as dual-energy X-ray absorptiometry at the lumbar spine (L), femoral neck (FN) and radius (R).

Results:

Logistic regression analysis adjusted for age, body mass index and L-BMD showed that the presence of T2DM was an independent risk factor for prevalent VFs in women [odds ratio (OR) = 1.86, $p = 0.019$] as well as men [OR = 4.73, $p < 0.001$]. BMD at any site, however, was not significantly associated with the presence of prevalent VFs in T2DM patients, in contrast to the significant association in controls (at least $p = 0.010$). Comparison of T2DM patients with and without VFs showed no significant differences in BMD values, bone markers, or diabetes status. Receiver operating characteristic analysis showed that the absolute L-, FN-, and R-BMD values for detecting prevalent VFs were higher in T2DM patients than controls, while their sensitivity and specificity were lower.

Conclusion:

T2DM patients may have an increased risk of VFs independent of BMD or diabetic complication status, suggesting that bone quality may define bone fragility in T2DM.

Keywords: type 2 diabetes, vertebral fracture, bone mineral density, osteoporosis

Introduction

Many clinical studies have investigated the association between diabetes and osteoporosis, given that these disorders affect a large proportion of the elderly population. Recent meta-analyses on accumulating studies showed that patients with type 2 diabetes (T2DM) had an increased risk of hip fracture for both genders (1). On the other hand, only a few studies documented the risk of vertebral fractures (VFs) in T2DM patients, and these presented conflicting results (2-4). Thus, it is still unclear whether or not VF risk is increased in T2DM patients. Assessing the risk of VF as well as hip fracture is especially important for men with T2DM, because their age-standardized mortality ratio after either fracture is known to be higher than that of women (5).

A recent meta-analysis also showed that T2DM patients had higher hip BMD than non-DM controls, despite an increased risk of hip fracture (6), suggesting that BMD values may not reflect bone fragility in T2DM. Recently, we also reported that spinal $L_{2\text{-}4}$ (L) BMD was not associated with the presence of prevalent VFs in T2DM women, suggesting that L-BMD was not sensitive enough to assess the risk of VFs in this group (7). T2DM is generally known to be accompanied by the presence of osteophytes and calcification of the abdominal aorta. Orwoll *et al.* suggested that osteophytic calcification exerted an important influence on the measurement of L-BMD in men, because the presence of osteophytes obscured the relationship of BMD to age (8). Liu *et al.* also showed that the prevalence of osteoporosis became lower when estimated by L-BMD instead of by femoral neck (FN) BMD in both genders (9), suggesting that L-BMD was insensitive to the diagnosis of osteoporosis. Thus, it is possible that FN- or radial (R) BMD, not but L-BMD, might be more suitable for evaluating the risk of VFs in T2DM patients.

In order to clarify these issues, we investigated whether the prevalence of VFs in T2DM men or women was higher or lower than in their non-DM counterparts. We also examined whether or not FN- and R-BMD could detect prevalent VFs in T2DM

men or women more efficiently than L-BMD.

Subjects and Methods

Subjects

We consecutively enrolled 298 Japanese patients with diabetes [137 postmenopausal women (age range 46-89 years) and 161 men (age range 50-83 years)] who underwent BMD measurements at the outpatient clinic of Shimane University Hospital. The patients had been referred to our hospital from community clinics for treatment of diabetes. We excluded patients who had higher than the normal range of serum creatinine (normal range for women, 0.44–0.83 mg/dl; men, 0.56–1.23 mg/dl) and higher than 300 mg albumin/g urine creatinine of urinary albumin excretion. We also excluded patients with primary hyperparathyroidism or a history of falls or traffic accidents in order to eliminate the possibility of injury-associated fractures. None of the patients were taking any drugs or hormones that affected bone metabolism, including sex steroids, warfarin and bisphosphonates. For the control group, 622 postmenopausal women and 132 men over 50 years of age (age range 47-88 years and 50-86 years, respectively) without underlying conditions affecting the skeleton were consecutively recruited. None were taking drugs or hormones that affected bone metabolism. Assessment by one-tailed tests ($\alpha = 0.05$) showed that 63 males and 560 females were needed for controls to reach sufficient statistical power ($1-\beta = 0.8$), assuming that the number of diabetic men and women was 150 each and that 30% of them had prevalent VFs. Thus, the number of subjects in each group in this study fulfilled this requirement. Baseline characteristics of all subjects are shown in Table 1. In the diabetic group, 57 (42%), 11 (8%), 37 (27%) and 38 (28%) women and 65 (40%), 15 (9%), 28 (17%) and 24 (15%) men had been taking sulfonylurea, pioglitazone, metformin, and insulin therapies, respectively. Sixty-three women (46%) and 66 men (41%) had diabetic retinopathy, whereas 100 women (73%) and 111 men (69%) had diabetic neuropathy. Eleven women (8%) and 115 men (71%) smoked more than 20 cigarettes per day, and eight (6%) women and 101 (63%) men consumed three or more units per day of alcohol.

This study was cross-sectional, approved by the ethical review board of our institution and in compliance with the Helsinki declaration. All subjects agreed to participate in the study and gave written informed consent.

Biochemical measurements

Fasting blood was obtained and the concentrations of fasting plasma glucose (FPG), hemoglobin A_{1c} (HbA_{1c}) and serum creatinine (Cr) were measured by automated techniques at the central laboratory of our hospital. C-peptide was assayed by radioimmunoassay. Serum bone-specific alkaline phosphatase (BAP) (normal range for men, 13.0-33.9 U/L; women, 9.6-35.4 U/L) and urinary levels of N-telopeptide (uNTX) (normal range for men, 13.0-66.2 nmolBCE/mmol · Cr; postmenopausal women, 14.3-89.0 nmolBCE/mmol·Cr) were commercially measured with specific antibodies against BAP and trivalent peptides derived from NTX, respectively, using enzyme–linked immunosorbent assays (ELISAs).

BMD measurements

BMD values of the L, the FN and one-third of the R (1/3R) were measured by dual-energy X-ray absorptiometry (DXA) using the QDR-4500 system (Hologic, Waltham, MA). BMD was automatically calculated from the bone area (cm^2) and bone mineral content (BMC, g) and expressed as an absolute value (g/cm^2). Values were also expressed relative to the standard deviation (SD) of age- and sex-matched normal Japanese mean values of BMD provided by the manufacturer (BMD Z score) (10). The coefficients of variation of measurement of the L-, FN- and R-BMD were 1.0, 1.0 and less than 1%, respectively.

Ascertainment of fractures

In all subjects, conventional thoracic and spinal radiographs in lateral and antero-posterior projections were obtained. We defined VFs as grades 1-3 according to

the classification by Genant et al (11). A VF was diagnosed if a reduction of 20% or more was observed by two investigators who were blinded to each other's reading. If judgment of VFs did not agree, the film was independently reassessed. If the re-evaluated findings were again different, we regarded that case as a non-fracture.

Statistical analysis

All data are expressed as the mean ± SD for each index. An unpaired t-test was used to compare parameters between subjects with and without VFs. Comparisons of categorical variables were made using chi-square test. To compare the strength of association between prevalent VFs and BMD values at each measurement point, we analyzed the area under the receiver operating characteristic (ROC) curve (12). For each of the groups with or without VFs and for each of the BMD measurements at the L, FN and R sites, possible cut-off points for BMD were defined, and the proportion of subjects with fractures below these points (the sensitivity) and the proportion of subjects without fractures above these points (the specificity) were calculated. This yielded an ROC curve that displayed the relationship between sensitivity and specificity for BMD at each skeletal site as a discriminator between the normal and fracture groups. Logistic regression analysis was performed using the statistical computer program StatView (Abacus Concept, Berkeley, CA). P values less than 0.05 were considered significant.

Results

Background data are shown in Table 1. There were 43 women (31.4%) and 61 men (37.9%) with VFs among the T2DM patients. T2DM women were significantly older, shorter in height, and heavier in weight than non-DM controls ($p < 0.01$). L-, FN-, and 1/3R-BMD values and their Z scores in T2DM women and men were significantly higher than in their non-DM counterparts ($p < 0.01$), except for the Z score of 1/3R in men. Multivariable logistic analysis was performed with the presence of vertebral fractures as a dependent variable and the presence of T2DM adjusted for age, body

mass index (BMI), and L-BMD as an independent variable. The results revealed that the presence of T2DM was a risk factor for prevalent VFs with odds ratios of 1.86 in women and 4.73 in men (Table 2).

Next, we compared demographic and biochemical parameters between T2DM subjects with and without VFs in both genders (Tables 3 and 4). T2DM women with VFs were significantly older than those without VFs ($p = 0.001$). T2DM men with VFs were significantly older, shorter in height, and had higher values of fasting C-peptide than those without VFs ($p = 0.003$, $p = 0.021$ and $p = 0.032$, respectively). There were no significant differences in BMD at any site, biochemical parameters, or the percentage of people with DM complications, habitual smoking, or alcohol consumption between those with and without VFs in either gender.

To clarify the association between VFs and BMD, we calculated cut-off values of BMD for VFs by ROC analysis (Table 5). The cut-off values of L-, FN-, and 1/3R-BMD for VFs in T2DM women and men were higher than those of non-DM controls, while the sensitivity and specificity for VFs in T2DM patients were lower than those in controls. Multivariable logistic analysis was performed with the presence of VFs as a dependent variable and each BMD value of L, N and 1/3R adjusted for age and BMI as a linear independent variable. Analyses of T2DM patients were also adjusted for HbA_{1c}, fasting C-peptide, Cr, duration of diabetes, presence of diabetic retinopathy or neuropathy, diabetic therapies (e.g., sulfonylurea, metformin, pioglitazone or insulin), risk factors for osteoporosis (e.g., smoking and habitual alcohol drinking) and history of non vertebral fractures. The results showed that L-, FN- and 1/3R-BMD values were not significantly associated with the presence of prevalent VFs in either T2DM women or men, in contrast to their significant associations in controls (at least $p = 0.010$) (Table 6). In T2DM patients, these DM-related conditions as well as risk factors for osteoporosis were not identified as risk factors associated with the presence of VFs in either gender (data not shown).

Discussion

The present study showed that the presence of T2DM in both genders was significantly associated with the presence of prevalent VFs, in spite of higher BMD values in the DM subjects than in controls. A recent meta-analysis showed that pooled estimates for L- and FN-BMD Z scores were increased in T2DM patients (6), and we confirmed this observation. Although accumulating studies have shown that hip fracture risk is increased in T2DM patients compared to non-DM subjects (1,6), only a few studies have investigated VF risk in T2DM patients. Vestergaard *et al.* reported that the relative risk of VFs in 9,598 T2DM patients was 1.34 (2), while Hanley *et al.* reported that the relative risk in 347 T2DM women and 182 T2DM men was 0.92 and 0.77, respectively (3). Gerdhem *et al.* documented that the ratio was 0.52 in 74 T2DM women (4). Thus, these studies presented conflicting results about VF risk in T2DM patients. We found that the relative risk of VF in 137 T2DM women and 161 T2DM men was 1.70 and 5.66, respectively, which seems to agree with the large-scale study by Vestergaard *et al.* (2).

We previously showed that L-BMD was not associated with the presence of prevalent VFs in T2DM women (7). In the present study, we also showed that not only L-BMD but also FN- and 1/3R-BMD were not significantly different between T2DM patients with and without VFs. In fact, BMD values at any site in T2DM patients were not identified as a risk factor for VFs in either gender by logistic regression analyses. These findings suggest that the insensitivity of L-BMD for the detection of VFs in T2DM may not be because L-BMD is affected by the presence of osteophytes or aortic calcification, but instead because bone fragility underlying VFs in T2DM is independent of BMD. Thus, we could extend our previous observation (7) to T2DM men as well as to FN- and R-BMD.

It is documented that poor nerve function is a cause of falls (13) and that an increased risk of non-VFs is associated with DM retinopathy, longer DM duration and insulin treatment (14). Thus, these DM-related complications and conditions might

partly explain the BMD-independent increased VF risk in our subjects. VF risk in T2DM, however, was not associated with the duration of diabetes or the presence of diabetic retinopathy or neuropathy by multivariable logistic analysis, suggesting that either BMD or diabetic complications were not associated with the increased VF risk in our T2DM populations. Recently, Saito *et al.* reported that increases in pentosidine content in bone in rats with spontaneous DM were linked to impaired mechanical properties in spite of normal BMD (15). We have also reported that serum pentosidine levels were positively associated with the presence of prevalent VFs in T2DM women (16). Pentosidine, one of the well known advanced glycation end-products, might change collagen properties by decreasing the hydroxylysine residues of collagen fibrils, which are necessary for the formation of intermolecular cross-links between collagen molecules. Since lysine is the source of hydroxylysine residues as well as pentosidine synthesis, the process of pentosidine synthesis leads to decreased hydroxylysine residues, shortening fibril diameter and decreasing the number of pyridinium crosslinks formed in collagen (17). Although we found the association between serum pentosidine and VFs only in T2DM women (16), these impaired mechanical properties of bone might partly explain the mechanism by which diabetic conditions lead to deterioration in bone strength despite higher BMD, presumably through decreasing bone quality.

Deterioration of diabetic conditions such as hyperglycemia and glucosuria was positively correlated with urinary excretion rates of calcium (18). McNair P et al. showed that intact PTH secretion in patients with diabetes was lower than that of controls and that low intact PTH levels were correlated with high glucosuria and significantly associated with a higher urinary calcium excretion rate (19). These observations suggest that parathyroid function in diabetes may affect bone metabolism with a negative net calcium balance and could be another causative factor for bone deteriorations in T2DM.

Insulin receptor sustrate-1 (IRS-1) and IRS-2 knockout mice have impaired bone healing (20) and osteopenia (21), implying that insulin is an anabolic agent for

bone. Insulin like growth factor -I (IGF-I), which is also an important anabolic regulator for bone cell function (22), requires insulin for hepatic expression and generation (23,24). Patients with type 1 diabetes (T1DM), whose chief pathogenesis is depletion of insulin secretion, have lower IGF-I level than non-diabetics (25) and decreased BMD Z scores in the spine and hip (6). In contrast, T2DM patients are characterized by insulin resistance accompanied by obesity, and they usually show higher serum levels of insulin and IGF-I than T1DM (25). The anabolic effects of these hormones on bone may explain the relatively maintained BMD in T2DM compared to T1DM. In clinical studies, we and others showed that serum IGF-I levels were positively correlated with BMD in non-DM populations (26-29) and that the hormone level was associated with reduced risk of VFs in postmenopausal women (26). In T2DM women, however, we previously showed that serum IGF-1 level was not significantly correlated with BMD, while its increase was associated with reduced VF risk (30). These findings also suggest that there may be dissociation between BMD and VF risk in T2DM.

Bone metabolic markers such as serum C-terminal cross-linked telopeptide of type I collagen and serum osteocalcin were reported to be decreased in elderly T2DM women in spite of their higher L- and FN-BMD values compared to non-DM women (4). Although low bone turnover could slow the rate of bone loss and make BMD higher than expected for age, it could also increase bone fragility because of the inability to repair accumulated damage. Bone turnover, however, may not be linked to bone fragility in our subjects since bone metabolic markers were not significantly different between T2DM patients with and without VFs.

This study showed that VF risk was relatively higher in T2DM men than in T2DM women. It is important to identify secondary causes of bone loss such as hypogonadism and steroid use in male osteoporosis, because these conditions may underlie in over 50% of men with symptomatic VFs (31). Several case control studies have shown that the relative risk of VFs in men is significantly increased with smoking, alcohol consumption, anticonvulsant treatment, physical inactivity and low free

androgen index (32,33). Cross-sectional studies have found that 21 to 64 % of T2DM men have hypogonadism, with higher prevalence rates in the elderly (34,35). Recently, large-cohort studies showed that T2DM men also had a higher relative risk for hip fractures than non-DM men (36,37). Thus, although we did not measure testosterone levels in our subjects and have no data, the presence of DM may cause hypogonadism and be one of the risk factors of secondary osteoporosis, especially in elderly men.

This study had some limitations. First, the sample size was not large enough to draw any definite conclusions. Second, we analyzed only subjects who attended our university hospital, a tertiary care center, for evaluation or treatment of T2DM. Therefore, the patients enrolled in this study might have had relatively severe level of T2DM and might not have been representative of normal Japanese T2DM patients. Third, subjects in this study received several DM treatments that affected bone mass and fracture risk. Thiazolidinedione may cause bone loss (38,39), while use of sulfonylurea and metformin has been associated with a significantly decreased risk of any fractures (2). Therefore, we were unable to totally exclude the effects of these drugs when estimating VF risk in our T2DM patients. We found that the results were almost the same, however, if we analyzed the data after excluding patients on pioglitazone (data not shown). Fourth, we did not measure vitamin D levels in the subjects, although vitamin D insufficiency is quite common and has implications for bone health and fracture risk (40-42). Finally, we found that there were significant differences in age and BMI between female controls and T2DM patients in this study. Although analyses were adjusted for these confounders, statistical adjustments may not be perfect, and these differences might affect the outcome of the study.

In conclusion, we found that T2DM patients had an increased risk for VFs independent of BMD or DM complications. We also found that not only L-BMD but also FN- and R-BMD were not sensitive enough to assess the risk of VFs in T2DM since none of these BMD values were significantly associated with the presence of VFs. Thus, bone quality, not reflected by BMD, seems to play an important role in

determining bone strength in T2DM patients.

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