Serum Pentosidine Levels Are Positively Associated with the Presence of Vertebral Fractures in Postmenopausal Women with Type 2 Diabetes

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Context: Although type 2 diabetic patients are at increased risk of fractures, bone mineral density (BMD) may not be useful for assessing the risk. Recent studies have reported that increased bone content of pentosidine (PEN) is associated with its plasma concentration and bone fragility.

Objective and Methods: To examine the association between serum PEN levels and vertebral fractures (VFs) in Japanese type 2 diabetic patients (77 males older than 50 yr and 76 postmenopausal females), we compared parameters including BMD, PEN, serum bone-specific alkaline phosphatase, and urinary levels of N-telopeptide between those with and without VFs.

Results: Comparison of diabetic subjects with and without VFs revealed no significant differences in BMD values or bone metabolic markers in either gender. In contrast, PEN levels in women with VFs were significantly higher than in those without VFs (0.0440 ± 0.0136 *vs.* 0.0321 ± 0.0118 μg/ml; $P < 0.001$). Multivariate logistic regression analysis adjusted for age, height, weight, hemoglobin A1c, estimated glomerular filtration rate, the presence of diabetic complications, histories of taking insulin or pioglitazone, risk factors for osteoporosis, and lumbar BMD identified PEN levels as a factor associated with the presence of VFs in postmenopausal diabetic women independent of BMD, risk factors for osteoporosis, diabetic status, and renal function (odds ratio 2.50, 95% confidential interval 1.09–5.73 per SD increase; $P = 0.0302$).

Conclusion: PEN levels, but not BMD, may be useful for assessing the risk of prevalent VFs in postmenopausal diabetic women and may reflect bone quality in this group. (***J Clin Endocrinol Metab*** **93: 1013–1019, 2008)**

The association between diabetes and osteoporosis has been extensively investigated because both disorders affect a large proportion of elderly people. Type 1 diabetes is associated with decreased bone mineral density (BMD) in the hip (1–4) and lumbar spine (2–4) and increased risks of fractures in the hip (5–7) and vertebra (7). In contrast, patients with type 2 diabetes mellitus have an increased risk of fractures (2, 8), although they have higher BMD than healthy subjects. We recently reported that lumbar BMD was not significantly associated with the presence of vertebral fractures in female diabetic patients and was not sensitive enough to assess the risk of vertebral fractures in the group (9). Because bone strength reflects the integration of bone density and bone quality, these findings suggested that patients with type 2 diabetes might have poor bone quality not defined by BMD. However, the nature of bone quality in patients with type 2 diabetes is still unclear.

Advanced glycation end products (AGEs) are generated by the sequential nonenzymatic glycosylation of protein amino groups (10) and accumulate in various tissues including kidney and coronary arteries (11), resulting in the development of diabetic vascular complications (11). Accumulation of AGEs has also been observed in bone and has been associated with deterioration of bone mechanical properties (12, 13). Pentosidine is one of the well-known AGEs, and the concentrations of pentosidine in cortical and trabecular bone are negatively associated with bone strength (12–14). Clinically, patients with femoral

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Abbreviations: AGE, Advanced glycation end product; BAP, bone-specific alkaline phosphatase; BCE, bone collagen equivalent; BMD, bone mineral density; BUN, blood urea nitrogen; CI, confidential interval; Cr, creatinine; FPG, fasting plasma glucose; GFR, glomerular filtration rate; HbA_{1c}, hemoglobin A_{1c}; OR, odds ratio; uNTX, urinary levels of N-telopeptide.

neck fractures had higher concentrations of pentosidine in cortical (15) and cancellous bone (16) than nonfracture controls.

We have recently shown that the combination of high glucose and AGEs could additionally or synergistically inhibit osteoblastic mineralization through glucose-induced increases in expression of receptors for AGE *in vitro* (17). In patients with diabetes, serum concentrations of pentosidine were shown to be significantly higher than those in control subjects (18). It has recently been reported that plasma pentosidine has a significant linear correlation with cortical bone pentosidine (19). Saito *et al.* (20) reported that increases in pentosidine content in bone from spontaneous diabetic rats were linked to impaired mechanical properties despite normal BMD. However, it is still unclear whether serum pentosidine level is associated with bone fragility in diabetic patients. In this study, to examine this issue, we compared serum pentosidine levels between diabetic patients with and without vertebral fractures and evaluated their association with the risk of vertebral fractures.

Subjects and Methods

Subjects

We enrolled 77 male and 76 postmenopausal female Japanese patients with type 2 diabetes (age range 50–80 and 50–85 yr; mean 65.3 and 66.7 yr, respectively), who underwent BMD measurements at the lumbar spine, femoral neck, and radius, of a total of 134 male and 119 female diabetic patients 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 (male, 0.56–1.23 mg/dl; female, 0.44–0.83 mg/dl) and higher than 300 mg/d of urinary albumin excretion. We also excluded patients with primary hyperparathyroidism and histories of falls or traffic accidents to eliminate the possibility of injury-associated fractures. We defined the onset of diabetes mellitus as the first time when glucosuria or hyperglycemia was found. None were taking any drugs or hormones that affect bone metabolism, including sex steroids, glucocorticoids, warfarin, and bisphosphonates. Baseline characteristics of subjects are shown in Table 1. Among diabetic patients, 12 males (16%) and 13 females (17%) had histories of nonvertebral fractures, but none had histories of Colles fractures or femoral neck fractures. Twenty-eight (36%), six (8%), and 11 (14%) males as well as 28 (37%), nine (12%), and 20 (26%) females had been taking sulfonylurea, pioglitazone, and insulin therapies, respectively. Thirty-two males (42%) and 40 females (53%) had diabetic retinopathy, whereas 46 males (60%) and 58 females (76%) had diabetic neuropathy. Fifty-six males (73%) and seven females (9%) smoked more than 20 cigarettes per day, and 49 males (64%) and 5 females (7%) consumed more than 1 U/d of alcohol. This study was cross-sectional and approved by the ethical review board of our institution and complied 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}), blood urea nitrogen (BUN), serum creatinine (Cr), and serum calcium were measured by automated techniques at the central laboratory of our hospital. Glomerular filtra-

TABLE 1. Background data of male and female diabetic patients

Variables	Male	Female
No. of subjects	77	76
No. of subjects with vertebral fractures	28 (36.4%)	20 (26.3%)
No. of subjects with nonvertebral fractures	12 (15.9%)	13 (17.1%)
Age (yr)	65.3 ± 8.0	66.7 ± 9.5
Body height (cm)	164.8 ± 6.3	150.8 ± 5.6
Body weight (kg)	61.7 ± 10.9	56.5 ± 11.4
BMI (kg/m^2)	22.7 ± 3.6	24.9 ± 4.8
L_{2-4} BMD (g/cm^2)	1.057 ± 0.182	0.876 ± 0.183
T score	0.1 ± 1.5	−1.2 ± 1.7
Z score	0.6 ± 1.1	0.6 ± 1.1
Neck BMD (g/cm^2)	0.768 ± 0.113	0.645 ± 0.121
T score	−0.8 ± 0.9	−1.3 ± 1.1
Z score	1.0 ± 0.1	0.6 ± 1.1
Radius one third BMD (g/cm^2)	0.708 ± 0.057	0.534 ± 0.096
T score	−2.0 ± 1.1	−2.4 ± 1.9
Z score	−0.9 ± 1.0	0.7 ± 1.7
BUN (mg/dl)	15.4 ± 4.1	15.2 ± 4.2
Estimated GFR (ml/min)	87.6 ± 29.5	86.0 ± 32.1
Serum calcium (mg/dl)	9.1 ± 0.3	9.2 ± 0.3
FPG (mg/dl)	174.5 ± 60.2	168.5 ± 49.7
HbA_{1c} (%)	9.1 ± 2.5	9.1 ± 1.8
Fasting C-peptide (ng/ml)	1.47 ± 0.68	1.60 ± 0.75
BAP (U/liter)	26.2 ± 7.8	31.6 ± 10.3
uNTX (nmol BCE/mmol Cr)	35.0 ± 20.6	57.1 ± 36.1
Pentosidine (μg/ml)	0.0366 ± 0.0125	0.0353 ± 0.0133
Duration of diabetes (yr)	13.2 ± 9.3	13.7 ± 9.8
OHA/pioglitazone/insulin	28/6/11	28/9/20
Diabetic retinopathy/neuropathy	32/46	40/58
Smoking/alcohol	56/49	7/5
Duration of postmenopausal state (yr)		18.0 ± 9.6

BMI, Body mass index; OHA, oral hypoglycemic agents.

tion rate (GFR) was estimated by a Cockcroft-Gault equation. Serum C-peptide was assayed by RIA. Serum bone-specific alkaline phosphatase (BAP) (normal range: males, 13.0–33.9 U/liter; females, 9.6–35.4 U/liter) and urinary levels of N-telopeptide (uNTX) (males, 13.0–66.2 nmol bone collagen equivalent (BCE)/mmol·Cr; postmenopausal females, 14.3–89.0 nmol BCE/mmol·Cr) were commercially measured with specific antibody against BAP and trivalent peptides derived from N-telopeptide, respectively, by ELISAs.

Serum pentosidine levels were detected using a competitive ELISA kit (FSK pentosidine ELISA kit; Fushimi Pharmaceutical, Kagawa, Japan) as previously described (21). In brief, proteolytic enzyme was used to expose the pentosidine molecule that is bound to plasma proteins. Sample was added to pronase and incubated at 55 C for 1.5 h. After the reaction, the mixture was heated in boiling water for 15 min to inactivate the enzyme. Pentosidine antibody and pretreated sample were added to each well and incubated at 37 C for 1 h after washing. Peroxidase-labeled goat antirabbit IgG polyclonal antibodies were added and incubated for 1 h at room temperature. A color development reagent was added to each well. The reaction was stopped 10 min later. The absorbance was measured within 10 min at 450 and 630 nm (main and reference wavelength, respectively). The inter- and intraassay coefficients of variations of absorbance were 6.6 and 8.0%, respectively. Reference concentration of serum pentosidine in normal subjects is 0.0261 ± 0.0007 μg/ml. This ELISA was highly correlated with the conventional HPLC method (r = 0.9356) (21).

BMD measurements

BMD values of the lumbar spine, femoral neck, and one third of the radius were measured by dual-energy x-ray absorptiometry using the QDR-4500 DXA system (Hologic Inc., Waltham, MA). These BMD values were automatically calculated from the bone area (square centimeters) and bone mineral content (grams) and expressed as an absolute value in grams per square centimeter. Z score indicates deviation from the normal age- and sex-matched mean in SD (22). The coefficients of variation (precision) of measurements of the lumbar spine, neck, and radius one third were 1.0, 1.0, and less than 1%, respectively.

Ascertainment of fractures

In all subjects, conventional thoracic and spinal radiographs in lateral and anteroposterior projections were obtained. We defined vertebral fractures as grades 1–3 according to the classification by Genant *et al.* (23). Grade 1 corresponds to a 20–25% reduction in at least one of anterior, middle, and posterior heights along the length of the same vertebrae, compared with the height of the nearest uncompressed vertebral body. Grade 2 corresponds to a 25–40% reduction in any height, and grade 3 corresponds to a more than 40% reduction in any height. A vertebral fracture was diagnosed if 20% or more reduction was observed by two investigators who were blinded to each other's readings. If judgment of vertebral fractures did not agree, the film was independently reassessed. If the reevaluated findings were again different, we regarded that case as nonfracture.

Statistical analysis

All parameters are expressed as mean ± SD for each group. The unpaired *t* test was used to compare parameters between subjects with and without vertebral fractures. Logistic regression analysis was performed using the statistical computer program StatView (Abacus Concepts, Inc., Berkeley, CA). $P < 0.05$ was considered significant.

Results

Background data are shown in Table 1. There were 28 males (36.4%) and 20 females (26.3%) with diabetes who had vertebral fractures. Serum pentosidine values in male (0.0366 ± 0.0125 μg/ml) and female (0.0353 ± 0.0133 μg/ml) patients were higher than that of normal subjects previously reported (0.0261 ± 0.0007 μg/ml) (18), but there was no significant difference between the sexes ($P = 0.649$). As shown in Table 2, simple regression analysis revealed that serum pentosidine levels were significantly increased with age in diabetic females (r = 0.319, $P = 0.006$). There were no correlations between serum pentosidine levels and body weight, body height, BMD at any site, renal function, bone metabolic markers, or duration of diabetes in either gender. When multiple regression analysis was performed with serum pentosidine levels as a dependent variable and age, body weight, body height, HbA_{1c}, GFR, fasting C-peptide, and duration of diabetes, L2–4 BMD, and duration of postmenopausal state (if female) as independent variables, age in females was positively correlated with serum pentosidine (r = 0.619, $P = 0.035$) but did not correlate in males (r = 0.045, $P = 0.772$).

Next, we compared biochemical parameters including serum pentosidine levels between diabetic subjects with and without vertebral fractures in both genders (Tables 3 and 4). Diabetic men with vertebral fractures were significantly older ($P = 0.009$) and shorter ($P = 0.036$) than those without fractures (Table 3). Diabetic women with vertebral fractures were significantly older ($P = 0.003$), had higher serum pentosidine levels ($P < 0.001$) and had a longer duration of both diabetes ($P = 0.023$) and the postmenopausal state ($P = 0.021$) than those without fractures (Table 4). There were no differences in BMD at any site or in biochemical parameters including bone metabolic markers between those with and without fractures in either gender.

When multivariable logistic regression analysis was performed with the presence of vertebral fractures as a dependent variable and serum pentosidine levels adjusted for age, body weight, body height, HbA_{1c}, estimated GFR, duration of diabetes, duration of postmenopausal state (if female), the presence of diabetic retinopathy or neuropathy, histories of using insulin or

TABLE 2. Simple regression analysis between pentosidine and independent variables.

Variables	Male		Female	
	r	*P*	r	*P*
Age (yr)	0.125	0.282	0.319	0.006[a]
Height (cm)	−0.117	0.316	0.064	0.587
Body weight (kg)	0.012	0.921	−0.023	0.844
L_{2-4} BMD (g/cm^2)	0.182	0.116	−0.013	0.913
Neck BMD (g/cm^2)	0.187	0.110	0.028	0.813
Radius one third BMD (g/cm^2)	−0.008	0.946	−0.119	0.322
BUN (mg/dl)	0.056	0.630	−0.084	0.479
Estimated GFR (ml/min)	−0.100	0.390	−0.163	0.160
Serum calcium (mg/dl)	0.201	0.081	0.004	0.975
FPG (mg/dl)	0.065	0.579	−0.045	0.704
HbA_{1c} (%)	−0.008	0.943	−0.057	0.630
Fasting C-peptide (ng/ml)	−0.130	0.274	−0.054	0.658
BAP (U/liter)	−0.049	0.673	−0.083	0.485
uNTX (nmol BCE/mmol Cr)	−0.020	0.866	0.176	0.133
Duration of diabetes (yr)	0.169	0.145	0.092	0.433
Duration of postmenopausal state (yr)			0.211	0.071

[a] $P < 0.01$.

TABLE 3. Comparison of various parameters between male diabetic patients with and without vertebral fractures

	Vertebral fractures		
	(−)	(+)	*P*
No. of subjects	49	28	
Age (yr)	63.5 ± 7.6	68.4 ± 7.4	0.009[a]
Body height (cm)	165.9 ± 6.2	162.8 ± 5.9	0.036[b]
Body weight (kg)	62.2 ± 11.2	60.9 ± 10.5	0.613
BMI (kg/m^2)	22.6 ± 3.7	22.9 ± 3.4	0.690
L_{2-4} BMD (g/cm^2)	1.069 ± 0.193	1.035 ± 0.163	0.432
T score	0.2 ± 1.6	−0.1 ± 1.4	0.431
Z score	0.6 ± 1.2	0.5 ± 0.9	0.674
Neck BMD (g/cm^2)	0.768 ± 0.108	0.769 ± 0.124	0.982
T score	−0.8 ± 0.9	−0.7 ± 1.0	0.862
Z score	0.2 ± 1.0	0.5 ± 1.0	0.309
Radius one third BMD (g/cm^2)	0.714 ± 0.054	0.697 ± 0.061	0.211
T score	−1.9 ± 1.0	−2.2 ± 1.1	0.341
Z score	−0.9 ± 1.0	−0.9 ± 1.1	0.914
BUN (mg/dl)	15.7 ± 3.0	14.7 ± 5.5	0.320
Estimated GFR (ml/min)	88.5 ± 30.1	86.1 ± 28.8	0.732
Serum calcium (mg/dl)	9.2 ± 0.3	9.1 ± 0.3	0.303
FPG (mg/dl)	174.5 ± 61.2	174.4 ± 59.5	0.992
HbA_{Ic} (%)	9.17 ± 2.74	8.88 ± 2.02	0.629
Fasting C-peptide (ng/dl)	1.44 ± 0.78	1.51 ± 0.50	0.687
BAP (U/liter)	26.7 ± 7.5	25.5 ± 8.3	0.542
uNTX (nmol BCE/mmol Cr)	36.5 ± 19.7	32.3 ± 22.3	0.404
Pentosidine (μg/ml)	0.0367 ± 0.0133	0.0364 ± 0.0113	0.909
Duration of diabetes (yr)	12.7 ± 9.2	14.2 ± 9.4	0.492
Pioglitazone	4	2	0.872
Diabetic retinopathy	20	12	0.861
Diabetic nephropathy	30	16	0.725
Smoking	35	21	0.735
Alcohol	31	18	0.929
Nonvertebral fractures	10	2	0.123

BMI, Body mass index.

[a] $P < 0.01$, unpaired *t* test.

[b] $P < 0.05$, unpaired *t* test.

pioglitazone, risk factors for osteoporosis (smoking and habitual alcohol drinking), histories of nonvertebral fractures, and lumbar BMD, serum pentosidine levels were identified as an independent factor associated with the presence of vertebral fractures in females [odds ratio (OR) 2.50, 95% confidential interval (CI) 1.09–5.73 per SD increase, $P = 0.0302$] but not in males (Table 5). The association between serum pentosidine levels and the presence of vertebral fractures in females was still significant after replacing lumbar BMD with femoral neck (OR 2.65, 95% CI 1.15–6.11, $P = 0.0225$) or radius one third BMD (OR 2.55, 95% CI 1.11–5.88, $P = 0.0227$). There was no association between serum pentosidine levels and gender by logistic analysis adjusted for the above variables (data not shown).

Discussion

The present study showed that serum pentosidine levels were positively and significantly associated with the presence of vertebral fractures in postmenopausal women with diabetes and that this association was independent of BMD. Pentosidine is a well-known AGE, which was a fluorescent protein cross-link discovered from aging human extracellular matrix and could be synthesized *in vitro* by the reaction of ribose, lysine, and arginine (24, 25) (Fig. 1). HbA_{1c} is also one of the early-stage glycation products used to evaluate metabolic control in diabetic patients and reflects the extent of exposure to glucose in the 4–8 wk before testing. HbA_{1c} levels after a 10-month period of insulin therapy decreased to near-normal range, whereas serum pentosidine levels were not normalized (26), indicating that serum pentosidine levels were not affected by short-term changes in diabetic metabolic conditions. Furthermore, pentosidine levels in skin of diabetic patients were shown to be higher than that of controls at baseline and were reduced by only 9% after 5 yr of intensive insulin therapy (27). Taken together, these studies suggested that blood levels or tissue content of pentosidine was a more stable marker not influenced by glycemic control (18, 26). Thus, the difference in serum pentosidine levels between diabetic postmenopausal female subjects with and without vertebral fractures in this study seemed not to arise from short-term changes in glycemic control.

It is possible that diabetes progression and risk factors for osteoporosis might cause bone fragility. Ivers *et al.* (28) reported that a significantly increased risk of nonvertebral fractures was associated with diabetic retinopathy, longer diabetes duration, and a history of insulin treatment. Recently thiazolidinedione,

TABLE 4. Comparison of various parameters between female diabetic patients with and without vertebral fractures

	Vertebral fractures		
	(−)	(+)	*P*
No. of subjects	56	20	
Age (yr)	64.8 ± 9.0	72.0 ± 8.8	0.003[a]
Body height (cm)	151.5 ± 5.9	148.7 ± 4.4	0.058
Body weight (kg)	56.7 ± 11.0	56.1 ± 12.4	0.829
BMI (kg/m^2)	24.7 ± 4.6	25.3 ± 5.2	0.660
L_{2-4} BMD (g/cm^2)	0.888 ± 0.182	0.845 ± 0.189	0.380
T score	−1.1 ± 1.7	−1.5 ± 1.7	0.402
Z score	0.6 ± 1.1	0.6 ± 1.1	0.938
Neck BMD (g/cm^2)	0.654 ± 0.130	0.621 ± 0.092	0.311
T score	−1.2 ± 1.2	−1.5 ± 0.8	0.313
Z score	0.6 ± 1.2	0.6 ± 0.9	0.949
Radius one third BMD (g/cm^2)	0.545 ± 0.101	0.504 ± 0.074	0.104
T score	−2.2 ± 1.9	−3.1 ± 1.4	0.060
Z score	0.7 ± 1.8	0.6 ± 1.3	0.890
BUN (mg/dl)	15.2 ± 4.3	15.1 ± 4.1	0.910
Serum Cr (mg/dl)	0.60 ± 0.13	0.63 ± 0.14	0.333
estimated GFR (ml/min)	89.8 ± 32.2	75.4 ± 30.3	0.085
FPG (mg/dl)	167.5 ± 51.7	171.4 ± 44.5	0.767
HbA1c (%)	9.16 ± 1.90	9.03 ± 1.19	0.791
Fasting C-peptide (ng/dl)	1.59 ± 0.75	1.63 ± 0.75	0.834
BAP (U/liter)	31.3 ± 9.3	32.5 ± 13.1	0.671
uNTX (nmol BCE/mmol Cr)	56.1 ± 32.2	59.8 ± 47.0	0.705
Pentosidine (μg/ml)	0.0321 ± 0.0118	0.0440 ± 0.0136	<0.001[a]
Duration of diabetes (yr)	12.2 ± 8.8	18.0 ± 11.4	0.023[b]
Duration of postmenopausal state (yr)	16.5 ± 9.0	22.2 ± 9.9	0.021[b]
Pioglitazone	6	3	0.611
Diabetic retinopathy	27	13	0.711
Diabetic nephropathy	43	15	0.197
Smoking	6	1	0.448
Alcohol	5	0	0.167
Nonvertebral fractures	10	3	0.711

BMI, body mass index.

[a] $P < 0.01$, unpaired *t* test.

[b] $P < 0.05$, unpaired *t* test.

one of the therapeutic drugs for diabetes, has been reported to cause bone loss (29), suggesting that this agent might increase the risk of fractures. In this study, however, multiple logistic regression analysis revealed that serum pentosidine levels were associated with the presence of vertebral fractures independent of diabetes complications, duration of diabetes, insulin or pioglitazone therapies, or known risk factors for osteoporosis such as smoking and habitual alcohol drinking, duration of postmenopausal state, or histories of nonvertebral fractures.

TABLE 5. Associations between the presence of vertebral fractures and serum pentosidine levels in male and female diabetic patients

	Presence of vertebral fractures		
Independent variables	**OR**	**95% CI**	***P***
Pentosidine			
Male	0.79	0.41–1.52	0.4746
Female	2.50	1.09–5.73	0.0302[a]

Serum pentosidine levels were adjusted for age, body weight, body height, HbA_{1c}, estimated GFR, duration of diabetes, duration of postmenopausal state (if female), the presence of diabetic retinopathy or neuropathy, histories of using insulin or pioglitazone, risk factors for osteoporosis (smoking and habitual alcohol drinking), histories of nonvertebral fractures, and lumbar BMD. Unit of change: SD per increase.

[a] $P < 0.05$.

Several studies have shown that serum pentosidine levels were increased in patients with chronic renal failure as well as diabetic nephropathy (30, 31) because renal insufficiency was a dominant determinant of serum pentosidine levels (32). Thus, in this study, to eliminate the influence of renal function, we recruited patients with type 2 diabetes who were within the normal range of serum creatinine and with no obvious proteinuria and performed all statistical analysis for pentosidine with multiple or logistic regressions adjusted for estimated GFR.

Several studies have revealed that pentosidine content in cortical or trabecular bone from vertebra or femur was negatively associated with mechanical properties (12–14) and that pentosidine content of cortical and trabecular bone derived from patients with femoral neck fracture were higher than those of age-matched controls (15, 16). However, the assessments of pentosidine content in bone were not easily done in clinical situations because invasive procedures like bone biopsy were needed for preparing specimens. A recent study revealed that content of pentosidine in plasma showed a significant linear correlation with that in cortical bone (19), suggesting that serum

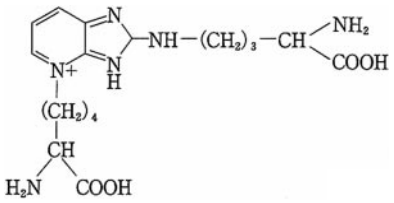

FIG. 1. Constitutional formula of pentosidine.

pentosidine level could be used as a surrogate marker for its content in bone and could evaluate bone strength.

The mechanism by which pentosidine affects bone strength is still unclear. One possible mechanism is that pentosidine might change collagen properties by decreasing its hydroxylysine residues. Pentosidine is known to consume lysine as well as arginine and ribose in its synthesis (24, 25). Because lysine is the source of hydroxylysine, which is necessary for forming intermolecular cross-linking of collagen molecules, the process of pentosidine synthesis leads to decreased hydroxylysine residues, shortening fibril diameter, and decreasing the number of pyridinium cross-link formation in collagen (33). Collagen fibers are a major component of bone matrix and provide the ductility and ability to absorb energy, and thus, deterioration of collagen properties can affect the mechanical properties of bone and increase fracture susceptibility (34), resulting in increased bone fragility.

Serum pentosidine levels are known to be enhanced by increased oxidative stress because prooxidizing conditions are necessary for its formation (35). Several *in vivo* human studies assessed the role of oxidative stress in osteoporosis. In healthy postmenopausal females, urinary excretion of 8-hydroxydeoxyguanosine, a product of oxidized DNA indicating oxidative cellular damage, was significantly higher than in age-matched males and premenopausal females (36), suggesting that postmenopausal women were more often exposed to oxidative stress than other groups. This may explain, in part, why serum pentosidine levels in diabetic patients were associated with the presence of vertebral fractures specifically in postmenopausal women but not men. Oxidative stress might be one of the causative factors for osteoporosis through inducing a high content of pentosidine in bone in postmenopausal diabetic women.

This study has some limitations. First, this study was not population based and the sample size was not large enough to make definite conclusions. Second, we analyzed only the subjects who attended Shimane University Hospital, a tertiary care center, for an evaluation or treatment of diabetes. Therefore, the patients enrolled in this study might have had a relatively severe state of the disorder and might not be representative of normal Japanese diabetic patients. Third, this was a cross-sectional study, and longitudinal design would be needed to evaluate the usefulness of serum pentosidine levels in predicting the occurrence of vertebral fractures in the diabetic population. Fourth, vertebral fracture rates in the present populations (36.4% in males and 26.3% in females) seem to be higher than those observed in Western counterparts. However, we found the similar fracture rate (31.6%) in 193 nondiabetic postmenopausal women in a previous study (37), and comparison of vertebral fracture rates between one Japanese and two European cohorts shows that Japanese have a higher fracture rate than Europeans (38–40). Finally, we did not confirm the correlation between serum pentosidine levels and vertebral content of pentosidine or mechanical bone strength assessed by loading tests, and thus, it is unclear whether serum pentosidine levels actually reflect pentosidine content in the spine or bone strength in the fractured site.

In conclusion, this is the first report that serum pentosidine levels were associated with prevalent vertebral fractures in postmenopausal women with diabetes. This association was independent of BMD, suggesting that it might reflect bone quality rather than bone density. We recently found that BMD is not sensitive enough to assess the risk of vertebral fractures in diabetic women because lumbar BMD was not significantly associated with the presence of vertebral fractures, and absolute lumbar BMD values were higher and their sensitivity and specificity for detecting vertebral fractures were lower in diabetic patients than in controls. Thus, bone quality seems to be more important than bone density for determining bone strength in diabetic patients. Although further studies are required, our findings suggest that serum pentosidine levels are more sensitive than BMD in assessing the risk of prevalent vertebral fractures in postmenopausal women with diabetes and may reflect bone quality in this group.

Acknowledgments

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