Journal of Bone and Mineral Metabolism (2019) 37:1067–1074
https://doi.org/10.1007/s00774-019-01017-9

ORIGINAL ARTICLE

The association of urinary pentosidine levels with the prevalence of osteoporotic fractures in postmenopausal women

Masataka Shiraki[1] · Shoji Kashiwabara[2] · Takumi Imai[3] · Shiro Tanaka[3] · Mitsuru Saito[4]

Received: 5 April 2019 / Accepted: 3 June 2019 / Published online: 18 June 2019

Abstract

To evaluate whether or not the urinary pentosidine level has clinical value in the assessment of the osteoporotic fracture risk, a novel ELISA for pentosidine was used in clinical samples. This study employed a cross-sectional design to analyze a subset of postmenopausal women in the Nagano Cohort Study. A total of 517 urine samples were analyzed using an ELISA system, which can measure urinary pentosidine without hydrolysis. Patients were asked about their history of non-vertebral osteoporotic fracture and the prevalence of vertebral fracture was semi-quantitatively assessed on X-ray films. A 10-year increase in age was related to a 1.09-fold increase in the urinary pentosidine level (95% CI 1.05–1.13, $P<0.001$), prevalent fracture (+) was related to a 1.10-fold increase in the urinary pentosidine level (95% CI 1.03–1.18, $P=0.006$). Patients with prevalent fracture who had a normal bone mineral density (BMD) showed higher pentosidine levels (median 34.3 pM/mg Cr) than patients with a low BMD without fracture (median 31.4 pM/mg Cr). A multivariable logistic regression analysis revealed that urinary pentosidine was significantly associated with the prevalence of fracture after adjustment for known risk factors for fracture (odds ratio 1.92, 95% CI 1.09–3.37, $P=0.023$). The present results indicated a significant association between urinary pentosidine and fracture after adjustment for age and BMD, suggesting that urinary pentosidine may be useful for assessing the fracture risk in postmenopausal women.

Keywords Advanced glycation end product (AGEs) · Urinary pentosidine · Fracture · Osteoporosis

Introduction

Osteoporosis, which is characterized by deteriorated bone strength, is a serious morbid state in elderly people, because it disturbs the patient's activity of daily life (ADL) and is associated with a low quality of life (QOL). Patients with osteoporosis develop non-traumatic fractures of the vertebrae, the proximal end of humerus, the distal end of radius, and the proximal end of femur. After the occurrence of an osteoporotic fracture, the patient requires long-term treatment or care for osteoporosis to prevent further fractures or maintain their ADL. Although the exact mechanism(s) underlying the development of the morbid state is complex, tissue oxidation or glycation is recognized as an important causal factor [1]. Biomarkers to evaluate tissue glyco-oxidation are urgently required in the clinical field. Among several biomarkers used to detect tissue glyco-oxidation, pentosidine is a well-established intermolecular crosslinking AGE that is used as a surrogate marker of total AGE formation [2, 3]. This compound is non-enzymatically formed in collagen fibers in bone and accumulates with advancing of age [4]. In fact, urinary pentosidine increases with age [4] and predicts future fracture in men [5], women [4, 5], and diabetic patients [5, 6]. These reports indicate that pentosidine plays an important role in the mechanism underlying the development of fractures. However, the measurement of pentosidine is not widely used in the clinical setting, because the HPLC method that is used for this purpose is time-consuming and difficult to apply, due to the massive sample size that

✉ Masataka Shiraki
ripid@bh.wakwak.com

[1] Research Institute and Practice for Involutional Diseases, 1610-1, Meisei, Misato, Azumino, Nagano Prefecture 399-8101, Japan

[2] Research and Development, SB Bioscience Co., Ltd., Osaka, Japan

[3] Department of Clinical Biostatistics, Graduate School of Medicine, Kyoto University, Kyoto, Japan

[4] Department of Orthopedic Surger, Jikei University School of Medicine, Tokyo, Japan

is required. Thus, an improved assay method has been desired. We developed a new ELISA system to measure urinary pentosidine without the hydrolysis process, which is required in HPLC, and used it to measure the pentosidine levels in clinical samples from postmenopausal Japanese women with or without an osteoporotic fracture.

Materials and methods

Subjects

The Nagano cohort study is an ongoing study consisting of ambulatory postmenopausal women at a primary care institute in Nagano Prefecture, Japan [4, 9, 10]. A subset of the Nagano cohort study participants, who were consecutively registered between May 2014 and March 2017, were included in the present study. A total of 600 subjects were registered during the 3 years. Among of them, participants with hyperparathyroidism, renal insufficiency, unstable diabetes mellitus, and those taking glucocorticoids were excluded from this analysis. Patients with serious illness such as terminal-stage malignant disease and bed bound patients were also excluded from the registration. A total of 517 participants were included in the present study.

Data collection for the characteristics of the participants

The patients' body weight, body height, and body mass index (BMI) were measured. The BMI was calculated using the formula: body weight (kg) divided by height (m^2). Non-fasting serum and urine samples were collected to measure biochemical markers. The serum levels of blood urea nitrogen (BUN), creatinine, uric acid, total protein, albumin, triglycerides, glycated hemoglobin (HbA1c), and total, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol were also measured. The total osteocalcin was measured by an EIA (Tosoh Osteocalcin kit, Tokyo, Japan). Serum levels of hCRP were measured by N-Latex CRP II kit (Siemens, Health Care Diagnostics, Tokyo, Japan) and total homocysteine in serum was measured by LC–MS/MS method (LSI Medience, Tokyo Japan). The estimated glomerular filtration rate (eGFR) was calculated using the following formula: $194 \times \text{serum creatinine}^{-1.094} \times \text{age}^{-0.287} \times 0.739$. The bone mineral density (BMD) at the lumbar spine (LBMD) and proximal femur (Total hip BMD) was measured using dual-energy X-ray absorptiometry (DXA) (Prodigy, GE Lunar, Madison, WI, USA).

Development of pentosidine ELISA

The ELISA consisted of polyclonal anti-pentosidine IgG and a secondary antibody. The accuracy, precision, and reliability of this ELISA system were evaluated. In brief, the LoB and LoD were 4.25 and 6.24 pmol/mL, respectively. The intra-assay and inter-assay coefficients of variation were < 5%. The spiking and dilution recoveries were 101.4 and 100.5%, respectively. An analysis of cross-reactivity against 7 compounds, representative for AGE and a substance with a structure close to pentosidine, revealed no significant cross-reactivity. The comparability between the values obtained from HPLC and the ELISA (in the same urine samples) was $r = 0.815$.

The diagnosis of fracture

Prevalent fracture was defined as non-traumatic fractures at the vertebrae, the rib, the pelvis, the proximal end of the humerus, the distal end of radius, the proximal end of the femur, and the lower extremity fractures. Subjects were asked about their history of long bone fracture. Prevalent vertebral fracture was semi-quantitatively evaluated on baseline X-ray films of thoracic and lumbar vertebrae [11].

The diagnosis and classification of osteoporosis

Osteoporosis was diagnosed in accordance with the diagnostic criteria of osteoporosis (2012 version) [12]. Briefly, patients with prevalent vertebral or proximal femur fracture were diagnosed as having osteoporosis, regardless of their bone mineral density [BMD (assessed as *T* score)]. Patients with a *T* score of ≤ -2.5 were diagnosed as having osteoporosis regardless of their fracture status. Patients with osteoporotic fracture at pelvis, proximal end of humerus, distal end of radius, or at lower extremities with their BMD within larger than -2.5 *T* Score to less than -1.0 *T* score (osteopenia), were also diagnosed as having osteoporosis. The osteoporosis was classified into three categories: low BMD (≤ -2.5 *T* score) without fracture, low BMD with fracture, and high BMD (> -2.5 *T* Score) with fracture.

Diagnosis of co-morbidities

Diabetes mellitus was diagnosed based on an HbA1c value of > 6.5% or treatment for diabetes and unstable diabetes was defined as HbA1c over 9%. Hypertension was diagnosed based on a persistent systolic blood pressure of 140 mmHg and/or a persistent diastolic blood pressure of 90 mmHg, or treatment with anti-hypertension drugs. The

diagnosis of renal insufficiency was defined as eGFR less than 30 ml/min/1.73 m^2.

Ethical considerations

The study protocol of the Nagano cohort study was reviewed by the ethical committee of Research Institute and Practice for Involutional Diseases and comprehensive written informed consent was obtained from the participants.

Statistical analysis

Patient characteristics are reported as the mean and standard deviation (SD), median, and inter-quartile range (IQR) or number and proportion, for all patients and for the patients stratified by prevalent fracture status. The differences in characteristics between these groups were evaluated using t tests for continuous variables or the Chi-squared test for categorical variables. To visualize the distribution of urinary pentosidine, the values of urinary pentosidine were plotted against age, BMD status (low or normal), and prevalent fracture status (− or +). The associations of these factors with the urinary pentosidine level were quantified by a multivariable linear regression model. To investigate whether the association between the prevalent fracture status and urinary pentosidine was independent of other variables known to be associated with prevalent fracture, a multivariable logistic regression analysis was performed. Based on medical consideration, the following variables (other than urinary pentosidine) were identified as risk factors for prevalent fracture and included as covariates in the multivariable logistic model: age, body weight, body height, BMD, smoking, alcohol drinking, urine NT*x*, serum hCRP, diabetes mellitus, and hypertension. Co-morbidities were included due to the high prevalence in the elderly study population. We input missing data by conducting multiple imputations with chained equations (50 rounds). All reported *P* values were two tailed, and *P* values of <0.05 were considered to indicate statistical significance. The statistical analysis was conducted using R the software program (version 3.5.2 R Foundation for Statistical Computing, Vienna, Austria).

Results

The background numerical and categorical data of the patients arelisted in Table 1. The study population was characterized by older age (mean age 74 years) that those in the previous our reports of urine pentosidine (63 years [4, 6]). However, the *Z* score of the lumbar and femoral bone mineral densities were − 0.36 and 0.24, respectively, suggesting that their mean BMDs were within ± 0.5 SD for Japanese women of the corresponding age. In addition, the mean eGFR (age-adjusted glomerular filtration) was 70 ml/min/1.73 m^2 and the IQR was 59.8–78.9 ml/min/1.73 m^2, suggesting that the present population did

Table 1 Patient characteristics

	All (*N* = 517)		
	Mean/median number	SD IQR %	NM
Age (years)	73.7	9.8	0
Years after menopausal (years)	16.0	9.7	12
Body weight (kg)	50.5	7.8	0
Body height (cm)	151.1	6.1	0
BMI (kg/m^2)	22.1	3.1	0
Lumbar BMD (g/cm^2)	0.9	0.2	0
T score	− 0.4	1.3	0
Z score	− 1.9	1.3	77
Total hip BMD (g/cm^2)	0.7	0.1	0
T score	− 1.4	1.0	1
Z score	0.2	1.0	1
Abdominal circumference (cm)	84.2	8.6	10
Smoker	16	3%	0
Alcohol drinker	60	12%	0
Laboratory test results (median, IQR)			
Albumin (mg/dl)	4.2	4.0–4.4	5
Total protein (g/dl)	7.3	7.0–7.6	0
Alkaline phosphatase (U/l)	206.0	164.8–258.0	1
Blood urea nitrogen (mg/dl)	16.0	13.0–18.8	4
Creatinine (mg/dl) (Cr)	0.6	0.6–0.7	0
Uric acid (mg/dl)	4.3	3.7–5.1	4
eGFR (ml/min/1.73 m^2)	70.3	59.8–78.9	0
Serum Ca (ml/dl)	9.3	9.0–9.6	3
Serum P (mg/dl)	3.5	3.2–3.8	3
Total cholesterol (mg/dl)	206.0	183.0–230.0	1
Triglyceride (mg/dl)	120.0	87.0–175.2	1
LDL cholesterol (mg/dl)	122.0	103.0–142.0	12
HDL cholesterol (mg/dl)	66.0	55.0–78.0	1
HbA1c (%)	5.4	5.1–5.7	0
Urine NT*x* (nmolBCE/mmol Cr)	45.0	32.1–61.9	5
Osteocalcin (ng/ml)	7.1	5.5–9.0	20
hCRP (mg/ml) × 10	0.5	0.2–0.9	11
Homocysteine (nmol/ml)	8.1	7.0–9.9	8
Pentosidine (pmol/mg Cr)	32.2	26.4–40.8	0
Co-morbidities			
Diabetes mellitus	82	16%	0
Hypertension	269	52%	0
Osteoporosis	317	61%	0
Prevalent fracture history	195	38%	0

SD standard deviation, *IQR* inter-quartile range, *NM* number of missingness

not include patients with severe renal failure, which may be associated with high urine pentosidine.

Since the present population included ambulant patients in a primary care institution, the prevalence of chronic diseases might have been high in comparison to a community-dwelling population. Approximately half of the patients had osteoporosis and hypertension, while the rate of osteoporosis in the previous study was around 20%, probably because the age of the previous studies was around 10 years younger than that in the present study [4, 6].

Both smoking and drinking are known risk factors for osteoporosis in Caucasian populations. However, the rates of patients with these habitual risk factors were very low (3% for smoking and 11% for drinking). All patients with osteoporosis were undergoing treatment with bisphosphonates ($n=161$), denosumab ($n=40$), SERM ($n=60$), vitamin D3 ($n=42$), or daily teriparatide ($n=6$) at the time of urinary pentosidine measurement.

Table 2 shows the patient characteristics stratified by the prevalent fractures status. One hundred and ninety-five patients were diagnosed with prevalent fracture. However, 211 osteoporotic fractures [forearm fracture, $n=29$; proximal femur, $n=8$; proximal humerus, $n=6$; and vertebral fracture, $n=168$ (16 patients had multiple fractures)] were observed in 195 patients. Significant differences were observed in the age, body height, total hip BMD, and urinary pentosidine levels of the subjects with ($n=195$) and without prevalent fracture ($n=322$). The percentage of the patients with hypertension in the prevalent fracture group was significantly higher (59%) than that in the patients without fracture (48%). The urinary NT*x* levels of the groups did not differ to a statistically significant extent, probably because the urine samples were obtained from patients undergoing osteoporosis treatment. The lumbar BMD was not significantly different, probably due to the presence of vertebral fractures and osteoarthritic deformity in the region of interest (L2–4 BMD). The total hip BMD of the prevalent fracture group was significantly lower than that of the patients without fracture. The other characteristics listed in Table 1 did not differ between the groups with or without prevalent fracture.

The urinary pentosidine values were plotted against age and prevalent fracture status (Fig. 1). A 10-year increase in age was related to a 1.09-fold increase in the urinary pentosidine level (95% CI 1.05–1.13, $P<0.001$), and prevalent fracture (+) was related to a 1.10-fold increase in the urinary pentosidine level (95% CI 1.03–1.18, $P=0.006$).

When the osteoporosis was categorized according to BMD status (low or normal) and the prevalent fracture status (− or +) (Fig. 2), the median urinary pentosidine values were 31.3 pmol/mg Cr for normal BMD (BMD *T* Score >-2.5) and prevalent fracture (−) (Non OP) group, 31.4 pmol/mg Cr for low BMD (*T* Score ≤ -2.5) and prevalent fracture (−) group, 33.8 pmol/mg Cr for low BMD and prevalent fracture (+) group, and 34.3 pmol/mg Cr for normal BMD and prevalent fracture (+) group. Based on the multivariable linear regression analysis for log-transformed urinary pentosidine, age and prevalent fracture were related with urinary pentosidine level, while the relationship between low BMD and urinary pentosidine was weak (0.98-fold decrease, $P=0.579$). These results

Table 2 Patient characteristics stratified by prevalent fracture status (for variables included in the analysis)

	Prevalent fracture (−) ($N=322$)			Prevalent fracture (+) ($N=195$)			*P* value*
	Mean median number	SD IQR %	NM	Mean median number	SD IQR %	NM	
Age (years)	71.1	9.6	0	78.1	8.6	0	<0.001
Year after menopausal	13.6	9.1	6	20.1	9.3	6	<0.001
Body weight (kg)	51	7.7	0	49.8	7.9	0	0.078
Body height (cm)	152.4	5.3	0	148.9	6.6	0	<0.001
BMI	22	3.2	0	22.4	3	0	0.112
Lumbar BMD × 10 (g/cm^2)	9.3	1.7	0	9.1	1.8	0	0.085
Total hip BMD × 10 (g/cm^2)	7.5	1.2	0	6.9	1.2	0	<0.001
Urine NT*x* (nmolBCE/mmol Cr)	44.8	31.2–59.6	4	45.8	33.9–65.0	1	0.135
hCRP × 10 (mg/ml)	0.4	0.2–0.8	10	0.6	0.3–1.2	1	0.350
Pentosidine (pmol/mg Cr)	31.4	25.6–38.4	0	34.3	27.9–44.9	0	<0.001
Homocysteine	8.1	6.8–9.9	8	8.2	7.2–9.9	0	0.464
Diabetes mellitus	54	17%	0	28	14%	0	0.546
Hypertension	154	48%	0	115	59%	0	0.018

SD standard deviation, *IQR* inter-quartile range, *NM* number of missingness

**T* tests for continuous variable and Chi-square tests for categorical variable

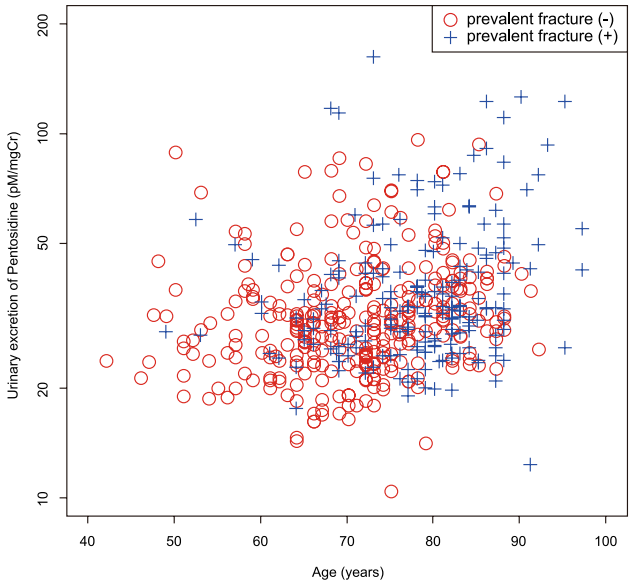

Fig. 1 The effect of aging on urinary pentosidine. The urinary pentosidine values were plotted against age and the prevalent fracture status. A 10-year increase in age was related to a 1.09-fold increase in the urinary pentosidine level (95% CI 1.05–1.13, $P<0.001$) and prevalent fracture (+) was related to a 1.10-fold increase in the urinary pentosidine level (95% CI 1.03–1.18, $P=0.006$)

clearly indicated that the urinary pentosidine levels of osteoporosis patients depended on age and the prevalent fracture status, irrespective of the BMD. All osteoporosis patients were under treatment at the time of urinary pentosidine measurement, and urinary pentosidine did not differ according to the mode of treatment for osteoporosis with the exception of denosumab treatment, which showed a small association comparing to that in the group without treatment (the median urinary pentosidine excretion values for patients with denosumab treatment and without any treatments were 32.8 pmol/mg Cr and 31.9 pmol/mg Cr, respectively, and $P=0.01$.). This may be a reflection of higher prevalence of fractures in the denosumab treatment group (65%) than that in the no treatment group (20.7%).

Table 3 shows the results of the multivariable logistic regression analysis to identify factors associated with a prevalent fracture status. Here, we select the variables, which have significant relation with fracture status (Table 2), and several characteristics, which may affect on urinary pentosidine level such as NTx, hCRP, or presence of diabetes. The estimated odds ratio for a prevalent fracture status per 1 increase in log-transformed urinary pentosidine was 1.92 (95% CI 1.09–3.37, $P=0.023$). Based on the estimated standardized odds ratios, the strength of the association between prevalent fracture status and urinary pentosidine was comparable to that of BMD (1.28 for urinary pentosidine and 0.73 for BMD).

Discussion

Assessing the risk of osteoporotic fracture is extremely important for preventing incident fracture. Among the various risk factors for fracture, prevalent fracture takes on special significance in predicting the future occurrence of osteoporotic fracture, along with aging and BMD. We previously reported that the collagen property of bone is an important factor for determining bone strength [1], because > 90% of bone matrix protein consists of collagen fiber [13]. Bone strength is determined by the enzymatic formation of collagen crosslinks [14–16]. On the other hand, the non-enzymatic formation of collagen crosslinks was produced by advanced glycation end products (AGE), which formed as a result of the accumulation of reducible sugars in bone tissue [17]. The increase in pentosidine, one of the AGE crosslinks in collagen, is connected to the occurrence of incident fracture [4–8]. In the present study, we reported that high urinary pentosidine was independently associated with prevalent fracture and that this association was the comparable to that of BMD (Table 3). Since the present study design was cross-sectional in nature, the causal relationship between prevalent fracture and pentosidine could not be discussed; however, a tight relationship between pentosidine and both incident and prevalent fractures was shown in the present study and a previous report [4]. It is possible that pentosidine is involved in the higher susceptibility to subsequent fracture observed in patients with prevalent fracture. The accumulation of fractures is known to severely disturb a patient's ADL and QOL. Thus, the prevention of secondary osteoporotic fractures is a matter of importance in osteoporosis treatment. The importance of prevalent fracture was recognized in the "Stop at One; Make your first break your last campaign of the International Osteoporosis Foundation" (https://www.iofbonehealth.org/stop-one-make-your-first-break-your-last). Thus, the measurement of urinary pentosidine is meaningful for assessing the risk of second fracture. The urinary pentosidine levels of patients with osteoporosis were higher than those of patients without osteoporosis. When we further stratified the patients according to the characteristics of their osteoporosis, higher pentosidine was observed in osteoporosis patients with prevalent fracture, regardless of their BMD (Fig. 2). Our previous report pointed out that the urinary pentosidine level, as measured by HPLC, is useful for fracture risk classification [6]. The clinical significance of pentosidine measurement in the classification of osteoporosis is, therefore, consistent.

The present study clearly indicated that the urinary pentosidine level, as measured by a newly developed ELISA, was useful for the assessment of fracture risk in addition to the measurement of BMD.

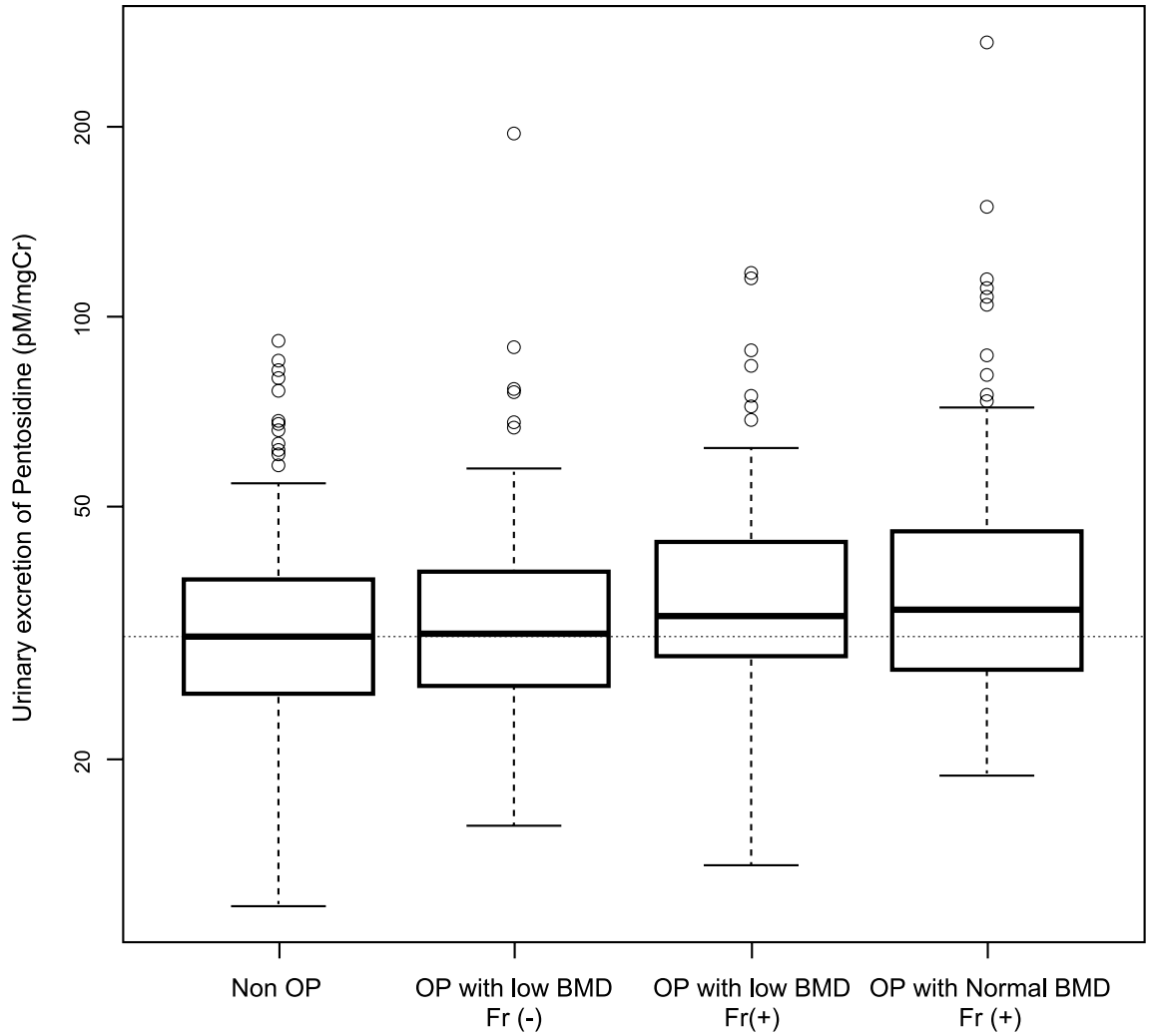

Fig. 2 Urinary pentosidine in patients without osteoporosis and osteoporosis patients with or without prevalent fractures. The urinary pentosidine levels in patients without osteoporosis (NonOP, $n=200$) and in patients with osteoporosis. Osteoporotic patients were further classified by their BMD status and the presence or absence of prevalent fractures. One hundred and twenty-one OP patients were diagnosed with low BMD, without fracture 121. Ninety-two OP patients were diagnosed with low BMD and fracture. One hundred and four OP patients were diagnosed without low BMD, and with fracture. The horizontal dotted line shows the median urinary pentosidine value in patients without osteoporosis (31.3 pmol/mg Cr). The thick line in the box is the median. The bottom end of the box indicates the first quartile; the top end indicates the third quartile. The whiskers indicate the minimum and maximum values at 1.5 boxes in length from the first and third quartiles

The antecedent ELISA kits have been available to measure plasma/serum level of pentosidine for assessment renal function. These kits are required pretreatment of sample with high-temperature acid hydrolysis [18] or enzymatic digestion with pronase E [19, 20]. Nakano et al. [21] reported that the pentosidine content was increased 1.1- to 4.2-fold by the heating process compared to unheated samples, and the increased rate was not identical for each sample. On the other hand, the blood was filtered by the renal glomerulus and is passed through molecular sieve at podocyte. Finally, urine contains small size molecules. This is a reason why urine sample is not necessary the heat pre-processing. The overestimation of pentosidine may not count even in the samples with high urinary glucose. In addition, the anti-pentosidine antibody is highly specific for pentosidine. We examined the antibody specificity to the other types of AGEs and the compounds resembling to pentosidine, and there was no significant cross-reactions. As a whole, the present kit is considered to be reliable.

However, the present study was associated with some limitations. First, the number of patients was relatively small; which may have affected the stability of the results. Second, the present population was composed of outpatients who were managed in a primary care institution, which might have produced a selection bias. The further evaluation of the pentosidine levels in a community-dwelling population

Table 3 Relation with prevalent fracture history (multivariable logistic regression)

Variables	Direction	Odds ratio	95% CI		Direction	Standardized Odds ratio	95% CI		*P* value
Age	10 year increase	1.58	1.11	2.25	1 SD	1.57	1.11	2.22	0.011
Year after menopausal	1 increase	1.02	0.99	1.06	1 SD	1.27	0.94	1.71	0.124
Body weight	10 kg increase	1.34	0.95	1.87	1 SD	1.25	0.96	1.63	0.092
Body height	10 cm increase	0.63	0.40	0.98	1 SD	0.75	0.57	0.99	0.042
Total hip BMD	0.1 g/cm^2 increase	0.78	0.63	0.96	1 SD	0.74	0.58	0.95	0.019
Log-urine NTx	1 increase	1.06	0.69	1.63	1 SD	1.03	0.83	1.26	0.800
Log-hCRP	1 increase	1.16	0.96	1.41	1 SD	1.18	0.95	1.47	0.130
Log-pentosidine	1 increase	1.93	1.09	3.41	1 SD	1.29	1.03	1.60	0.024
Log-homocysteine	1 increase	0.68	0.34	1.35		0.89	0.72	1.09	0.266
Diabetes mellitus	Yes/no	0.62	0.35	1.09		0.62	0.35	1.09	0.097
Hypertension	Yes/no	0.81	0.52	1.26		0.81	0.52	1.26	0.354

will be required to evaluate generalizability. Third, the osteoporotic fracture prevalence may be under estimated, because the narrative information of prevalent rib fracture from the patients has an inclination to forget, so that the prevalent rib fracture information is inaccurate comparing to the other osteoporotic fractures.

In conclusion, the urinary pentosidine level, as measured by an ELISA, was useful for evaluating the risk of fracture in osteoporosis.

Acknowledgements The authors would like to thank the patients who agreed to participate in the present project. The authors would also like to thank Dr. Brian Quinn for editing the English of this paper.

Compliance with ethical standards

Conflict of interest MS received consultant fee from Teijin pharma and Asahi Kasei Pharma, a manufacturer of drugs to treat osteoporosis. SK is an employee of SB Bioscience Co., Ltd. TI, ST, and MiS declare no conflicts of interest in association with the present study.

References

1. Saito M, Marumo K (2010) Collagencross-links as a determinant bone quality: a possible explanation for bone fragility in ageing, osteoporosis, and diabetes mellitus. Osteoporosis Int 21:195–214
2. Saito M, Kida Y, Nishizawa T, Arakawa S, Okabe H, Seki A, Marumo K (2015) Effects of 18-month treatment with bazedoxifene on enzyme immature and mature cross-links and non-enzymatic advanced glycation end products, mineralization, and trabecular microarchitecture of vertebra in ovariectomized monkeys. Bone 81:573–580
3. Saito M, Grynpas MD, Burr DB, Allen MR, Smith SY, Doyle N, Amizuka N, Hasegawa T, Kida Y, Marumo K, Saito H (2015) Treatment with eldecalcitol positively affects mineralization, microdamage, and collagen crosslinks in primate bone. Bone 73:8–15
4. Shiraki M, Kuroda T, Tanaka S, Saito M, Fukunaga M, Nakamura T (2008) Nonenzymatic collagen cross-links induced by glycoxidation (pentosidine) predicts vertebral fractures. J Bone Miner Metab 26:93–100
5. Tamaki J, Kouda K, Fujita Y, Iki M, Yura A, Miura M, Sato Y, Okamoto N, Kurumatani N (2018) Ratio of endogenous secretory receptor for advanced glycation end products to pentosidine predicts fractures in Men. J Clin Endocrinol Metab 103:85–94
6. Tanaka S, Kuroda T, Saito M, Shiraki M (2011) Urinary pentosidine improves risk classification using fracture risk assessment tools for postmenopausal women. J Bone Miner Res 26:2778–2784
7. Schwartz AV, Garnero P, Hillier TA, Sellmeyer DE, Strotmeyer ES, Feingold KR, Resnik HE, Tylavsky FA, Black DM, Cummings SR, Harris TB, Bauer DC, For the health, aging, and body composition study (2009) Pentosidine and increased fracture risk in older adults with type 2 diabetes. J Clin Endocr Metab 94:2380–2386
8. Yamamoto M, Yamaguchi T, Yamauchi M, Yano S, Sugimoto T (2008) Serum pentosidine levels are positively associated with the presence of vertebral fractures in postmenopausal women with type 2 diabetes. J Clin Endocrinol Metab 93:1013–1019
9. Urano T, Shiraki M, Ouchi Y, Inoue S (2012) Association of circulating sclerostin levels with fat mass and metabolic disease- related markers in Japanese postmenopausal women. J Clin Endocrinol Metab 97:E1473–E1477
10. Kuroda T, Uenishi K, Ohta H, Shiraki M (2018) Multiple vitamin deficiencies additively increase the fracture risk of incident fractures in Japanese postmenopausal women. Osteoporos Int. https://doi.org/10.1007/s00198-018-4784-2
11. Genant HK, Wu CY, van Kuijk C et al (1993) Vertebral fracture assessment using a semiquantitative technique. J Bone Miner Res 8:1137–1148
12. Soen S, Fukunaga M, Sugimoto T, Sone T, Fujiwara S, Endo N, Gorai I, Shiraki M, Hagino H, Hosoi T, Ohta H, Yoneda T, Tomomitsu T, Japanese Society for Bone and Mineral Research and Japan Osteoporosis Society Joint Review Committee for the Revision of the Diagnostic Criteria for Primary Osteoporosis (2013) Diagnostic criteria for primary osteoporosis: year 2012 revision. J Bone Miner Metab 31:247–257
13. NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy (2001) Osteoporosis prevention, diagnosis, and therapy. JAMA 2001(285):785–795
14. Wang X, Shen X, Li X, Agrawal CM (2002) Age-related changes in collagen network and toughness of bone. Bone 31:1–7

15. Vashishth D, Gibson GJ, Khoury JI, Schaffler MB, Kimura J, Fyhrie DP (2001) Influence of nonenzymatic glycation on biochemical properties of cortical bone. Bone 28:195–201
16. Uzawa K, Grezesik WJ, Nishimura T, Kuznetssov SA, Robey PG, Brenner DA, Yamauchi M (1999) Differential expression of human lysyl hydroxylase genes, lysyl hydroxylation, and cross-linking of type I collagen during osteoblastic differentiation in vitro. J Bone Miner Res 14:1270–1280
17. Sell DR, Monnier VM (1989) Structure elucidation of a senescence crosslink from human extracellular matrix: implication of pentose in aging process. J Biol Chem 264:21597–21602
18. Izuhara Y, Miyata T, Ueda Y, Suzuki D, Asahi K, Inagi R, Sakai H, Kurokawa K (1999) A sensitive and specific ELISA for plasma pentosidine (in eng). Nephrol Dial Transplant 14:576–580
19. Taneda S, Monnier VM (1994) ELISA of pentosidine, an advanced glycation end product, in biological specimens (in eng). Clin Chem 40:1766–1773
20. Sanaka T, Funaki T, Tanaka T, Hoshi S, Niwayama J, Taitoh T, Nishimura H, Higuchi C (2002) Plasma pentosidine levels measured by a newly developed method using ELISA in patients with chronic renal failure (in eng). Nephron 91:64–73. https://doi.org/10.1159/000057606
21. Nakano M, Kubota M, Owada S, Nagai R (2013) The pentosidine concentration in human blood specimens is affected by heating. Amino Acids 44:1451–1456. https://doi.org/10.1007/s00726-011-1180-z