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esRAGE-to-Pentosidine Ratio and Fracture Events

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**Context:** Although the endogenous secretory receptor for advanced glycation end products (esRAGE) has been associated with reduced activity of pentosidine (PEN), the association between PEN, esRAGE, and fracture is poorly understood.

**Objectives:** To evaluate the ability of serum PEN and esRAGE levels to predict fragility fractures.

**Methods:** A cohort of 1,285 Japanese men aged ≥65 years old participated in a 2007-2008 FORMEN Study baseline survey, as part of the Fujiwara-kyo prospective cohort study. Those participants provided information regarding any fractures they experienced over 5 years. The baseline bone mineral density (BMD) was measured (QDR4500A, Hologic, USA). Hazard ratios (HR) per one standard deviation increase of log-transformed serum levels of PEN, esRAGE, and esRAGE-to-PEN ratio were estimated at baseline.

**Results:** Twenty-five participating men suffered incident clinical fragility fractures. The crude HRs (95% confidence interval) for PEN, esRAGE, and esRAGE-to-PEN ratio were 1.56 (1.05–2.31), 0.79 (0.54–1.15), and 0.65 (0.44–0.95), respectively. HRs for PEN adjusted for age, esRAGE, and T-score of BMD at femoral neck (FN) and lumbar spine (LS) were 1.48 (1.00–2.18) and 1.51 (1.03–2.21), respectively. The marginal significance adjusted for BMD at FN and the statistical significance adjusted for BMD at LS were attenuated after additional
adjustment for HbA1c level (p = 0.111 and 0.072, respectively). The HRs for esRAGE-to-PEN ratio adjusted for age, HbA1c, and T-score of BMD at FN and LS were 0.67 (0.45–0.98) and 0.64 (0.43–0.95).

Conclusions: Higher esRAGE-to-PEN ratios were associated with decreased risk of fragility fractures independent of BMD among elderly Japanese men.

We found that higher esRAGE-to-PEN ratios were associated with decreased risks of fragility fracture during five years even after adjusting for bone mineral density in community-dwelling elderly men.

Introduction

Fragility fractures are now becoming a major public health issue, as the number of hip fracture events in both men and women has been increasing in the aging population of Japan (1). Although age-adjusted hip fracture incidence rates in North America, Northern Europe, and Central Europe have been declining (2), the age-adjusted incidence rates in a certain prefecture of Japan have been reported to be increasing (3). Hip fracture incidence rates throughout Japan are reported to have plateaued, with no sign of declining (1).

Patients with types 1 and 2 diabetes mellitus have been reported to have increased fragility fracture risk in a recent meta-analysis (4), despite type 2 diabetic patients having relatively high bone mineral density (BMD) values. One potential mechanism for higher fracture risk in these patients has been explained by their increased level of pentosidine (PEN), an advanced glycation end-product (AGE) (5, 6). In studies of human and diabetic rats, accumulation of PEN has been indicated in bone, which has been correlated with deterioration in bone strength (7-11). Previous epidemiologic studies of the general population and elderly patients with type 2 diabetes demonstrated that increased urinary PEN levels could predict fragility fracture events (12-15). Those findings have supported that increased PEN levels are associated with an increased fracture risk.

Intercellular signal pathways of AGEs have been reported to be transmitted by the receptor for advanced glycation end products (RAGE) on a cell surface (16). RAGE has been observed to engage in diabetes-induced vascular injury, yielded by AGEs. Diabetic RAGE knockout mice showed improvement of nephropathy (17), while diabetic mice overexpressing RAGE showed exaggerated nephropathy (18). Human endogenous secretory RAGE (esRAGE), a splice variant of RAGE that lacks the transmembrane and cytoplasmic domains, has been reported to bind AGEs as a decoy receptor and block the intracellular pathways of AGEs (19).

Plasma esRAGE levels in diabetic and nondiabetic subjects have indicated an inverse correlation with levels of carotid or femoral atherosclerosis (20, 21). Additionally, esRAGE levels have shown an inverse correlation with measurement levels of metabolic syndrome.
components (20). Additionally, a recent review suggested that serum sRAGE or esRAGE levels should be considered in conjunction with serum AGEs levels to utilize as biomarkers for disease, and that a combined quantity such as the AGEs-to-sRAGE or AGEs-to-esRAGE ratio would provide a better biomarker than sRAGE or esRAGE alone for all AGE-RAGE-associated diseases (22).

Given those numbers, the ability of PEN levels to predict fragility fractures should be investigated, including the role of esRAGE levels in this process. However, at the time of this study, there is no existing research that examines the associations between circulating PEN and esRAGE levels, and fragility fracture events within the general population. A cross-sectional study indicated that esRAGE to PEN ratios were associated with prevalent vertebral fracture in type 2 diabetes patients (23). In another cross-sectional study with type 1 diabetes patients, serum pentosidine levels, but not esRAGE levels were associated with prevalent fractures (24). Therefore, an investigation of whether or not esRAGE antagonizes the effects of PEN for fracture events would be valuable.

Given that a combination of PEN and esRAGE could provide increased information about the risk of fractures, the aim of this study was to examine whether a high esRAGE-to-PEN ratio is associated with a reduced risk of osteoporotic fractures. This is the first large-scale Asian study to evaluate the ability of PEN and esRAGE to predict fragility fracture events in a prospective cohort study. Analysis was based on a sample population from the Fujiwara-kyo Osteoporosis Risk in Men (FORMEN) study, a large-scale community-based single-center study for elderly Japanese men.

Methods

Study setting
The baseline survey for the FORMEN study was conducted during 2007-2008 as part of the larger Fujiwara-kyo prospective cohort study (Primary Investigator: Norio Kurumatani, MD, PhD, Professor, Nara Medical University School of Medicine), which was conducted as a collaboration between Nara Medical University and four cities in the Nara prefecture. Details of the Fujiwara-kyo study and the FORMEN study have been described elsewhere (25). Participants aged 65 years or older at the baseline survey were recruited by the Administrative Center of the Fujiwara-kyo Study, with the cooperation of local resident associations and elder organizations in each of the four cities. The FORMEN study examined the bone health of male participants from the Fujiwara-kyo study among whom 2,012 men completed the baseline survey during 2007-2008 (Figure 1). In 2008 and 2009, the mail and telephone surveys were administered to obtain information about osteoporotic fracture events occurring after the enrollment period. After excluding men who could not be located (n=12) or had died (n=137),
1,863 were invited to participate in the five-year follow-up study in 2012. A total of 1,431 participants completed the follow-up survey (76.6% of the 1,863 men recruited for follow-up, Figure 1).

**Study Population**

Among the total 1,580 men (follow-up rate for information of fracture events, 84.8% of the 1,863 men), 1,285 men with complete available data for PEN, esRAGE, HbA1c, and femoral neck (FN) BMD values, were selected for the analysis (Figure 1).

This study protocol as well as the baseline survey was approved by both the Ethics Committee of Kinki University School of Medicine and the Medical Ethics Committee of Nara Medical University. Study procedures were explained to all participants and written informed consent was obtained prior to participation in baseline and follow-up surveys.

**Ascertainment of fragility fracture events**

Fracture locations, timing of fracture events, situations in which fractures occurred, and X-ray diagnoses of fractures (yes/no) were reported during the baseline survey, the 2010 mailed questionnaire, and the five-year follow-up survey. Fragility fracture was defined as a painful fracture that was diagnosed by a medical doctor with radiographic reports. Detailed interviews were conducted by trained nurses or medical doctors to confirm each participant’s questionnaire answers during the surveys. Detailed methods of ascertaining fracture events in the FORMEN study have been described elsewhere (26). We validated this method using fracture events during the first 2 years of follow-up obtained via a mailed questionnaire survey. Of the 21 subjects with self-reported fracture events, 19 provided consent to validate fracture information through hospitals. We conducted the survey for the attending surgeon who examined the 19 subjects to confirm the fracture information through medical records. The surgeon confirmed the occurrence of all self-reported fractures, while differences between the fracture date obtained by the questionnaire and the description of medical records were all within 6 months. There were seldom differences between the self-reported fracture site and the site found in the medical records.

**Baseline bone density characteristics**

BMD was measured by dual X-ray absorptiometry of the second through fourth lumbar vertebrae (L2-4) and total hip (TH) (QDR4500A, Hologic, Bedford, MA, USA) (25, 27). Short-term precision (coefficient of variance, CV) values of BMD measurements *in vivo* were found to be 1.6%, 1.2%, and 1.2% for the FN, TH, and lumbar spine (LS), respectively (27). Densitometric data of the spine from participants with either vertebral fractures or grade four osteophytes, according to Nathan's classification, were excluded from the analysis (28).
Explanatory variables

Height and weight were measured using an automatic scale (Tanita TBF-215, Tanita Inc., Japan). Body mass index (BMI) was calculated as body weight (kg) divided by body height squared (m$^2$). Variables from the study questionnaire and baseline survey included history of fracture events, disease history, prescribed medications, smoking and drinking habits, and the participant’s parental history of hip fractures that occurred at $\geq$50 years of age. Trained nurses, public health nurses, or medical doctors confirmed each participant’s answers with a detailed interview during the survey.

Biochemical measurements

Fasting venous blood samples from all subjects were drawn into serum separator tubes during the baseline survey. Serum was extracted after centrifugation at room temperature, and was stored at -80 degrees centigrade until it was assayed for PEN and esRAGE.

Fasting plasma glucose (FPG) levels (mg/dl) and glycated hemoglobin A$\text{lc}$ (HbA1c) levels (%) were measured using the hexokinase-glucose-6-phosphate dehydrogenase method (L-type Glu 2, Wako Pure Chemical Industries, Ltd., Osaka, Japan), and the latex aggregation immunoassay (Determiner L HbA1C, Kyowa Medex Co., Tokyo, Japan), respectively. HbA1c values were converted to National Glycohemoglobin Standardization Program values, according to guidelines established by the Japan Diabetes Society (29).

Serum PEN levels (µg/ml) were measured using competitive enzyme-linked immunosorbent assay (ELISA) kits (FSK pentosidine ELISA kit; Fushimi Pharmaceutical Co., Marugame, Japan) with sensitivity of 0.00915 µg/ml. Intra-assay, inter-assay, and overall precision were determined to have CV values of 3.9%, 2.4%, and 4.6%, respectively. Pentosidine levels measured using an FSK pentosidine ELISA kit were highly correlated with values measured by high-performance liquid chromatography ($r=0.936$) (30).

Serum esRAGE (ng/ml) values were measured using ELISA kits (B-Bridge esRAGE ELISA Kit; B-Bridge International, Mountain View, USA). Intra-assay, inter-assay, and overall precision of this measurement were determined to have CV values of 1.66%, 4.52%, and 4.61%, respectively.

Statistical analysis

esRAGE-to-pentosidine ratio was calculated as esRAGE (ng/ml) divided by pentosidine (µg/ml). The log-transformation for the values of PEN, esRAGE, and esRAGE-to-PEN ratio was used to stabilize the variance. Those values were represented as geometric means with standard deviations (SDs) under log-normal distribution. The chi-square test was used for categorical data. Continuous data was analyzed with a t test if normally distributed or a Mann–Whitney–Wilcoxon test if the distribution was skewed. Kaplan–Meier cumulative
incidence rates of fracture were compared between the lowest tertile and the middle and highest tertiles after checking the proportional hazards assumption (p value for the interaction term of covariate and time in each model, 0.461–0.794), a Cox proportional hazards model was used to estimate hazard ratios (HRs) per one SD increase in log-transformed PEN, esRAGE, and esRAGE-to-PEN ratio baseline values. The Cox proportional hazard model was applied for the total subjects (n=1285), those with present illness of diabetes mellitus (n=232), and those without present illness of diabetes mellitus (n = 1053). Subjects with diabetes mellitus were defined as those who had been diagnosed with diabetes mellitus by physicians, or whose test results in the baseline survey met the cut-off values given by the guidelines of the American Diabetes Association (31) and the Japan Diabetes Society (32) (FPG ≥ 126 mg/dl or HbA1c ≥ 6.5%). The first fracture events found during follow-up were used to measure the abilities of PEN, esRAGE, and esRAGE-to-PEN ratio at baseline to predict incident fractures. For evaluation of the goodness of fit of the regression equations to the data, the Akaike Information Criterion (AIC) was calculated using the following equation: AIC=-2(logM–k). In this equation, M represents the maximum likelihood of a model, and k represents the number of independently-adjusted parameters in the model (33). When a difference in AIC between two models is more than one, the model with the smaller AIC is assessed as showing a significantly better fit to the data (34).

A logistic regression analysis for osteoporotic fracture events was conducted using esRAGE-to-PEN ratio to estimate the expected probability (P) for osteoporotic fracture events. ROC analysis was then performed to identify appropriate cut-off values of P for detecting osteoporotic fracture events. The value that maximized the difference between sensitivity and the false positive rate (the Youden index) was selected as the cut-off value (35). Sensitivity, specificity, and likelihood ratio for a positive result (LR) of osteoporotic fracture events were calculated using the cut-off values of P obtained by the Youden index, along with 95% confidence intervals (95% CI). Analyses were performed using statistical software packages (SPSS version 15.0, SPSS, Tokyo, Japan).

Results

Fragility fracture events and participant characteristics

Over a median follow-up period of 4.1 years (interquartile range (IQR), 3.8–4.6 years; total person years (PY), 5,149.9PY), 25 men sustained fragility fractures (incidence rate, 4.9/1,000PY) at the following locations: hip (n=2); clinical vertebrae (n=10); forearm (n=6); proximal humerus (n=1); clavicle (n=1); rib (n=4), and pelvis (n=2). One subject suffered an event in which fractures at forearm and rib occurred simultaneously. The median time (IQR) from baseline to the first fracture event was 1.4 years (0.9–3.4 years). The incidence rate for fracture events was 3.3/1,000PY (with an event number of 16) for the 1,156 participants in the
A five-year follow-up survey, and 31.8/1,000PY (with an event number of 9, including two hips) for the 129 respondents to the mailed questionnaire (p<0.001).

Characteristics of the 1,285 male study participants are shown in Table 1. PEN levels were significantly higher in men who experienced fracture events, while esRAGE-to-PEN ratio values were significantly lower in those who experienced fracture events. Men with fracture events were older and had lower TH and LS BMDs, as well as significantly higher levels of HbA1c. No significant differences were observed with regard to other characteristics or prevalence rates of clinical risk factors, as shown in Table 1.

A comparison of characteristics to those of the 1,156 participants in the five-year follow-up survey and the 129 subjects who did not participate in the follow-up survey, but who responded to the mailed questionnaire in 2010 revealed that the respondents were older and had lower FN and TH BMD values and higher PEN values than the participants (74.8±6.1 vs. 72.9±5.0 years, p=0.001; 0.709±0.122 vs. 0.746±0.115g/cm², p<0.001; 0.843±0.132 vs. 0.885±0.125g/cm², p<0.001; geometric mean (geometric SD), 0.053 (0.080) vs. 0.049 (0.070) µg/ml, p=0.007).

There were no significant differences in characteristics among the respondents when compared to the participants except for higher smoking rate in the respondents (24.8 vs. 14.2%, p=0.003).

PEN, esRAGE, and esRAGE-to-PEN ratio and fracture prediction

Kaplan–Meier analysis indicated no significant differences in the incidence of fractures between the lowest tertile of PEN levels and the middle and highest tertiles of PEN levels (Supplemental Figure 1). However, the lowest tertile of esRAGE levels was associated with a significantly higher number of fractures than were the middle and highest tertiles combined (Figure 2). The crude HRs per one SD increase in PEN and esRAGE values were 1.56 (95% CI, 1.05–2.31; p = 0.027) and 0.79 (95% CI, 0.54–1.15; p = 0.220), respectively. In the model with PEN and esRAGE as the explanatory variables, those HRs were 1.57 [1.06, 2.33] with p-values of 0.026 and 0.78 [0.54, 1.15] with p-values of 0.207, respectively. Adjusted HRs for PEN in Model 1 were indicative of an association with increased risk of fracture events after adjusting for age, esRAGE, and T-score of BMD at FN, TH, or LS (Table 2). Statistical significance or marginal significance is shown in Table 2. However, the significance attenuated after adjusting for HbA1c levels, as shown in Model 2 (Table 2).

Incidence rates between the lowest tertile of esRAGE-to-PEN ratio levels and the middle and highest tertiles of esRAGE-to-PEN ratio levels indicated a significant divergence shown in Figure 3. The crude HR per one SD increases for esRAGE-to-PEN ratio levels was significantly associated with a decreased fracture risk, with a value of 0.65 (95%CI, 0.44, 0.95; p=0.025). The significance of HRs for esRAGE-to-PEN ratios remained after adjusting for age and T-score of BMD at any three skeletal sites shown in Model 3 (Table 2). This significance additionally
remained after adjusting for HbA1c levels shown in Model 4 (Table 2). The HR values [95%CI] for esRAGE-to-PEN ratios, when adjusted for age, HbA1c, smoking rate, and T-score of BMD at FN, TH, and LS, were 0.66 [0.45, 0.98], 0.65 [0.44, 0.96], 0.64 [0.44, 0.95], respectively, while the smoking rate in responders to the mail questionnaire was higher than that of the participants in the follow-up survey.

According to AIC, each model depicting the esRAGE-to-PEN ratio afforded significantly better fit to the data than did the corresponding model depicting PEN and esRAGE values, as shown in Table 2. The AIC values for Models 1 and 3, when adjusted for FN BMD, were 329.1 and 327.5, respectively. The AIC values of each model with TH BMD indicated significantly better fit to the data than the corresponding model with FN BMD.

Fracture prediction in participants with present illness of diabetes mellitus

The 232 men with present illness of diabetes mellitus at the time of the baseline survey was used for the analysis (median [IQR] follow-up period, 4.3 [3.8–4.6] years; total PY, 930.3PY). Fracture events were ascertained in eight men, with a median time from baseline survey to fracture event of 1.5 years (IQR, 0.4–2.3 years). The characteristics and findings shown in Supplemental Table, while no significant difference was found in PEN, esRAGE, or esRAGE-to-PEN ratio levels between men with and without fracture events (Supplemental Table). The crude HR per one SD increase in PEN, esRAGE, and esRAGE-to-PEN ratio levels was 1.13 (95% CI, 0.57–2.25; p = 0.720), 0.85 (95% CI, 0.43–1.68; p = 0.648), and 0.81 (95% CI, 0.41–1.62; p = 0.559), respectively.

Fracture prediction in participants without present illness of diabetes mellitus

This analysis was used to evaluate the fracture event risk of 1,053 men without present illness of diabetes mellitus at the time of the baseline survey. From the time of the baseline survey to a median follow-up period of 4.1 years (IQR, 3.8–4.6 years; total PY, 4,215.5PY), fracture events were discovered in 17 men, with a median time from baseline survey to fracture event of 1.4 years (IQR, 0.9–3.7 years).

The results obtained among the 1,053 men without present illness of diabetes mellitus were similar to the results obtained in the overall cohort of 1,285 men with regard to characteristics and findings shown in Table 3, while no significant difference was found in HbA1c levels between men with and without fracture events (median values [IQR]; 5.6 [5.4, 5.9] vs. 5.4 [5.2, 5.7]%, respectively) in those 1,053 men. The Kaplan–Meier analysis indicated marginal significant differences in incidence rates between the lowest tertile of esRAGE-to-PEN ratio levels and the middle and highest tertiles of esRAGE-to-PEN ratio levels (log rank test, p = 0.053). The crude HR per one SD increase in esRAGE-to-PEN ratio levels was 0.62 (95% CI,
The HRs for esRAGE-to-PEN ratios in Model 4 indicated marginal significance or significance after adjusting for age, HbA1c, and FN or TH BMD (Table 3).

The AIC values of each model that included esRAGE-to-PEN ratio did not indicate significantly better fit to the data than the corresponding models that included PEN and esRAGE values (Table 3). There was no significant difference in goodness-of-fit between models with TH BMD, and corresponding models with FN BMD (Table 3).

**The sensitivity, specificity, and LR findings for esRAGE-to-PEN ratio**

The formula was derived from the logistic regression analysis using esRAGE-to-PEN ratio as an explanatory variable to estimate the expected probability of osteoporotic fracture events (P) (Supplemental figure 2). Using the Youden index, the cut-off value for P was set at 0.0197 (corresponding value of esRAGE-to-PEN ratio, 0.430) to most accurately predict osteoporotic fracture events as found in the ROC analysis (Supplemental figure 2). The sensitivity was marginally significant using the cut-off value, while the sensitivity and the LR indicated statistically significant (Supplemental figure 2).

**Discussion**

The present study found that serum PEN levels and serum esRAGE-to-PEN ratio values were associated with a risk of fracture events over a five-year period in community-dwelling elderly men. This risk occurred independently of BMD. The effect of esRAGE-to-PEN ratio values was associated with a decreased risk of fracture events independently of HbA1c levels. These associations demonstrated statistical or marginal significance in participating men without present illness of diabetes mellitus.

To date, this is the first longitudinal study to indicate that serum esRAGE-to-PEN ratio values are associated with the risk of fragility fracture events independently of BMD and HbA1c values in a community-dwelling population. Serum PEN levels itself did not indicated the statistical significance to predict fracture events after adjusting for age, esRAGE, BMD, and HbA1c in our findings. A previous report has indicated that PEN improves risk classification obtained by the FRAX score to predict fracture risk (15). We would surmise, therefore, that the usage of PEN and esRAGE values improves the fracture risk classification. The association between esRAGE-to-PEN ratio and fracture risk is consistent with biological plausibility (19). Previous reports indicated that accumulates of PEN cross-linking, instead of enzymatic cross-linking, within collagen fibers are thought to impair matrix properties; the tensile strength, toughness, and postyield properties in bone (36). Also, it has been reported that the interaction of esRAGE with pro-inflammatory proteins suppresses the signal transduction pathways induced by the RAGE ligands that propagate inflammation (37). In addition, an increase in reactive oxygen
species by the ligation of AGEs with RAGE (22) would be associated increased risk of fractures, as in a case-control study using elderly women with hip fractures as cases, mRNA expression of reactive oxygen species (SOD2 and Gpx3) in the fractured hip was higher than in the control (38).

These findings suggest that higher esRAGE-to-PEN ratio values are significantly associated with a decreased risk of fracture events, independent of age, BMD levels, and HbA1c levels, among elderly men dwelling in communities, though the significance was almost marginal in our findings after additional adjustment for HbA1c among subjects without present illness of diabetes mellitus. In previous studies using patients with type 2 diabetes, urine PEN (13) or serum esRAGE-to-PEN levels (23) were associated with prevalence or incidence of fracture independently of HbA1c and BMD values. On the other hand, the increased PEN levels were not associated with an increase in the risk of fracture after adjustment for age, esRAGE, the T-score of BMD, and HbA1c levels. In our findings, either of PEN, esRAGE, or esRAGE-to-PEN ratio did not indicated statistical significance to predict for osteoporotic fractures. We thought we have not enough sample size for the analysis in subjects with diabetes mellitus. In previous studies using non-diabetic subjects, it is indicated that the content of PEN in bone was negatively associated with reduced mechanical properties and toughness (39). Thus, the deterioration in bone strength induced by increased PEN or decreased esRAGE-to-PEN levels would be associated with fracture risk independently of HbA1c and BMD values. No comparative studies have been conducted to date. We also admit that HbA1c would mediate the relationship between PEN and fracture risks, as the significance of the HRs for PEN were attenuated after adjustment for HbA1c in our findings.

There are several limitations to the present study. First, the follow-up rate of our study was relatively low, which induced lower observed fracture event rates compared to those in the corresponding general population (1). Second, all study participants are volunteers, rather than a random sample of the general population. Thus, subjects with severe diabetes mellitus may have been less likely to participate in the study. Third, the diagnosis of diabetes mellitus would not be accurate, as it was based on self-reported data. Fasting blood glucose and HbA1c levels were determined only once, and glucose tolerance was not tested. The possibility of the underestimation of diabetes mellitus may reduce the accuracy of findings, though the misclassification of diabetes mellitus would be infrequent, as no significant difference in HbA1c levels was observed between subjects with and without fracture events. Fourth, the measurement of PEN using ELISA has been reported to involve some degree of uncertainty (40). Some factors in human serum can prevent AGE-antibody reactions in ELISA (40). The systematic error that might be yielded by the above issue would be related with underestimating the effect of PEN and overestimating esRAGE-to-PEN ratio for fracture events. However, the lower accuracy of the
PEN levels, obtained by ELISA, compared with those obtained by ultra-performance liquid chromatography tandem mass spectrometry would underestimate our findings that esRAGE-to-PEN ratio was associated with a fracture risk. Finally, this study did not confirm all of self-reported fracture events with medical record data. We also recognize that we have not investigated the relationship between the ratio of esRAGE to pentosidine and morphometric vertebral fractures. However, previous studies, in which both false-positive and false-negative rates of self-reporting were available (41, 42), indicated that self-reported data are relatively accurate for forearm, vertebral, and hip fractures. In the present study, the number of fractures at corresponding sites accounted for 72% of the total number of fractures. We also confirmed that 76% of all fracture events with medical records. Thus, we believe that the data regarding the fracture events was worthy of analysis, although we recognize this as a limitation of our study.

The FORMEN study, a large-scale, longitudinal study of community-dwelling elderly men, found that serum esRAGE-to-PEN ratio values were significantly and inversely associated with fragility fracture events. These associations were independent of BMD in both the diabetic and non-diabetic populations. Study findings indicated that the risk of fragility fracture events may be predicted by the balance between PEN and esRAGE levels. Usage of PEN and esRAGE values are expected to improve fracture risk classification, the improved values of which may be useful for fracture prevention.

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References


Figure 1. Participant flow diagram, FU: Follow up, a At baseline survey

Figure 2. esRAGE levels at baseline and cumulative incidence rates of fragility fracture during subsequent 5 years, esRAGE; endogenous secretory receptor for advanced glycation end products

Figure 3. esRAGE-to-pentosidine ratio at baseline and cumulative incidence rates of fragility fracture during subsequent 5 years, esRAGE; endogenous secretory receptor for advanced glycation end products, esRAGE-to-pentosidine ratio was calculated as esRAGE (ng/ml) divided by pentosidine (µg/ml)

Table 1  Participant characteristics from the baseline FORMEN Study, 2007.

<table>
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<tr>
<th>Characteristics and clinical risk factors</th>
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<th>Subjects with Fx event (n=25)</th>
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<tr>
<td>Height (cm), mean (SD)</td>
<td>162.8 (5.7)</td>
<td>162.9±5.7</td>
<td>161.5±6.2</td>
<td>0.239</td>
</tr>
<tr>
<td>Weight (kg), mean (SD)</td>
<td>60.9(8.3)</td>
<td>60.9±8.4</td>
<td>58.5±7.2</td>
<td>0.150</td>
</tr>
<tr>
<td>Body mass index (kg/m²), mean (SD)</td>
<td>22.9 (2.7)</td>
<td>22.9±2.7</td>
<td>22.5±3.2</td>
<td>0.521</td>
</tr>
<tr>
<td>Total hip BMD (g/cm²), mean (SD)</td>
<td>0.881 (0.126)</td>
<td>0.882 (0.125)</td>
<td>0.822 (0.149)</td>
<td>0.017</td>
</tr>
<tr>
<td>Femoral neck BMD (g/cm²), mean (SD)</td>
<td>0.743(0.116)</td>
<td>0.744 (0.116)</td>
<td>0.701 (0.126)</td>
<td>0.069</td>
</tr>
<tr>
<td>Lumbar spine BMD (g/cm²), mean (SD)</td>
<td>1.024(0.191)</td>
<td>1.027±0.192</td>
<td>0.915±0.150</td>
<td>0.004</td>
</tr>
<tr>
<td>Diabetes mellitus b, no. (%)</td>
<td>235 (18.3%)</td>
<td>227 (18.0%)</td>
<td>8 (32.0%)</td>
<td>0.110</td>
</tr>
<tr>
<td>Prediabetes (HbA1c 5.7-6.4%)</td>
<td>305 (23.7%)</td>
<td>296 (23.5%)</td>
<td>9 (36.0%)</td>
<td>0.156</td>
</tr>
<tr>
<td>Rheumatoid arthritis, no. (%)</td>
<td>8 (0.6%)</td>
<td>8 (0.6%)</td>
<td>0 (0%)</td>
<td>0.999</td>
</tr>
<tr>
<td>Oral glucocorticoid therapy c, no. (%)</td>
<td>11 (0.9%)</td>
<td>11 (0.9%)</td>
<td>0 (0.0%)</td>
<td>0.999</td>
</tr>
<tr>
<td>Previous fragility fractures, no. (%)</td>
<td>53 (4.1%)</td>
<td>52 (4.1%)</td>
<td>1 (4.0%)</td>
<td>0.999</td>
</tr>
<tr>
<td>Parental history of hip fractures, no. (%)</td>
<td>84 (6.5%)</td>
<td>83 (6.6%)</td>
<td>1 (4.0%)</td>
<td>0.999</td>
</tr>
<tr>
<td>Current smoker, no. (%)</td>
<td>197 (15.3%)</td>
<td>194 (15.4%)</td>
<td>3 (12.0%)</td>
<td>0.999</td>
</tr>
<tr>
<td>Ethanol intake (≥3 unit/day), no. (%)</td>
<td>318 (24.7%)</td>
<td>312 (24.8%)</td>
<td>6 (24.0%)</td>
<td>0.999</td>
</tr>
<tr>
<td>HbA1c (%), (Interquartile range)</td>
<td>5.5 (5.2, 6.0)</td>
<td>5.5 (5.2, 5.9)</td>
<td>5.8 (5.5, 6.5)</td>
<td>0.011</td>
</tr>
</tbody>
</table>
Pentosidine (µg/ml), geometric mean (SD) | 0.049 (1.427) | 0.049 (1.426) | 0.057 (1.402) | 0.031
esRAGE (ng/ml), geometric mean (SD) | 0.240 (1.683) | 0.240 (1.684) | 0.205 (1.580) | 0.128
esRAGE-to-pentosidine ratio, geometric mean (SD) | 4.9 (1.9) | 4.9 (1.9) | 3.6 (1.8) | 0.013

esRAGE; endogenous secretory receptor for advanced glycation end products

Data for lumbar spine BMD were obtained from 1,237 participants with no deformities at the second, third or fourth lumbar vertebrae.

Table 1 (Cont.)

Physician-diagnosed, or one of the following test results (FPG level ≥126 mg/dL or HbA1c levels ≥6.5%) obtained in the present study.

Oral glucocorticoid therapy for which potency of prednisolone was 5 mg/day for more than 3 months.

esRAGE-to-pentosidine ratio was calculated as esRAGE (ng/ml) divided by pentosidine (µg/ml).

Table 2. Hazard ratios of fragility fractures in the FORMEN Study.

<table>
<thead>
<tr>
<th></th>
<th>Adjusted for T-score of FN BMD (N=1285)</th>
<th>Adjusted for T-score of TH BMD (N=1285)</th>
<th>Adjusted for T-score of LS BMD (N=1237)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95%CI) p-value</td>
<td>HR (95%CI) p-value</td>
<td>HR (95%CI) p-value</td>
</tr>
<tr>
<td>Model 1b (including age)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pentosidine per SD 1.48 (1.00,2.18)</td>
<td>0.05 (95%CI) 0.01,2.18</td>
<td>0.04 (95%CI) 0.01,2.18</td>
<td>0.037 (95%CI) 0.01,2.18</td>
</tr>
<tr>
<td>esRAGE per SD 0.70 (0.48,1.05)</td>
<td>0.08 (95%CI) 0.00,1.05</td>
<td>0.07 (95%CI) 0.00,1.05</td>
<td>0.065 (95%CI) 0.00,1.05</td>
</tr>
<tr>
<td>T-score of BMD 0.73 (0.49,1.10)</td>
<td>0.13 (95%CI) 0.00,1.10</td>
<td>0.02 (95%CI) 0.00,1.10</td>
<td>0.008 (95%CI) 0.00,1.10</td>
</tr>
<tr>
<td>AIC</td>
<td>329.1</td>
<td>326.4</td>
<td>321.1</td>
</tr>
<tr>
<td>Model 2c (including age and HbA1c)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pentosidine per SD 1.36 (0.93,1.99)</td>
<td>0.11 (95%CI) 0.04,1.99</td>
<td>0.09 (95%CI) 0.04,1.99</td>
<td>0.072 (95%CI) 0.04,1.99</td>
</tr>
<tr>
<td>esRAGE per SD 0.76 (0.51,1.13)</td>
<td>0.17 (95%CI) 0.05,1.13</td>
<td>0.15 (95%CI) 0.05,1.13</td>
<td>0.158 (95%CI) 0.05,1.13</td>
</tr>
<tr>
<td>T-score of BMD 0.69 (0.46,1.04)</td>
<td>0.07 (95%CI) 0.02,1.04</td>
<td>0.01 (95%CI) 0.02,1.04</td>
<td>0.003 (95%CI) 0.02,1.04</td>
</tr>
<tr>
<td>AIC</td>
<td>327.8</td>
<td>324.7</td>
<td>319.0</td>
</tr>
</tbody>
</table>
Model 3\(^a\) (including age)

<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>esRAGE-to-pentosidine ratio(^d) per SD</td>
<td>0.6</td>
<td>(0.41,0.89)</td>
<td>0.01</td>
<td>0.6</td>
<td>(0.41,0.88)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td></td>
<td>1</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>T-score of BMD</td>
<td>0.7</td>
<td>(0.48,1.10)</td>
<td>0.12</td>
<td>0.6</td>
<td>(0.42,0.94)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td></td>
<td>3</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>AIC</td>
<td>327.5</td>
<td></td>
<td>324.7</td>
<td></td>
<td>319.5</td>
</tr>
</tbody>
</table>

Model 4\(^a\) (including age and HbA1c)

<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>esRAGE-to-pentosidine ratio(^d) per SD</td>
<td>0.6</td>
<td>(0.45,0.98)</td>
<td>0.04</td>
<td>0.6</td>
<td>(0.44,0.96)</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td></td>
<td>1</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>T-score of BMD</td>
<td>0.6</td>
<td>(0.45,1.04)</td>
<td>0.07</td>
<td>0.6</td>
<td>(0.40,0.89)</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td></td>
<td>4</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>AIC</td>
<td>326.1</td>
<td></td>
<td>322.9</td>
<td></td>
<td>317.4</td>
</tr>
</tbody>
</table>

Table 2 (Cont.)

BMD; bone mineral density, HR; hazard ratio, FN; femoral neck, TH; total hip, AIC; Akaike’s information criterion, esRAGE; endogenous secretory receptor for advanced glycation end products, per SD; per one standard deviation increase of log-transformed values of pentosidine, esRAGE, esRAGE-to-pentosidine ratio, or T-score of BMD.

In models 1 and 2, the values of the dependent variables (first row) were obtained after adjustment for the variables in the second and third rows.

\(^a\)The number of fragility fracture events was 25 in the model with 1,285 subjects, and 25 in the model with 1,237 subjects.

\(^b\)Explanatory variables of Models 1 or 3 are variables shown in the table and age.

\(^c\)Explanatory variables of Models 2 or 4 are variables shown in the table and age and dummy variables of quartiles of HbA1c values with the first quartile as a reference; the 2nd, 3rd, and the 4th quartile range was 5.3-5.5%, 5.6-5.9%, and 6.0-10.4% for the model using the 1285 subjects, and 5.3-5.5%, 5.6-5.8%, and 5.9%-10.4% for the model using the 1237 subjects, respectively.

\(^d\)esRAGE-to-pentosidine ratio was calculated as esRAGE (ng/ml) divided by pentosidine (µg/ml).

Table 3 Hazard ratios of fragility fractures\(^a\) in men without present illness\(^b\) in the FORMEN Study.
### Table 3 (Cont.)

<table>
<thead>
<tr>
<th>Model</th>
<th>esRAGE-to-pentosidine ratio per SD</th>
<th>T-score of BMD</th>
<th>AIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1° (including age)</td>
<td>0.6 (0.34 ,0.95 )</td>
<td>0.9 (0.56 ,1.50 )</td>
<td>216.3</td>
</tr>
<tr>
<td></td>
<td>0.5 (0.37 ,0.94 )</td>
<td>0.7 (0.48 ,1.21 )</td>
<td>215.7</td>
</tr>
<tr>
<td></td>
<td>0.5 (0.36 ,0.92 )</td>
<td>0.6 (0.37 ,1.04 )</td>
<td>211.5</td>
</tr>
<tr>
<td>Model 2° (including age and HbA1c)</td>
<td>0.6 (0.34 ,0.95 )</td>
<td>0.9 (0.56 ,1.50 )</td>
<td>216.8</td>
</tr>
<tr>
<td></td>
<td>0.5 (0.37 ,0.94 )</td>
<td>0.7 (0.48 ,1.21 )</td>
<td>214.7</td>
</tr>
<tr>
<td></td>
<td>0.5 (0.36 ,0.92 )</td>
<td>0.6 (0.37 ,1.04 )</td>
<td>211.9</td>
</tr>
<tr>
<td>Model 3° (including age)</td>
<td>0.6 (0.34 ,0.95 )</td>
<td>0.9 (0.56 ,1.50 )</td>
<td>215.6</td>
</tr>
<tr>
<td></td>
<td>0.5 (0.37 ,0.94 )</td>
<td>0.7 (0.48 ,1.21 )</td>
<td>214.9</td>
</tr>
<tr>
<td></td>
<td>0.5 (0.36 ,0.92 )</td>
<td>0.6 (0.37 ,1.04 )</td>
<td>210.6</td>
</tr>
<tr>
<td>Model 4° (including age and HbA1c)</td>
<td>0.6 (0.39 ,1.01 )</td>
<td>0.9 (0.55 ,1.49 )</td>
<td>215.9</td>
</tr>
<tr>
<td></td>
<td>0.6 (0.39 ,1.00 )</td>
<td>0.7 (0.48 ,1.29 )</td>
<td>215.10</td>
</tr>
<tr>
<td></td>
<td>0.6 (0.39 ,0.99 )</td>
<td>0.6 (0.36 ,1.05 )</td>
<td>211.0</td>
</tr>
</tbody>
</table>

BMD; bone mineral density, HR; hazard ratio, FN; femoral neck, TH; total hip, AIC; Akaike’s information criterion, esRAGE; endogenous secretory receptor for advanced glycation end products, per SD; per one standard deviation increase of log-transformed values of pentosidine, esRAGE, esRAGE-to-pentosidine ratio, or T-score of BMD.
In models 1 and 2, the values of the dependent variables (first row) were obtained after adjustment for the variables in the second and third rows.

- The number of fragility fracture events was 17 in the model with 1,053 subjects and 17 in the model with 1,018 subjects.
- Men without present illness were included, except for those who were diagnosed with diabetes mellitus by physicians, or who indicated one of the following test results (FPG level ≥ 126mg/dl or HbA1c levels ≥ 6.5%) obtained in the baseline survey.
- Explanatory variables of Models 1 or 3 are variables shown in the table and age.
- esRAGE-to-pentosidine ratio was calculated as esRAGE (ng/ml) divided by pentosidine (µg/ml).
- Explanatory variables of Models 2 or 4 are variables shown in the table and age and dummy variables of tertile of HbA1c values with the first tertile as a reference; the 2nd and 3rd tertile range was 5.2-5.5% and 5.6-6.4% for the models using 1053 or 1018 subjects shown in the table.