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ORIGINAL ARTICLE

Decreased cortical thickness, as estimated by a newly developed ultrasound device, as a risk for vertebral fracture in type 2 diabetes mellitus patients with eGFR of less than 60 mL/min/1.73 m^2

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Abstract
Summary Cortical porosity is increasingly recognized as an important risk for fracture in DM patients. The present study demonstrated that decreased cortical thickness, assessed using a newly developed quantitative ultrasonic bone densitometry, is a significant risk factor for vertebral fractures in type 2 diabetes mellitus patients with stage 3 or higher chronic kidney disease, but not in those without.
Introduction Cortical porosity is increasingly recognized as an important risk factor for fracture in type 2 diabetes mellitus (T2DM) patients as well as in stage 3 chronic kidney disease (CKD) patients in whom serum parathyroid hormone (PTH) starts to increase. The present study aimed to clarify whether the coexistence of CKD might affect the relationship of decreased cortical thickness (CoTh) in the development of vertebral fractures (VF) in T2DM patients.
Methods In this cross-sectional study, trabecular bone mineral density (TrBMD), elastic modulus of trabecular bone (EMTb), and CoTh were estimated with a new quantitative ultrasound bone densitometry in 173 T2DM patients. VFs were identified radiographically.
Results Thirty-nine patients (22.5 %) had VF. Those with estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m^2 (low eGFR) showed a significantly higher VF rate (32.4 %) than those with eGFR ≥60 mL/min/1.73 m^2 (high eGFR, 16.2 %). Serum PTH was significantly higher with low eGFR than with high eGFR. In those with high eGFR, EMTb was significantly lower in VF(+) than VF(−). In those with low eGFR, TrBMD, EMTb, and CoTh were significantly lower in VF(+) than in VF(−). In a multivariate logistic regression analysis, EMTb was independently and significantly associated with VF in T2DM patients with a high eGFR, in contrast to those with only CoTh with VF in T2DM with low eGFR.
Conclusion This study demonstrated CoTh as a factor independently associated with VF in T2DM patients with low eGFR and increasing serum PTH levels.

Keywords Bone fracture · Bone mineral density · Chronic kidney disease · Cortical thickness · Type 2 diabetes

Introduction

It is increasingly recognized that patients with diabetes mellitus (DM) are at higher risk of bone fracture relative to their bone mineral density (BMD) [1, 2]. A number of reports, including ours [3], indicate a higher prevalence of vertebral fracture (VF) in those with type 2 DM (T2DM) than in those without, irrespective of the insignificant difference in BMD between patients with or without T2DM [4]. Meta-analysis studies recently reported that T2DM patients exhibited a higher fracture rate, particularly in appendicular bones, despite their comparable BMD [5, 6], suggesting the possible involvement of impaired bone quality, but not BMD, in the development of bone fragility [7, 8]. It is increasingly recognized that

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the higher fracture rate in T2DM patients is explained by increased cortical porosity, as shown by high-resolution peripheral quantitative computed tomography (HR-pQCT) [9]. We have recently reported validation of a newly developed quantitative ultrasound (QUS) device, the LD-100 system (Oyo Electric, Kyoto, Japan); this system can estimate both trabecular and cortical bone components [10, 11], as illustrated by a significant correlation of bone parameters estimated by the new QUS with the respective parameters as measured by Stratec pQCT [12].

It has been recently recognized that serum parathyroid hormone (PTH) plays an important role in the development of cortical porosity, which starts to increase in chronic kidney disease (CKD) patients as they progress to stage 3 [13, 14], for whom evidence shows a higher fracture rate at the femur neck [15–18]. Since T2DM patients are complicated with stage 3 CKD more often than non-DM patients, it is important to examine whether DM by itself or in association with CKD might be a more important contributing factor to the development of cortical porosity.

Therefore, the present study aimed to determine (i) trabecular and cortical bone components in T2DM patients using the new QUS device and (ii) to determine the involvement of CKD complications in the development of decreased cortical thickness in T2DM patients.

Subjects and methods

Subjects

A total of 173 Japanese T2DM patients (98 men and 75 women) admitted to the Diabetes Center at Osaka City University Hospital were enrolled consecutively in the study. Written informed consent was obtained from all patients prior to participation. This cross-sectional study was approved by the Ethics Review Committee of Osaka City University Graduate School of Medicine (no. 164).

Patients diagnosed as T2DM according to Japan Diabetes Society (JDS) criteria were on either dietary therapy only, oral agents (30 % sulfonylurea, 35 % dipeptidyl peptidase-4 inhibitor, 2.1 % glinide, 32.1 % metformin, 5.7 % thiazolidinedione, or 7.1 % glucagon-like peptide-1 agonist), or insulin (39 %) for glycemic control. Mean values for age and estimated glomerular filtration rate (eGFR) were 62.3± 12.2 years and 64.2±25.2 mL/min/1.73 m^2, respectively.

All patients were free of significant acute illness or malignancy. Patients with severe renal failure such as stage 5 CKD and abnormal calcium metabolism such as primary hyperparathyroidism or a history of falls or traffic accidents were excluded to eliminate the possibility of injury-associated fractures. To avoid confounding results due to the treatment, patients who had a history of taking any drugs or hormones that affect bone metabolism, such as corticosteroids, warfarin, anti-osteoporotic drugs such as bisphosphonates, PTH (1–34), vitamin D derivatives, estrogen and selective estrogen receptor modulators, supplementary calcium, phosphate binders, or thyroid hormone, were excluded from the present study.

Biochemical parameters

Venous blood samples were obtained in the morning after an overnight fast. Fasting plasma glucose (FPG) was measured by the enzymatic method using an autoanalyzer in the hospital (GA-1171; ARKRAY, Inc., Kyoto, Japan), and glycated hemoglobin (HbA1c) was measured using high-performance liquid chromatography (HA-8180; ARKRAY, Inc., Kyoto, Japan). Calcium (Ca), phosphate (P), and creatinine (Cr) were measured by enzymatic methods using an autoanalyzer in the hospital (BM6070; JEOL Ltd., Tokyo, Japan). Measurement of serum PTH levels was performed simultaneously to avoid inter-assay variance. Serum active PTH (1–84) levels were measured using the whole PTH assay (LSI Medience Co., Tokyo, Japan) with a two-site IRMA assay as described previously [19]. Serum 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) was determined by radioimmunoassay (LSI Medience Co., Tokyo, Japan). Tartrate-resistant acid phosphatase-5b (TRACP-5b) activity was measured by using the enzyme-linked immunoassay (novel fragment absorbed immunocapture enzymatic assay [FAICEA]) method using two monoclonal antibodies (BML, Inc., Tokyo, Japan). Serum bone alkaline phosphatase (BAP) and intact osteocalcin (OC) were measured with an enzyme immunoassay. To assess renal function, eGFR was calculated using the equation proposed by the Japanese Society of Nephrology [20]: $\text{eGFR (mL/min/1.73 m}^2) = 175 \times \text{serum creatinine}^{-1.154} \times \text{age}^{-0.203}$. For women, this value was multiplied by 0.742. HOMA-IR was calculated from the FPG and serum insulin as follows: HOMA-IR=(FPG [mg/dL])×(fasting insulin [μU/mL])/405 [21].

Bone densitometry

Ultrasonic measurements were performed on the nondominant, ultradistal radius using the new QUS device, allowing estimation of trabecular BMD (TrBMD) of the radius, elastic modulus of the trabecular component (EMTb), and cortical thickness (CoTh) [12, 22, 23]. Validation of TrBMD and CoTh estimated by the new QUS device was done by demonstrating a significant correlation with values measured by Stratec pQCT [12]. Breban reported reproducibility values of 4.8, 10.3, and 3.5 % for TrBMD, EMTb, and CoTh, respectively [10].

As reported previously, the new QUS device consists of two ultrasonic transducers, located coaxially in the forward direction and it is equipped with a computer system. The transducers move simultaneously for scanning: one transmits

ultrasound through the objective region and the other receives ultrasonic signals. TrBMD, which is estimated by quantifying attenuation of ultrasound waves transmitted through the bone [22], is expressed in milligrams per cubic centimeter. In the field of structural physics in particular, it has been established that the EMTb (structural elasticity) can be quantified by the transmission velocity of the fast wave propagated through the trabecular bone structures [11, 24]; values are expressed in gigapascal (GPa). The new QUS device can also estimate CoTh by analyzing the reflected and transmitted ultrasonic signals as previously described [23, 25]. A pair of broadband ultrasonic transducers are faced and coaxially aligned in the water. The distance between the two transducers (distance A) and the ultrasonic signal level is checked automatically by the transmission mode through the water as a reference medium.

As shown in Supplemental Fig. 1, the total radial thickness (distance B) is estimated by the echo measurements of both transducers; that is, both transducers are used to measure each distance between the transducer surface and the cortical bone surface by the echo method. The total radial thickness (distance B) is estimated by subtracting the distances of the transducer and the cortical surface of both sides (distance C1+C2) from the distance between the two transducers (distance A). The total radial thickness can be expressed as B=A–(C1+C2) (Supplemental Fig. 1a). It is not possible to apply the echo measurements to cortical thickness. The inner boundary of cortical bone is connected to the trabecular network through a rough, random, and arbitrary boundary layer. Incident ultrasound is scattered in the boundary region. The backscattered ultrasound or the reflected signal to the transducer cannot be used to measure cortical thickness because of considerable waveform deformation and amplitude reduction.

The new QUS device measures the fast and slow waves, transmitted through trabecular bone, to estimate bone parameters. The propagation speed of the fast wave and the amplitudes of both the fast and the slow waves depend on the trabecular bone state, whereas the propagation speed of the slow wave is always constant, independent of trabecular bone density. The propagation speed of the slow wave corresponds to that of the bone marrow.

CoTh (the sum of cortical bone thicknesses on the input and output sides of the ultrasonic wave) is estimated by the total radial thickness, and its propagation time is measured by the slow wave at nominal propagation speeds in the bone marrow (1,400 m/s) and in cortical bone (3,300 m/s; Supplemental Fig. 1b).

The validity of estimates was confirmed by comparing with measurements from pQCT; the new QUS system has been approved as medical equipment in Japan. CoTh is expressed in millimeters.

Assessment of fractures

In all subjects, VFs were identified on lateral and anterior-posterior X-ray films of the thoracic and lumbar spine according to the semiquantitative method by two investigators who were blinded to the other's readings. VFs were defined as grades 1–3 according to the classification advocated by Genant HK et al. [26]. When the grade was different between the two investigators, they were advised to reevaluate X-ray findings. When the result was still different, the milder grade was used.

Statistical analysis

All data were analyzed with the Stat View 5.0J program. Numerical parameters with normal distribution are shown as mean±SD, and those with non-normal distribution are expressed as median with interquartile range (IQR). The difference between T2DM patients with and without VFs was analyzed by Student's *t* test or the Mann-Whitney *U* test. Chi-square tests were used to compare fractures between men and women. Multivariate logistic regression analysis was performed to estimate the independent risk of VF. Odds ratios (ORs) and 95 % confidence intervals (95 % CIs) were collectively calculated. $p<0.05$ was defined as statistically significant.

Results

Clinical characteristics of enrolled patients

The baseline characteristics of the enrolled subjects are shown in Table 1. Age and duration of T2DM were 62.3 and 13.0 years, respectively, with a BMI of 26.1 kg/m^2. Parameters for glycemic control, such as FPG and HbA1c, were above their respective normal upper limits. The parameter for insulin resistance, HOMA-R, was well above the normal upper limit.

Serum Cr was 0.84 (0.64–1.04)mg/dL, and eGFR was suppressed to 64.2±25.2 mL/min/1.73 m^2. Serum Ca, phosphate, and whole PTH levels were all within their respective normal ranges. Other serum parameters for Ca metabolism, such as 1,25$(OH)_2$D, TRACP-5b, and BAP, were all within their respective normal range; in contrast, serum intact OC was significantly reduced to 3.1 ng/mL. Among 173 T2DM patients, 39 patients (22.5 %) had X-ray findings, indicating prevalent VF on a lateral view of the vertebrae.

Comparison of clinical parameters between T2DM patients with and without VF

As shown in Table 2, the VF(+) group had a significantly greater age and duration of T2DM than the VF(−) group. There was no

Table 1 Clinical profiles of 173 T2DM patients

Clinical variables	
N (men/women)	173 (98/75)
Age (year)	62.3±12.2
Duration of T2DM (year)	13.0 (5.5–20.5)
BMI (kg/m^2)	26.1±5.5
Cr (mg/dL)	0.84 (0.64–1.04)
eGFR (mL/min/1.73 m^2	64.2±25.2
FPG (mg/dL)	116±35
HbA1c (%)	8.9±1.5
HOMA-R	2.00 (1.04–2.96)
Ca (mg/dL)	9.3±0.5
Phosphate (mg/dL)	3.9 (3.5–4.3)
Whole PTH (pg/mL)	19.2 (18.6–19.8)
1,25(OH)$_2$D (pg/mL)	40.0 (28.0–52.0)
TRACP-5b (mU/dL)	343 (231–455)
BAP (μg/L)	12.7 (9.1–16.3)
Intact OC (ng/mL)	3.1 (1.8–4.4)
TrBMD (mg/cm^3)	172±54
EMTb (Gpa)	2.96 (2.68–3.24)
CoTh (mm)	3.69±1.28
VF, *n* (%)	39 (22.5)

Data are expressed as *n*, *n* (%), mean±SD, or median (interquartile range)

BMI body mass index, *eGFR* estimated glomerular filtration rate, *FPG* Fasting plasma glucose, *HOMA-R* homeostasis model assessment ratio (insulin resistance), *IQR* interquartile range

Table 2 Comparison of clinical parameters between T2DM patients with and without VF

	VF(+) (*n*=39)	VF(−) (*n*=134)	*p* value
Age (year)	60.5±12.3	58.5±10.0	<0.001*
Gender (men/women)	20/19	78/56	0.467
Duration of T2DM (year)	19 (11–27)	11 (3–19)	0.016*
BMI (kg/m^2)	25.2±5.3	26.2±5.5	0.153
Cr (mg/dL)	0.86 (0.70–1.02)	0.81 (0.59–1.03)	0.123
eGFR (mL/min/1.73 m^2)	55.0±20.0	67.0±26.1	0.005*
FPG (mg/dL)	117±37	120±36	0.499
HbA1c (%)	7.7±1.6	8.4±1.6	0.045*
Ca (mg/dL)	9.2±0.6	9.3±0.5	0.677
Phosphate (mg/dL)	3.9 (3.6–4.2)	3.8 (3.4–4.2)	0.280
BAP (μg/L)	14.7 (10.9–18.5)	12.2 (8.8–15.6)	0.035*
Intact OC (ng/mL)	4.3 (3.0–5.6)	2.9 (1.8–4.0)	0.007*
1,25(OH)$_2$D (pg/mL)	34.0 (22.5–45.5)	41.0 (29.0–53.0)	0.775
Whole PTH (pg/mL)	20.8 (16.2–25.4)	18.2 (12.2–24.4)	0.036*
TRACP-5b (mU/dL)	427 (282–572)	329 (219–439)	0.006*
TrBMD (mg/cm^3)	142±49	182±53	<0.001*
EMTb (Gpa)	2.72 (2.57–2.87)	3.06 (2.78–3.34)	<0.001*
CoTh (mm)	3.23±1.07	3.85±1.31	0.014*

Data are expressed as mean±SD and were analyzed by Student's *t* test or median (IQR) and were analyzed by Mann-Whitney *U* test. A χ^2 test was used to analyze the difference between men and women

*$p<0.05$ (statistical significance)

difference in the prevalence of VF between men and women. Of interest, the eGFR in the VF(+) group was 55.0±20.0 mL/min/1.73 m^2, which was significantly lower than that in the VF(−) group (67.0±26.1 mL/min/1.73 m^2). Serum BAP, intact OC, whole PTH, and TRACP-5b were significantly higher in the VF(+) group than in the VF(−) group. All bone parameters, such as TrBMD, EMTb, and CoTh, were significantly lower in the VF(+) group.

Comparison of various clinical parameters between T2DM patients with and without VF, after segregation by eGFR < and ≥60 mL/min/1.73 m^2

To analyze the effect of excess PTH, which is involved in the stimulation of bone turnover in stage 3 CKD or higher [13, 14], enrolled patients were analyzed separately on the basis of eGFR, ≥ and <60 mL/min/1.73 m^2. PTH was significantly higher with eGFR <60 mL/min/1.73 m^2 (23.6 [15.8–31.4]pg/mL vs 17.2 [13.0–21.4]pg/mL for eGFR ≥60 mL/min/1.73 m^2, $p<0.001$).

As shown in Table 3, 105 (60.7 %) had eGFR ≥60 mL/min/1.73 m^2. The VF(+) group had a significantly lower eGFR (71.0±8.5 mL/min/1.73 m^2) than the VF(−) group (81.9±15.7 mL/min/1.73 m^2). Age and duration of T2DM were significantly greater in the VF(+) group than in the VF(−) group. VF prevalence did not differ between men and women. Although TrBMD and CoTh were not lower in the VF(+) group, EMTb was significantly lower in the VF(+) group than in the VF(−) group.

In 68 of the T2DM patients with an eGFR <60 mL/min/1.73 m^2, VF occurred in as many as 32.4 %, significantly higher than the respective value of 16.2 % in those with eGFR ≥60 mL/min/1.73 m^2. The VF(+) group had a significantly higher age and BAP than the VF(−) group. In addition, serum whole PTH and intact OC tended to be higher in the VF(+) group. TrBMD, EMTb, and CoTh were significantly reduced in the VF(+) group compared to the VF(−) group. Women exhibited higher fracture rate than men but not significantly.

Association between the presence of VF and the bone parameters in T2DM patients

To elucidate factors significantly associated with VF separately in the two patient groups based on eGFR, a multivariate logistic regression analysis was performed. As shown in Table 4, in patients with an eGFR ≥60 mL/min/1.73 m^2, among independent variables including age, gender, duration of T2DM, TrBMD, EMTb, and CoTh, duration of T2DM and

Table 3 Comparison of clinical parameters of T2DM patients with eGFR ≥ and eGFR <60 mL/min between VF(+) and VF(−) groups of patients (n=173)

	eGFR ≥60 (mL/min/1.73 m^2)			eGFR <60 (mL/min/1.73 m^2)		
	VF(+) (n=17)	VF(−) (n=88)	p value	VF(+) (n=22)	VF(−) (n=46)	p value
eGFR (mL/min/1.73 m^2)	71.0±8.5	81.9±15.7	0.010*	41.9±16.6	38.2±14.7	0.245
Age (year)	65.5±12.1	58.0±12.2	0.035*	71.0±6.6	65.5±10.8	0.025*
Gender (men/women)	9/8	45/43	0.891	11/11	33/13	0.079
Duration of T2DM (year)	21.0 (16.7–25.3)	8.0 (1.5–14.5)	<0.001*	18.0 (8.5–27.5)	15.0 (7.0–23.0)	0.573
HbA1c (%)	8.1±1.6	8.5±1.7	0.247	7.4±1.5	8.0±1.5	0.134
Ca (mg/dL)	9.3±0.4	9.2±0.4	0.362	9.0±0.8	9.2±0.6	0.182
Phosphate (mg/dL)	3.9 (3.6–4. 2)	3.9 (3.5–4.3)	0.766	3.8 (3.5–4.1)	3.7 (3.3–4.1)	0.318
BAP (μg/L)	14.6 (11.0–18.2)	12.5 (8.7–16.3)	0.322	16.0 (12.1–19.9)	12.0 (8.2–15.8)	0.015*
Intact OC (ng/mL)	3.0 (0.6–5.4)	2.8 (1.8–3.8)	0.350	5.3 (3.7–6.9)	3.5 (1.8–5.2)	0.070
$1,25(OH)_2D$ (pg/mL)	42.0 (33.2–50.8)	46.0 (33.4–58.6)	0.569	28.0 (39.5–36.5)	29.0 (18.5–39.5)	0.700
Whole PTH (pg/mL)	17.7 (12.8–22.6)	17.2 (13.1–21.3)	0.647	24.9 (15.1–34.7)	23.7 (14.8–32.6)	0.064
TRACP-5b (mU/dL)	396 (238–554)	325 (228–422)	0.165	456 (367–545)	361 (223–499)	0.196
TrBMD (mg/cm^3)	155±55	177±52	0.134	129±36	188±56	<0.001*
EMTb (Gpa)	2.66 (2.46–2.86)	3.05 (2.77–3.33)	0.007*	2.72 (2.56–2.88)	3.07 (2.78–3.36)	0.004*
CoTh (mm)	3.07±1.16	3.71±1.21	0.060	2.86±1.10	4.19±1.32	0.001*

Data are expressed as mean±SD and were analyzed by Student's t test or median (IQR) and were analyzed by Mann-Whitney U test. χ^2 tests were used to analyze the differences between men and women

*p<0.05 (statistical significance)

EMTb emerged as independent factors significantly associated with VF. In contrast, when the same analysis was performed in those with eGFR <60 mL/min/1.73 m^2, CoTh was the only independent factor significantly associated with VF.

Discussion

The present study demonstrated that multivariate regression analysis, including age, gender, duration of T2DM, TrBMD, EMTb, and CoTh as independent variables, longer duration of T2DM and lower EMTb, emerged as independent factors significantly associated with VF in T2DM patients with eGFR ≥60 mL/min/1.73 m^2, while CoTh was the only significant factor associated with VF in T2DM patients with eGFR <60 mL/min/1.73 m^2. This suggests the importance of impaired cortical bone components as the main factor for VF in T2DM patients with stage 3 CKD or higher; this is in contrast with the importance of trabecular bone in T2DM patients with eGFR ≥60 mL/min/1.73 m^2.

When comparing clinical parameters between two groups of T2DM patients with and without VF, T2DM VF(+) patients exhibited significantly longer duration of T2DM and significantly lower eGFR than T2DM VF(−) patients (Table 2). Of importance, T2DM VF(+) patients had a significantly higher PTH and bone turnover markers, BAP and TRACP-5b, which are not affected by renal dysfunction [27, 28], and significantly lower values of bone parameters, TrBMD, EMTb, and CoTh, as measured with the new QUS device, than T2DM VF(−) patients. Serum bone metabolic markers, such as serum BAP and TRACP-5b, were within their respective normal ranges, indicating that bone turnover was at normal levels.

Table 4 Associations between the presence of vertebral fractures and bone parameters in T2DM patients

	Presence of VF		
Independent variables	OR	95 %CI	p value
eGFR ≥60 mL/min/1.73 m^2			
Age (per 1 year increase)	0.99	0.92–1.07	0.863
Gender (women)	3.14	0.57–17.47	0.190
Duration of T2DM (per 1 year increase)	1.11	1.04–1.20	0.003*
TrBMD (per −1 quartile)	0.31	0.09–1.11	0.071
EMTb (per −1 quartile)	6.66	1.84–24.10	0.004*
CoTh (per −1 quartile)	1.84	0.64–5.28	0.260
eGFR <60 mL/min/1.73 m^2			
Age (per 1 year increase)	1.04	0.96–1.13	0.344
Gender (women)	1.41	0.27–7.43	0.683
Duration of T2DM (per 1 year increase)	0.99	0.95–1.06	0.945
TrBMD (per −1 quartile)	1.37	0.31–5.94	0.678
EMTb (per −1 quartile)	2.31	0.52–10.24	0.272
CoTh (per −1 quartile)	6.28	1.38–28.56	0.050*

95 % CI 95 % confidence interval, *OR* odds ratio

*p<0.05 (statistical significance)

Serum intact OC alone was reduced below its normal lower limit, which is characteristic of diabetic osteopathy [29] and might be accounted for by suppression due to poor glycemic control, independent of bone turnover [30].

These findings suggested the prevalence of CKD and the resultant development of secondary hyperparathyroidism with higher bone turnover as a possible mechanism for VF development in T2DM, as VF(+) patients displayed a significant decline in eGFR along with significant increases in whole PTH and bone metabolic markers. In CKD patients, it is known that serum PTH starts to increase in those with eGFR <60 mL/min/1.73 m^2 [13, 14]. We have previously reported that PTH is a major factor causing cortical osteoporosis in hemodialysis (HD) patients; this was accomplished by demonstrating a significant improvement in cortical bone histomorphometric parameters by serial bone biopsy of the iliac crest of patients with refractory secondary hyperparathyroidism before and after parathyroidectomy [31]. There was a strong correlation between serum PTH and BMD at the distal one third of the radius in HD patients [32].

Indeed, several studies have demonstrated that fracture rate at the femur neck, which is enriched in the cortical bone components, was significantly higher in CKD patients with eGFR <60 mL/min/1.73 m^2 [15–18]. This notion is supported by a report demonstrating that continuous PTH (1–34) injection could cause cortical porosity as well as a reduction of CoTh in dogs [33]. Furthermore, although a variety of mechanisms underlying impaired bone quality in T2DM patients has been proposed [3, 8, 34, 35], it has been increasingly recognized that cortical porosity might be a major mechanism explaining the higher fracture rate in T2DM patients [9, 36], irrespective of the insignificant reduction in BMD measured by dual-energy X-ray absorptiometry [2–4]. These notions led to a comparison of clinical characteristics of the enrolled patients after dividing them by eGFR ≥ or <60 mL/min/1.73 m^2 to examine the effect of hyperparathyroidism on bone metabolism in patients with low eGFR (Table 3). VF(+) patients with eGFR ≥60 mL/min/1.73 m^2 showed significantly lower EMTb and longer duration of T2DM. In contrast, VF(+) patients with eGFR <60 mL/min/1.73 m^2 showed reduced TrBMD and CoTh. The mechanism by which these bone parameters were significantly lower in VF(+) patients with eGFR <60 mL/min/1.73 m^2 might be explained by the occurrence of hyperparathyroidism with the concurrent development of high turnover bone disease.

In addition to duration of T2DM, multivariate regression analysis elucidated EMTb as an independent factor associated with VF in those with eGFR ≥60 mL/min/1.73 m^2, while CoTh emerged as the only independent factor in those with eGFR <60 mL/min/1.73 m^2. With lower eGFR, particularly when <60 mL/min/1.73 m^2, the effect of PTH on bone became greater. Since PTH acts preferentially on cortical bone components, the greater effect of PTH excess in those with eGFR <60 mL/min/1.73 m^2 might make parameters of cortical bone components, such as CoTh, more important for bone fragility in those with renal dysfunction.

In a study of these groups, we found that the proportion of CKD patients with eGFR <60 mL/min/1.73 m^2 in a Japanese population was as high as 15 % in their sixties and 27 % in their seventies (data unpublished), which agrees well with the 2012 CKD guide [37]. Due to the development of diabetic nephropathy, it is likely that the prevalence rate of CKD in T2DM patients is much higher [38]. Therefore, the more common occurrence of cortical osteoporosis with DM might be confounded by the coexistence of CKD in T2DM patients.

The mechanism by which CoTh may be associated with VF might have several explanations. First, since PTH enhances bone turnover in trabecular bone as well as cortical bone [39], the reduction of CoTh might reflect bone loss at trabecular bone-enriched vertebral bone. Second, since it was recently recognized that vertebral bone strength is also supported by the cortical bone-enriched vertebral cage [40], a greater deleterious effect of PTH on the cortical cage might cause VF in T2DM patients with eGFR <60 mL/min/1.73 m^2.

The strength of the present study is that this is the first report suggesting the involvement of diabetic nephropathy in decreased CoTh in DM patients; this was shown by dividing T2DM patients into two groups: those with eGFR ≥ or <60 mL/min/1.73 m^2, the point at which serum PTH is reported to increase [13, 14]. Therefore, the present study raised the possibility that protection against cortical osteoporosis, which is a definitive risk for femur neck fracture [41], might be obtained by treatment for diabetic nephropathy. Second, the validation of the new QUS device to determine parameters of cortical bone separate from trabecular bone may help distribute this apparatus more widely as a screening tool for cortical osteoporosis without any exposure to X-ray [11], although it is established that HR-pQCT is definitely the most sophisticated apparatus to precisely measure multiple bone parameters, including trabecular and cortical bone components [42, 43].

The limitations of the present study are as follows. First, this is a cross-sectional study and is insufficient to disentangle potential relationships among EMTb, CoTh, TrBMD, and the prevalence of VF since all parameters were estimated at a single point. Second, since our study examined a rather small number of Japanese T2DM patents, the results cannot be extended to other ethnicities. A large-scale, multiethnic study is needed to confirm these results. Third, since the T2DM patients had been treated with various drugs for DM, hypertension, and dyslipidemia, some drugs, which might affect bone metabolism, were prescribed. Therefore, the effect of such drugs on bone metabolism may have affected the results.

In conclusion, this is the first report demonstrating that the bone parameter CoTh is a significant factor independently associated with VF in T2DM patients with eGFR <60 mL/min/1.73 m^2 but not ≥60 mL/min/1.73 m^2, thus suggesting the involvement of DM nephropathy in increased serum PTH and the development of bone fragility in T2DM patients.

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Conflicts of interest None.

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