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Serum calcification propensity and its association with biochemical parameters and bone mineral density in hemodialysis patients

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Background: T_{50} is a novel serum-based marker that assesses the propensity for calcification in serum. A shorter T_{50} indicates a greater propensity to calcify and has been associated with cardiovascular disease and mortality among patients with chronic kidney disease. The factors associated with T_{50} and the correlation between T_{50} and bone mineral density (BMD) are unknown in hemodialysis (HD) patients.

Methods: This cross-sectional study included 184 patients undergoing HD. Individuals were grouped into tertiles of T_{50} to compare the demographic and disease indicators of the tertiles. Linear regression was used to evaluate the association between T_{50} and hip and spinal BMD in a multivariate model.

Results: Mineral and inflammatory parameters, including serum phosphate ($r = -0.156$, $p = 0.04$), albumin ($r = 0.289$, $p < 0.001$), and high-sensitivity C-reactive protein ($r = -0.224$, $p = 0.003$) levels, were associated with T_{50}. We found a weak association between T_{50} and BMD in the total hip area in the unadjusted model ($\beta = 0.030$, $p = 0.04$) but did not find a statistically significant association with the total hip ($\beta = 0.017$, $p = 0.12$), femoral neck ($\beta = -0.001$, $p = 0.96$), or spinal BMD ($\beta = 0.019$, $p = 0.33$) in multivariable-adjusted models.

Conclusion: T_{50} was moderately associated with mineral and inflammatory parameters but did not conclusively establish an association with BMD in HD patients. Broad-scale future studies should determine whether T_{50} can provide insights into BMD beyond traditional risk factors in this population.

Keywords: Bone mineral density, Hemodialysis, T_{50}, Vascular calcification

Introduction

Chronic kidney disease (CKD)-mineral bone disease (MBD) is a common complication of CKD that is associated with morbidity and mortality. Several studies have suggested an interconnection between vascular calcification, impaired bone and mineral metabolism, and increased mortality [1–3]. Recent studies have found that bone mineral density (BMD) measurement in patients with advanced CKD predicts the risk of fracture, which can be expected to provide

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nephrologists with skeletal fragility and targeted fracture prevention strategies [4–6].

T_{50} has been proposed as a potential novel serum-based marker for assessing calcification propensity [7]. With the initiation of calcium and phosphate precipitation in the serum, primary calciprotein particles (CPP) are formed that are rich in calcium and phosphate and contain small amounts of protein, including albumin and fetuin-A [7]. Over time, these primary CPPs are converted into larger secondary CPPs with different calcium, phosphate, and protein content. CPP maturation time (T_{50}) is a measurement of the *in vitro* conversion time from primary CPP to secondary CPP in the serum [7]. The balance of calcification enhancing and inhibitory factors in each serum sample is a critical factor in determining transformation time [8]. The shorter the T_{50}, the greater the tendency for calcification. A shorter T_{50} has been reported to be associated with increased risk of cardiovascular disease (CVD) and all-cause mortality in CKD patients [8,9].

Although vascular calcification and bone health are intercorrelated and are known risk factors for predicting cardiovascular events (CVE) in dialysis patients, the association between T_{50} and BMD in dialysis patients with a high CVE risk is not well understood. As renal function decreases in CKD patients, mineral parameters are perturbed and related to bone and vascular health, which is an important pathophysiology of CKD-MBD [10]. T_{50} also tends to accompany mineral parameters in serum in the CKD environment, indicating the tendency for vascular calcification [11].

In the past, routine BMD evaluation was not recommended in CKD patients [12], but fractures can be predicted by measuring BMD in non-dialysis-dependent CKD (CKD-ND) patients [13] and end-stage kidney disease (ESKD) patients on hemodialysis (HD) [6]. In addition, as osteoporosis treatments for patients with impaired renal function have been developed, BMD measurement is being actively performed. However, in the pathophysiology of CKD-MBD, it is difficult to reflect bone quality because the bone density of the trabecular bone may be overestimated [12,14]. A recent study reported that CKD showed a correlation with low BMD measured at the hip, but not with BMD measured at the spine [13]. Until now, osteopenia and osteoporosis have been diagnosed using the same cut-off values as in the general population [14], but follow-up studies on bone health are needed in ESKD patients.

Therefore, in this study, we aimed to provide the first analysis of the clinical and biochemical parameters of T_{50} in patients undergoing HD. We also examined the relationships between T_{50}, BMD from the various sites, and mineral and inflammatory parameters, to evaluate the potential of T_{50} as a predictor of the CKD-MBD association in HD patients.

Methods

Study design and setting

This study was based on maintenance HD patients from a single center in Korea. We investigated the associations between T_{50}, BMD, and biochemical parameters using a cross-sectional design.

Study population

A total of 184 patients who visited our HD unit at the Gachon University Gil Medical Center between March 2020 and February 2021 were analyzed. Patients were enrolled in the study if they 1) had been on HD for at least 3 months, 2) agreed to participate in the study with written informed consent, and 3) were free of any complications that could affect serum T_{50} and other biochemical parameters such as an indwelling catheter, any underlying malignancy, active liver disease, current infection, or previous parathyroidectomy.

This study adheres to the Declaration of Helsinki and was approved by the Institutional Review Board at the Gachon University Gil Medical Center (No. GBIRB2020-342). Written informed consent was obtained from all participants.

Clinical and laboratory parameters

All demographic and clinical data, comorbidities, laboratory values, and medications were collected at the time of enrollment from participants' medical records by a well-trained study coordinator. The following baseline demographic and clinical characteristics were collected: age, sex, body mass index, smoking, and HD duration. Data on comorbidities, including hypertension (HTN), diabetes mellitus (DM), CVD such as angina pectoris, myocardial infarction, heart failure (HF), transient ischemic attack (TIA), stroke, and peripheral arterial disease, were also collected. Angina pectoris and myocardial infarction were defined as

the presence of coronary artery disease as documented by angiography, an acute coronary syndrome, angina requiring percutaneous coronary intervention, or coronary artery bypass grafting surgery. Stroke and TIA were defined as cases where magnetic resonance imaging was performed on patients with suspected symptoms that were diagnosed by a neurologist. Systolic HF was defined as left ventricular ejection fraction of <40%, and diastolic HF was defined as E/é of >15. All blood samples were obtained prior to a mid-week HD session after overnight fasting and microcentrifugation for measurements. Serum was separated from blood samples within 1 hour of collection and stored at −70 °C until analysis. Laboratory data included the single-pool Kt/V (spKtV), hemoglobin, albumin, protein, calcium, phosphorus, 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D, parathyroid hormone, alkaline phosphatase (ALP), total cholesterol, triglyceride (TG), and high-sensitivity C-reactive protein (hsCRP). Medication data included the use of renin-angiotensin-aldosterone system blockers calcium channel blockers, β-blockers, phosphate binders, statin, vitamin D analogues, and cinacalcet.

Determination of the serum calcification propensity (T_{50})

T_{50} was determined using a nephelometer (Nephelostar; BMG Labtech, Offenburg, Germany), which measures the time-point transformation from primary to secondary CPP, as described in a previous study [7]. To this end, patient serum (80 μL) was first exposed to NaCl solution (20 μL), followed by high and supersaturated concentrations of calcium (50 μL) and phosphate (50 μL) solutions. The experiment was performed in triplicate in a 96-well plate. The Nephelostar was operated and controlled using Galaxy software. Nonlinear regression curves were calculated for the determination of T_{50}. The analytical coefficients of variation of standards precipitated at 120, 240, and 360 minutes were 9.8%, 8.7%, and 8.4%, respectively.

Measurement of bone mineral density and abdominal aortic calcification score

The BMD was estimated using a dual-energy X-ray absorptiometry system (Hologic, Marlborough, MA, USA). The BMD of the total hip, femoral neck, and lumbar spine (L1–L4) were measured at baseline, and the results were expressed as density (g/cm^2) and T-scores (standard deviation [SD] from the average BMD value in a healthy young population).

Plain X-ray images of the lateral lumbar spine from all subjects were studied to calculate semiquantitative abdominal aortic calcification (AAC) scores, as described by Kauppila et al. [15]. The AAC score was graded on a 0 to 3 scale at each segment (L1–L4) of the lumbar vertebrae based on the severity of calcification as follows: 0, no aortic calcific deposits; 1, small scattered calcific deposits less than 1/3 of the longitudinal wall of the aorta; 2, 1/3 or more but less than 2/3; and 3, 2/3 or more. The anterior and posterior wall scores were separately graded and summed, resulting in a total score of 0 to 24. All X-ray images were analyzed by two independent observers having no knowledge of the clinical history of each subject, and consensus was reached on the interpretation of all radiographs.

Statistical analyses

Continuous variables were tested for normality using the Shapiro-Wilk test before further statistical analysis. Variables without a normal distribution were either transformed into a logarithmic scale and then subjected to parametric tests or analyzed using a non-parametric test. Values with a normal distribution are expressed as mean ± SD, while those without a normal distribution are presented as median and interquartile range. Comparisons between the groups were performed using the chi-square test, Student t test, or analysis of variance with Tukey multiple comparison test as appropriate. Correlation between two continuous variables was analyzed using Pearson correlation test. Variables that do not show a normal distribution were analyzed by converting them to logarithmic values. Independent variables associated with T_{50} were identified using multiple stepwise linear regression analysis. All statistical analyses were conducted using R software, version 3.5.3 with packages (The Comprehensive R Archive Network; http://cran.r-project.org). For all statistical analyses, statistical significance was set at $p < 0.05$.

Results

Characteristics of the study population

Participant demographics and clinical characteristics strat-

ified by tertiles of T_{50} concentration are shown in Table 1. The mean T_{50} was 296 ± 85 minutes. Ninety-six participants (52.2%) were men, mean age was 61 ± 12 years, mean dialysis duration was approximately 107 months, and there was a high prevalence of comorbidities such as DM (47.3%), HTN (58.7%), and previous CVD (40.8%). Descending tertiles of serum T_{50} were associated with lower serum albumin (3.9 ± 0.4, 4.0 ± 0.3, 4.1 ± 0.3; p < 0.001) and TG (85.4 ± 68.8, 106.2 ± 74.6, 113.6 ± 72.8; p = 0.03) levels as well as higher serum hsCRP (0.2 [0.1–0.5], 0.2 [0.0–0.3], 0.1 [0.0–0.3]; p = 0.03), phosphate (5.6 ± 1.8, 5.3 ± 1.3, 5.0 ± 1.0; p = 0.02), and ALP (125.4 ± 95.7, 98.3 ± 41.2, 102.0 ± 37.5; p = 0.045) concentrations (Table 1).

Table 1. Baseline characteristics of the study group according to tertiles of serum T_{50}

Characteristic	Total	T_{50}			p-value
		T1	T2	T3	
No. of patients	184	61	62	61	
T_{50} (min)	296.3 ± 85.3	204.1 ± 39.0	290.9 ± 25.3	394.1 ± 40.3	
Age (yr)	61.1 ± 12.3	61.8 ± 12.5	58.7 ± 13.1	62.8 ± 10.9	0.65
Male sex	96 (52.2)	33 (54.1)	32 (51.6)	31 (50.8)	0.93
HD duration (mo)	107 (64–139)	120 (71–147)	92 (69–139)	104 (52–127)	0.10
BMI (kg/cm^2)	23.5 ± 3.8	23.1 ± 3.9	23.8 ± 3.8	23.5 ± 3.8	0.56
Smoking	28 (15.2)	10 (16.4)	9 (14.5)	9 (14.8)	0.95
Diabetes mellitus	87 (47.3)	25 (41.0)	29 (46.8)	33 (54.1)	0.35
Hypertension	108 (58.7)	36 (59.0)	37 (59.7)	35 (57.4)	0.97
CVD	75 (40.8)	27 (44.3)	21 (33.9)	27 (44.3)	0.40
spKtV	1.6 (1.4–1.8)	1.6 (1.4–1.9)	1.6 (1.4–1.8)	1.6 (1.4–1.8)	0.96
RAS blockade	79 (42.9)	26 (42.6)	28 (45.2)	25 (41.0)	0.90
CCB	81 (44.0)	29 (47.5)	28 (45.2)	24 (39.3)	0.64
β-blocker	80 (43.5)	29 (47.5)	28 (45.2)	23 (37.7)	0.52
Phosphate binder	131 (71.2)	44 (72.1)	42 (67.7)	45 (73.8)	0.75
Statin	71 (38.6)	24 (39.3)	22 (35.5)	25 (41.0)	0.81
Vitamin D analogues	123 (66.8)	41 (67.2)	37 (59.7)	45 (73.8)	0.25
Cinacalcet	17 (9.2)	8 (13.1)	5 (8.1)	4 (6.6)	0.42
Hemoglobin (g/dL)	10.8 ± 1.3	10.7 ± 1.3	10.7 ± 1.2	11.1 ± 1.2	0.07
Albumin (g/dL)	4.0 ± 0.3	3.9 ± 0.4	4.0 ± 0.3	4.1 ± 0.3	<0.001
Cholesterol (mg/dL)	137.0 ± 34.5	128.7 ± 28.6	141.9 ± 37.6	140.2 ± 35.6	0.07
Triglyceride (mg/dL)	101.9 ± 72.7	85.4 ± 68.8	106.2 ± 74.6	113.6 ± 72.8	0.03
hsCRP (mg/dL)	0.1 (0.0–0.4)	0.2 (0.1–0.5)	0.2 (0.0–0.3)	0.1 (0.0–0.3)	0.03
Calcium (mg/dL)	8.2 ± 0.9	8.3 ± 0.9	8.2 ± 1.0	8.2 ± 0.9	0.70
Phosphate (mg/dL)	5.3 ± 1.4	5.6 ± 1.8	5.3 ± 1.3	5.0 ± 1.0	0.02
VD_{25} (ng/mL)	17.2 ± 9.8	17.4 ± 10.4	17.9 ± 10.1	16.4 ± 8.9	0.61
$VD_{1,25}$ (pg/mL)	5.9 ± 7.1	5.7 ± 7.4	5.7 ± 7.0	6.4 ± 7.1	0.61
PTH (pg/mL)	564.2 ± 380.9	649.1 ± 501.2	504.2 ± 293.5	541.7 ± 306.9	0.12
ALP (U/L)	108.5 ± 64.6	125.4 ± 95.7	98.3 ± 41.2	102.0 ± 37.5	0.045
BMD (g/cm^2)					
Lumbar spine	1.034 ± 0.214	0.998 ± 0.218	1.045 ± 0.205	1.059 ± 0.217	0.13
Femoral neck	0.713 ± 0.145	0.693 ± 0.159	0.729 ± 0.147	0.715 ± 0.129	0.42
Total hip	0.749 ± 0.164	0.717 ± 0.165	0.753 ± 0.185	0.775 ± 0.135	0.06
AAC	4.0 (0.0–12.0)	4.0 (0.0–10.5)	3.5 (0.0–8.0)	5.0 (0.0–11.0)	0.54

Data are expressed as number only, mean ± standard deviation, number (%), or median (interquartile range).
AAC, abdominal aortic calcification; ALP, alkaline phosphatase; BMD, bone mineral density; BMI, body mass index; CCB, calcium channel blocker; CVD, cardiovascular disease; HD, hemodialysis; hsCRP, highly selective C-reactive protein; PTH, parathyroid hormone; RAS, renin-angiotensin-aldosterone; spKtV, single-pool Kt/V; T1, 1st tertile; T2, 2nd tertile; T3, 3rd tertile; $VD_{1,25}$, 1,25-dihydroxyvitamin D; VD_{25}, 25-hydroxyvitamin D.

Correlation between serum T_{50} and related parameters

T_{50} showed a significant correlation with the total hip T-score (r = 0.158, p = 0.038) (Table 2). However, there was no significant correlation between the T_{50} and AAC scores on plain radiographs (r = 0.064, p = 0.401) (Table 2). With respect to medication use, there was no significant correlation between the descending tertiles of serum T_{50} and medications for CKD-MBD, including phosphate binders, vitamin D analogues, and cinacalcet. Serum T_{50} was positively correlated with serum albumin concentration (r = 0.289, p < 0.001) (Fig. 1). In addition, it was inversely correlated with serum hsCRP (r = –0.224, p = 0.003) and phosphate (r = –0.156, p = 0.040) concentrations (Fig. 1).

Association between bone mineral density and related parameters

We compared the mean T-score of BMD according to the sites at which it was assessed (Fig. 2). The mean T-score for BMD measured at the femur neck was relatively lower than that for the BMD assessed at the total hip or lumbar spine (–1.9 ± 1.2, –1.6 ± 1.3, and –1.1 ± 1.8, respectively). There was a significant difference between the three groups (p < 0.001). In the multiple comparison test by Tukey method, there were also significant differences between femur neck and L spine (p < 0.001) and between total hip and L spine (p = 0.001), but the difference between femur neck and total hip was not significant (p = 0.26).

BMD showed an inverse correlation with age and the spKtV (lumbar: r = –0.310, p < 0.001; femoral neck: r = –0.403, p < 0.001; total hip: r = –0.440, p < 0.001) and a positive correlation with albumin (lumbar: r = 0.094, p = 0.218; femoral neck: r = 0.201, p = 0.008; total hip: r = 0.219, p = 0.004). Only the L spine BMD showed an inverse correlation with ALP (r = –0.225, p = 0.003). Femoral neck (r = –0.267, p < 0.001) and total hip BMD (r = –0.176, p = 0.021) also showed an inverse relationship with AAC scores (Table 2).

Evaluating the usefulness of T_{50} as a predictor of bone mineral density

We used linear regression to evaluate the cross-sectional association between T_{50} and femoral neck, hip, and spinal BMD. We found no statistically significant associations between T_{50} and femoral neck or lumbar spine BMD in either the unadjusted models (femoral neck: β = 0.005, p = 0.708; lumbar spine: β = 0.032, p = 0.101) or in the adjusted models (femoral neck: β = –0.001, p = 0.956; lumbar spine: β = 0.019, p = 0.331) (Table 3) for variables including age, sex, smoking, HD duration, spKtV, inflammatory and mineral parameters (albumin and ALP), and medications (phosphate binders, vitamin D analogues, and cinacalcet).

We found a weak association between T_{50} and BMD in the

Table 2. Cross-sectional correlation analyses between serum T_{50} and BMD and other variables

Variable	Age	spKtV	Albumin	hsCRP[a]	Calcium	Phosphate	PTH	ALP	BMD_LS	BMD_FN	BMD_TH	AAC[a]	T_{50}
Age	1.000												
spKtV	0.226*	1.000											
Albumin	–0.245*	–0.015	1.000										
hsCRP[a]	0.025	–0.120	–0.205*	1.000									
Calcium	0.020	0.037	0.242*	–0.107	1.000								
Phosphate	–0.350*	–0.213*	0.191*	0.097	0.063	1.000							
PTH	–0.193*	–0.215*	0.113	0.010	0.193*	0.342*	1.000						
ALP	0.089	–0.029	0.058	0.011	0.085	–0.032	0.339*	1.000					
BMD_LS	–0.180*	–0.310*	0.094	0.056	–0.026	0.105	–0.069	–0.225*	1.000				
BMD_FN	–0.513*	–0.403*	0.201*	–0.003	0.028	0.132	0.011	–0.019	0.599*	1.000			
BMD_TH	–0.353*	–0.440*	0.219*	0.053	0.044	0.061	–0.005	–0.103	0.599*	0.800*	1.000		
AAC[a]	0.443*	–0.009	–0.126	0.089	0.063	–0.051	–0.072	0.126	0.024	–0.267*	–0.176*	1.000	
T_{50}	0.042	–0.006	0.289*	–0.224*	–0.005	–0.156*	–0.081	–0.156*	0.123	0.034	0.158*	0.064	1.000

AAC, abdominal aortic calcification; ALP, alkaline phosphatase; BMD, bone mineral density; FN, femoral neck; hsCRP, highly selective C-reactive protein; LS, lumbar spine; PTH, parathyroid hormone; spKtV, single-pool Kt/V; TH, total hip.
[a]Data for hsCRP and AAC were log-transformed.
*p < 0.05.

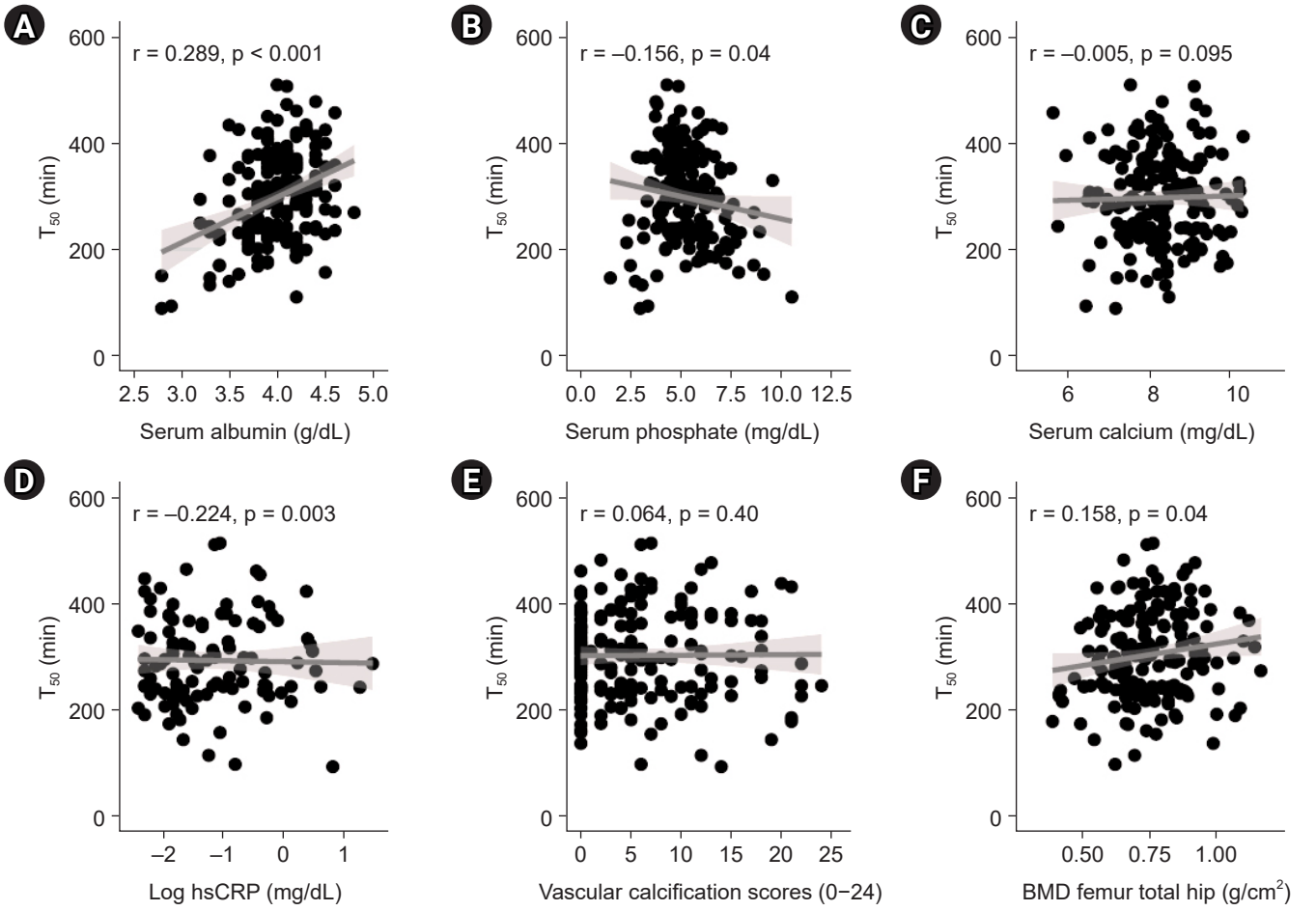

Figure 1. Association of serum T_{50} with mineral and inflammatory markers in hemodialysis patients. Bivariate correlation analysis of serum T_{50} with (A) albumin, (B) phosphate, (C) calcium, (D) hsCRP, (E) AAC, and (F) BMD total hip. Serum T_{50} was positively correlated with serum albumin concentration (r = 0.289, p < 0.001) and inversely correlated with serum hsCRP (r = –0.224, p = 0.003) and phosphate (r = –0.156) concentrations.
AAC, abdominal aortic calcification; BMD, bone mineral density; hsCRP, high-sensitivity C-reactive protein.

total hip area in the unadjusted model (β = 0.030, p = 0.043) but did not find a statistically significant association in the multivariate-adjusted models (β = 0.017, p = 0.188) (Table 3).

Discussion

In this cross-sectional study of HD patients, T_{50} was associated with mineral and inflammatory parameters but not with AAC score or BMD.

CKD-MBD is a common complication of CKD and is associated with morbidity and mortality. The interconnection between vascular calcification and bone health has been reported as a significant inverse relationship between vascular calcification and bone fragility (low BMD) [16–18]. Impaired bone metabolism, particularly low bone turnover, may promote vascular calcification [1]. Several factors have been suggested as possible links between bone and soft tissue calcification; however, the key elements of the cross-talk mechanism are yet to be elucidated [1].

Reduced serum T_{50} is associated with a lack of inhibitors and abundant promoters of vascular calcification [19]. Therefore, it is assumed that the action of these factors and the effect of T_{50} on the overall tendency of serum calcification will be in the same direction.

The main determinants of T_{50} in this study were inflammatory (serum albumin and hsCRP), mineral (serum

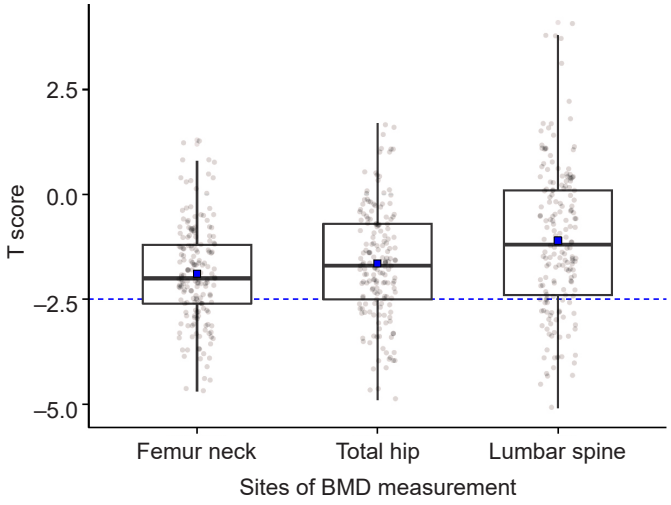

Figure 2. T-scores at the sites of BMD measurement. The mean T-score for BMD measured at the femur neck was relatively lower than that for the BMD assessed at the total hip or lumbar spine (−1.9 ± 1.2, −1.6 ± 1.3, and −1.1 ± 1.8, respectively; p < 0.001). In the multiple comparison test by Tukey method, there were also significant differences between femur neck and lumbar spine (p < 0.001) and between total hip and lumbar spine (p = 0.001), but the difference between femur neck and total hip was not significant (p = 0.27). The thick line within the box represents the median, the upper and lower boundaries of the box represent the interquartile range, the solid square inside the box represents the mean, the upper and lower whiskers represent the maximum and minimum values, respectively, and the gray dots in each group represent individual data.
BMD, bone mineral density.

Table 3. Linear regression of the association between T_{50} (every 100 minutes increase) and BMD (g/cm^2)

BMD	β (95% confidence interval)	p-value
Spine L1–L4		
Crude	0.03 (−0.01 to 0.07)	0.10
Model 1	0.03 (−0.002 to 0.07)	0.07
Model 2	0.02 (−0.02 to 0.06)	0.32
Model 3	0.02 (−0.02 to 0.06)	0.33
Femur neck		
Crude	0.01 (−0.02 to 0.03)	0.71
Model 1	0.01 (−0.01 to 0.03)	0.42
Model 2	−0.001 (−0.02 to 0.02)	0.94
Model 3	−0.001 (−0.02 to 0.02)	0.96
Total hip		
Crude	0.03 (0.001 to 0.06)	0.04
Model 1	0.03 (0.01 to 0.06)	0.01
Model 2	0.02 (−0.01 to 0.04)	0.19
Model 3	0.02 (−0.01 to 0.04)	0.19

Model 1: adjusted for age, sex, and smoking. Model 2: model 1 + adjustment for hemodialysis duration (mo), single-pool Kt/V, albumin, and alkaline phosphatase. Model 3: model 2 + adjustment for phosphate binders, vitamin D receptor activators, and cinacalcet.
BMD, bone mineral density.

phosphate), and the bone turnover marker (ALP). Only the values measured in the BMD total hip joint area showed a weak correlation in the unadjusted model, but there was no association with BMD measured in all regions in the multivariable-adjusted models. This finding is consistent with epidemiological data in advanced CKD-ND cohorts, where reduced T_{50} has been correlated with increased phosphate, decreased albumin, and CPP-associated fetuin-A concentration [8]. These results are also consistent with those of a dialysis cohort in which low BMD was related to mineral deposition in arterial walls and soft tissues [20,21].

Recently, an association between T_{50} and BMD was reported in 150 non-CKD participants from an elderly male cohort [22]. Subjects with a shorter T_{50} were likely to be older, and there was a nonlinear trend with a higher prevalence of diabetes, but T_{50} did not show any association with total hip or spine BMD. Moreover, there was no correlation with mineral parameters such as calcium and phosphate. The lack of association between T_{50} and BMD were consistent with our results, but the association between T_{50} and serum albumin from this and previous studies [8,9] could not be assessed.

A lower T_{50} was significantly associated with the severity and progression of coronary artery calcification in patients with CKD-ND; however, T_{50} was not associated with the incidence of coronary artery calcification [11]. In the present study, T_{50} was not associated with vascular calcification. The difference between these results is that although the range of our measurements using simple plain radiographs is limited, their correlation could be shown in their measurement methods using electron beam computed tomography. In addition, the fact that T_{50} is not related to the incidence of vascular calcification but correlates with its progression is thought to reflect a dynamic change in the progression of vascular calcification once it has occurred. This finding suggests that a significantly longer observation period is required to observe an association between T_{50} and vascular calcification.

The propensity for serum calcification reflects the degree of activity of numerous humoral and cellular factors that af-

fect the formation and growth of calcified crystals in blood vessels [7]. Calcification mechanisms require functional and direct measurements targeting calcium phosphate precipitation more comprehensively rather than focusing on the individual molecular components of the calcification process, as their developmental processes are multifactorial [8]. In this regard, it is considered that the contribution of T_{50} could be large, and in the case of CKD patients, the results of a study comparing reduced T_{50} and cardiovascular complications and death have been reported [8,9]. In patients with ESKD, an association between lower T_{50}, CVE, and mortality was reported in the EVOLVE (Effect of Cinacalcet on Cardiovascular Disease in Patients Undergoing Dialysis) study cohort [23]. However, in ESKD patients, dialysis itself and medications to maintain mineral parameters in the target range associated with CKD-MBD may affect T_{50}. Therefore, further studies are needed to evaluate its value as a predictor of clinical prognosis in patients with ESKD.

The T-score of BMD showed slightly different results depending on the measurement location, with the lowest values at the femur neck, the highest values at the lumbar spine, and a moderate level at the total hip. When studying the relationship between BMD and vascular calcification, there is currently no consensus as to which specific bone location should be the representative for BMD measurement [24]. This uncertainty is due to heterogeneity between the population and bone sites selected for BMD measurements in previous studies [16,25]. Depending on the severity of atherosclerosis, calcium deposition in the intima can affect the measurement of the spinal BMD. Our findings are consistent with those of previous studies that reported that peripheral BMD was lower than central BMD [26,27]. Lumbar BMD may be relatively overestimated in patients with ESKD with severe AAC. Therefore, peripheral BMD measurements may be more appropriate than central BMD measurements in these patients.

Osteoporosis causes both cortical and trabecular bone loss, whereas CKD-MBD results in primarily cortical bone loss [10,28]. In a recent study on the relationship between vascular calcification and BMD in CKD patients [29], cortical or trabecular bone loss was observed in CKD patients, but not all patients showed a simultaneous loss. In particular, the cortical bone loss did not show an association with vascular calcification, unlike trabecular bone loss [29]. Vascular calcifications are strongly associated with CKD-MBD [10,30]; however, the correlation between cortical bone and vascular calcification is not yet clear. In this respect, in this study, it is insufficient to explain the weak association between T_{50} and BMD in the femur and the lack of association in the lumbar region. To evaluate the relationship between the pathophysiology of vascular calcification and bone density in HD patients with both MBD and osteoporosis components, consensus through follow-up studies on quantitative BMD measurement methods and sites according to pathophysiology is required.

This study has some limitations. First, a causal relationship could not be confirmed by conducting a cross-sectional study. However, we performed correlation analyses with various mineral parameters; in particular, we evaluated BMD in a relatively large number of HD patients, described its distribution, and analyzed its association with T_{50}. Second, we were unable to control the dialysis protocol and medications that affected T_{50} measurements. However, considering that the characteristics of dialysis patients are always affected by medication as well as dialysis itself, we need to carefully consider the evaluation value of T_{50} in future.

In summary, for the first time in Korea, we have provided a stable measurement method for T_{50} and applied it to clinical research. T_{50} was correlated with mineral and inflammatory parameters but not with AAC. BMD was correlated with T_{50} in the case of total hip but was not correlated with BMD measured at other sites (femoral neck and lumbar spine). To evaluate the value of T_{50} as a predictor of CKD-MBD diagnosis and treatment in ESKD patients, a study on its association with hard outcomes, including fracture, CVE, and mortality, should be prioritized. In addition, to confirm the association between T_{50} and dynamic changes such as vascular calcification or BMD changes, a large-scale study that includes a larger number of patients and a longer observation period is needed.

Conflicts of interest

All authors have no conflicts of interest to declare.

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Data sharing statement

The data presented in this study are available on request from the corresponding author.

Authors' contributions

Conceptualization: JYJ
Data curation, Formal analysis: HK, JYJ
Investigation: AJK, HR, JHC, HHL, WC
Methodology, Funding acquisition, Supervision: JYJ
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