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Association Among Serum Fetuin-A Level, Coronary Artery Calcification, and Bone Mineral Densitometry in Maintenance Hemodialysis Patients

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Abstract: Patients with end-stage renal disease have a very high prevalance and extent of arterial calcification. A number of studies suggest that similar pathophysiologic mechanisms are responsible for development and progression of calcification of atherosclerotic plaque and bone formation. Fetuin-A is a potent calcification inhibitor and is expressed in bone, with not-yet well-defined functions. The aim of this study was to investigate the relation between bone mineral densitometry parameters, coronary artery calcification, and serum fetuin-A levels. In a cross-sectional design, we included 72 maintenance hemodialysis (HD) patients and 30 age- and gender- matched healthy controls. Serum fetuin-A levels were studied both in maintenance HD patients and healthy controls. Maintenance HD patients had radius, hip, and lumbar spine bone mineral density (BMD) assessed by dual-energy X-ray absorptiometry and coronary artery calcification score (CACS) measured by electron-beam computed tomography. The associations between site-specific BMD parameters, CACS, and serum fetuin-A levels were studied in maintenance HD patients. CACS, mass, and volume of plaques in coronary arteries were significantly higher in patients with a T-score below –2.5 than above in the proximal region of the radius, neck and trochanter of the femur, and the lumbar spine. Mean serum fetuin-A concentration was 0.636 ± 0.118 g/L in maintenance HD patients and it was less than healthy controls (0.829 ± 0.100 g/L, $P < 0.0001$). CACS, mass, and volume of plaques in coronary arteries correlated significantly with the serum fetuin-A levels. Moreover, significant positive correlations were shown between the serum fetuin-A levels, BMD values, and T-scores of proximal radius, neck, and trochanter of the femur, but not with the lumbar spine. The present study demonstrates an association between serum fetuin-A levels, coronary artery calcification, and bone mineral densities—except for the lumbar spine, in maintenance HD patients. However, the results should be interpreted with caution because of the cross-sectional design of the study. **Key Words:** Coronary artery calcification—Bone mineral density—Fetuin-A—Hemodialysis.

Several studies have clearly established that the prevalence and extent of cardiovascular calcification are increased in patients with end-stage renal disease (ESRD) (1–4). In such patients, the extents of vascular calcification were predictive of subsequent cardiovascular disease and mortality beyond established conventional risk factors (5). The pathogenesis of cardiovascular calcification is complex and it does not consist of a simple precipitation of calcium and phosphate, but is instead an active and modifiable process (6). Moreover, recent studies have shown that vascular calcification is a regulated process with plasma constituents maintaining minerals in solution and inhibiting their deposition in tissues (7).

When compared with the general population, a significant decrease in bone mineral density (BMD) has been reported in ESRD patients, and this decline of BMD becomes more marked as the duration of dialysis increases (8). Recently, growing evidence from basic research and from studies in experimental animals suggested that similar pathophysiologic mechanisms are responsible for development and progression of calcification of atherosclerotic plaque

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and bone formation. An inverse relationship between vascular calcification and bone density has been documented in both postmenopausal women (9) and uremic patients (2).

Fetuin-A is a liver-derived negative acute-phase protein present in all extracellular fluids (10). It is a major inhibitor of vascular calcification and serum concentrations of fetuin-A are depressed in patients with ESRD (11). Due to its high affinity for calcium phosphates, fetuin-A accumulates in the atherosclerotic plaques, and in pathologically mineralized tissues (12–14). Fetuin-A also accumulates in bone, with not-yet well-defined functions. It can be expressed in cartilage tissue (15) and is considered to be an inhibitor of the transforming growth factor-β/bone morphogenetic protein family (16). Furthermore, accelerated trabecular bone remodeling with an increase in number of osteoblastic cells and impairment of growth plate chondrocyte maturation and retardation in the longitudinal growth of femurs have been shown in fetuin-A knockout mice (17).

Given the important role of fetuin-A in preventing vascular calcification and the structural role as a major noncollagenous component of mineralized bone, the aim of this study was to investigate the association between serum fetuin-A levels, coronary atherosclerosis, and bone mineral densitometry in maintenance hemodialysis (HD) patients.

SUBJECTS AND METHODS

Subjects

This study was a cross-sectional study involving maintenance HD patients from Hemodialysis Unit, Hacettepe University Hospital, Ankara, Turkey. Of the 93 adult HD patients, 11 patients refused to participate and we excluded three patients due to clinical evidence of heart failure (defined as dyspnea in addition to two of the following conditions: increased jugular pressure; bibasilar crackles; pulmonary venous hypertension; or interstitial edema on chest X-ray requiring hospitalization or extra ultrafiltration [18]), two patients due to known malignancies, two patients due to autoimmune diseases, and three patients due to clinically evident active infections. The remaining 72 maintenance HD patients were the eligible study population (39 women and 33 men, mean age 54 ± 19 years, mean HD time 48 ± 12 months, on HD thrice a week).

Patients were receiving conventional 4-h HD with polysulfone dialysers F6HPS and F7HPS (Fresenius AG, Bad Homburg, Germany) thrice a week, with bicarbonate dialysate, and unfractioned heparin or low molecular weight heparin for standard anticoagulation. Mean blood flow rate was 250 mL/min during HD session (range 200 to 290 mL/min). Dialysate fluid composition was sodium 140 mEq/L, potassium 1–3 mEq/L, calcium 3 mEq/L, magnesium 1.8 mg/dL, and bicarbonate 33 mEq/L. Urea (Kt/V) values were calculated according to Daugirdas second generation formula (19).

Fifty patients were on treatment with recombinant human erythropoietin, and 29 patients were administered antihypertensive drugs (19 patients, monotherapy with angiotensin-converting enzyme inhibitors, calcium channel blockers, and β-blockers; the remaining 10 patients, double therapy with various combinations of these drugs). Fifty-nine patients were administered calcium salts (either calcium carbonate or calcium acetate). Thirty patients were under treatment with intravenous calcitriol, 2 to 8 μg/week, which had been started at least 6 months before the enrollment.

The study was approved by the Hacettepe University Hospital Ethics Board. All patients gave their informed consent in writing before they were submitted to any procedures related to the study.

Biochemical assays

Blood samples were collected after an overnight fasting directly through an arteriovenous fistula at a single midweek dialysis session (as part of our routine patient follow-up). Complete blood counts were assessed by the ultraviolet assay (Beckman-Coulter, Krefeld, Germany) whereas biochemical analyses were done by kinetic ultraviolet assay (Roche, Hitachi System, Indianapolis, IN, USA). Serum C-reactive protein (CRP) level was determined by rate nephelometry (IMMAGE Immunochemistry system, Beckerman Coulter, Inc., Fullerton, CA, USA). Serum intact parathormone (PTH) level was measured by chemiluminescance immunassay method with the Nichols-Advantage kits (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). Low-density lipoprotein cholesterol level was calculated using Friedewald's formula (20). Time-averaged values of biochemical parameters were calculated as the mean of the routine four weekly measurements of the previous 6 months.

Serum fetuin-A level

Serum fetuin-A level was measured using an enzyme-linked immunosorbent assay kit (Biovendor Laboratory Medicine, Inc., Modrice, Czech Republic). The 30 age- and gender-matched controls consisted of healthy staff members (mean age 48 ± 9 years, men 44%, mean body mass index 25 ± 3 kg/m^2,

smoking 19%). Control subjects had to have a negative cardiovascular medical history, including presence or history of ischemic heart disease, peripheral vascular disease and/or a cerebrovascular event, and hypertension. Hypertension was defined as a blood pressure ≥140 mm Hg systolic and/or ≥90 mm Hg diastolic, according to the Joint National Committee VII criteria (21) and/or the current use of antihypertensive medication. Intra-assay and interassay coefficients of variation were 4.8 and 7.4%, respectively.

Coronary artery calcification score (CACS)

All of the examinations were performed with a 16-MDCT Scanner (Sensation 16, Siemens Medical Solutions, Erlangen, Germany). The area between carina to apex of the heart was scanned in the craniocaudal direction. Calcium scoring parameters were tube voltage 120 Kv, an effective tube current-time product of 133 mAseff, a collimation of 12 × 0.75 mm, a table feed of 2.8 mm per rotation, and a tube rotation time of 420 ms. No tube current modulation has been applied. In each patient, 60% of the R-R reconstruction was prepared at 512 × 512 reconstruction matrix and a medium smooth convolution kernel (B35f). All reconstructed images were transferred to an external workstation (Leonardo, Siemens Medical Solutions) for CACS (Syngo Calcium Scoring CT, Siemens). The CACS was determined by applying the method described by Agatston et al. (22), using a threshold of 130 HU.

Bone mineral densitometry

The bone mineral densitometry of the proximal one-third of the radius, anterior-posterior lumbar vertebrate and femur were measured by dual-energy X-ray adsorptiometry (DEXA) (Hologic QDR-2000, Hoxologic, Inc., Waltham, MA, USA). Machine calibration was performed daily. T-scores (comparisons with a normal reference population of young adults) were also calculated in addition to BMD. The investigator performing the clinical and laboratory follow-up, the DEXA technician, and the radiologist performing the DEXA analysis were all blinded to the patient characteristics.The definitions recommended by the World Health Organization are based on T-scores (T-score = measured BMD – young adult BMD/young adult standard deviation [SD]). Based on this, patients may be divided into normal (T > –1), osteopenia (T-score > –2.5 and T-score ≤ –1), and osteoporosis (T-score ≤ –2.5) (23,24).

Statistical analysis

All data were first analyzed for normality of distribution using the Kolmogorov–Smirnov test. Data were expressed as mean ± SD whereas CACS, serum intact PTH, and CRP levels were expressed as median (range). Continuous variables were compared with a *t*-test or the Mann–Whitney *U*-test as appropriate. The linear correlations between study parameters were investigated by Pearson's correlation or Spearman rank correlation test as appropriate. The Cox proportional hazards model was used to estimate the relative risks of CACS for different variables. Factors with $P < 0.05$ on univariate analysis for coronary artery calcification that met the assumptions of proportional hazards were considered further in the multivariate Cox regression analysis for coronary artery calcification. A stepwise multiple regression analysis was used to investigate relationships between bone mineral densitometry parameters and risk factors, or serum biochemical markers. A two-tailed value $P < 0.05$ was considered to be statistically significant. Statistical analysis was performed using the SPSS 10.0 software program (Statistical Package for the Social Sciences, SPSS, Chicago, IL, USA).

RESULTS

Patient characteristics, CACS, and bone mineral densitometry

The demographic and clinical characteristics of the study population are summarized in Table 1. The patients suffered from ESRD due to chronic glomerulonephritis ($n = 22$), hypertensive nephrosclerosis ($n = 17$), diabetic nephropathy ($n = 7$), chronic pyelonephritis ($n = 11$), and polycystic disease ($n = 6$). The renal diagnosis was unknown in nine patients. Overall, patients had median CACS of 156 (range 5–1397). CACS correlated with age ($r = 0.33$, $P = 0.017$), serum CRP levels ($r = 0.423$, $P = 0.009$), and intravenous calcitriol treatment ($r = 0.28$, $P = 0.024$).

The data regarding mean bone mineral densities and T-scores in the proximal radius, neck, and trochanter region of the femur and L1–L4 vertebrate region are also presented in Table 1. There are statistically significant differences between radial T-scores and femoral neck T-scores ($P < 0.05$); and between radial T-scores and femoral trochanter T-scores ($P < 0.05$); but not between femoral neck T-scores and femoral trochanter T-scores. Significant correlations were observed between HD vintage and radius BMD ($r = –0.79$, $P < 0.0001$), femoral neck BMD ($r = –0.68$, $P < 0.0001$), femoral trochanter BMD ($r = –0.65$, $P < 0.0001$), and L1–L4 vertebrate region BMD ($r = –0.57$, $P < 0.0001$). Serum intact PTH levels

TABLE 1. *Baseline characteristics of the study patients* (n = *72)*

Parameter	
Age, years†	54 ± 19
Men, n (%),	33 (%46)
Body mass index (kg/m^2)†	21.4 ± 3.2
Dialysis vintage (months)†	48 ± 12
Ca, mg/dL†	9.4 ± 0.6
P, mg/dL†	4.4 ± 1.2
Ca × P mg^2/dL^2,†	42.7 ± 9
PTH,* pg/mL‡	195 (54–467)
CRP, mg/dL‡	1.67 (0.2–7.1)
Albumin, g/dL†	3.67 ± 0.28
Serum fetuin-A (g/L)†	0.636 ± 0.118
Coronary artery calcium score‡	156 (5–1397)
Diabetes, n (%)	7 (9.7%)
Smoking, n (%)	18 (25%)
Hypertension, n (%)	40 (55.5%)
Dyslipidemia, n (%)	18 (25%)
Kt/V†	1.32 ± 0.15
Radius BMD (g/cm^2)†	0.46 ± 13
Radius T-score†	–3.60 ± 2.26
Femur neck BMD (g/cm^2)†	0.62 ± 0.13
Femur neck T-score†	–2.94 ± 1.23
Femur trochanter BMD† (g/cm^2)	0.52 ± 13
Femur trochanter T-score†	–2.35 ± 1.35
Lumbar vertebrate BMD† (g/cm^2) (L1–4 region)	0.92 ± 20
Lumbar vertebrate T-score† (L1–4 region)	–1.48 ± 1.94

* Serum intact parathormone level.
† Values are mean ± SD.
‡ Values are median (range).

correlated significantly with radius BMD ($r = –0.593$, $P < 0.0001$) and T-score ($r = –0.585$, $P < 0.0001$); femoral neck BMD ($r = –0.429$, $P < 0.0001$) and T-score ($r = –0.409$, $P < 0.0001$); femoral trochanter BMD ($r = –0.588$, $P < 0.0001$) and T-score ($r = –0.586$, $P < 0.0001$); and L1–L4 vertebrate region BMD ($r = –0.518$, $P < 0.0001$) and T-score ($r = –0.482$, $P < 0.0001$). However, serum CRP levels did not correlate significantly with bone mineral densitometry parameters. Intravenous calcitriol use correlated significantly with radial ($r = 0.33$, $P = 0.02$), femoral neck ($r = 0.29$, $P = 0.03$) and trochanter ($r = 0.30$, $P = 0.026$), and lumbar spine BMDs ($r = 0.28$, $P = 0.03$).

Association between CACS and bone mineral densitometry

CACS, volume, and mass of plaques in right coronary artery, circumflex artery, left main coronary artery, and left anterior descending artery were significantly higher in patients with a T-score below –2.5 than above that in the proximal region of the radius, in the neck and trochanter of femur (hip), and in the lumbar spine (Table 2). Significant negative correlations were observed between CACS and radius BMD ($r = –0.505$, $P < 0.0001$) and T-score ($r = –0.491$,

TABLE 2. *Association between coronary artery calcification score, mass, and volume of coronary plaques and bone mineral densitometry**

Parameter	Proximal radius T-score <–2.5	Proximal radius T-score >–2.5	Femur neck T-score <–2.5	Femur neck T-score >–2.5	Femur trochanter T-score <–2.5	Femur trochanter T-score >–2.5	Lumbar spine T-score <–2.5	Lumbar spine T-score >–2.5
CACS†	424 (245–1397)	145 (5–259)	419 (238–1397)	139 (16–346)	412 (189–1397)	346 (34–523)	416 (134–1397)	114 (5–223)
Volume of plaques RCA‡ (mm^3)	150 ± 91	87 ± 28	143 ± 63	74 ± 40.6	159 ± 99	67 ± 33	182 ± 100	92 ± 46
Mass of plaques RCA‡ (g)	36 ± 23	24 ± 11	26 ± 11	16 ± 9	29.6 ± 15	12 ± 6	32 ± 15	18 ± 7
Volume of plaques (circumflex artery; mm^3)	94 ± 51	49 ± 23	90 ± 48	48 ± 24	90 ± 44	45 ± 23	95 ± 47	49 ± 22
Mass of plaques (circumflex artery; g)	28 ± 13	14 ± 4	27 ± 14	13 ± 6	26 ± 15	13 ± 4	29 ± 14	15 ± 8
Volume of plaques (LMCA)§ (mm^3)	174 ± 69	113 ± 29	182 ± 60	109 ± 28	144 ± 52	110 ± 33	179 ± 9	137 ± 4
Mass of plaques (LMCA)§ (g)	32 ± 22	23 ± 9	35 ± 19	25 ± 8	30 ± 22	21 ± 8	35 ± 2	24 ± 1.4
Volume of plaques (LAD¶ artery) (mm^3)	144 ± 112	93 ± 31	140 ± 99	89 ± 35	138 ± 102	93 ± 29	140 ± 99	89 ± 35
Mass of plaques (LAD¶ artery) (g)	40 ± 12	23 ± 12	37 ± 13	22 ± 9	37 ± 10	22 ± 13	37 ± 13	22 ± 9

* All of the coronary parameters are significantly different in patients with a T-score below –2.5 than above that in the mentioned anatomical sites.
† CACS, coronary artery calcification score; values are median (range).
‡ RCA, right coronary artery; related values are mean ± SD.
§ LMCA, left main coronary artery; related values are mean ± SD.
¶ LAD, left anterior descending artery; related values are mean ± SD.

$P < 0.0001$); femoral neck BMD ($r = –0.695$, $P < 0.0001$) and T-score ($r = –0.618$, $P < 0.0001$); femoral trochanter BMD ($r = –0.541$, $P < 0.0001$) and T-score ($r = –0.556$, $P < 0.0001$); and L1–L4 vertebrate region BMD ($r = –0.424$, $P = 0.007$) and T-score ($r = –0.394$, $P = 0.013$).

Serum fetuin-A level

The mean ± SD serum fetuin-A concentration was 0.636 ± 0.118 g/L and it was significantly less than healthy controls (0.829 ± 0.10 g/L, $P < 0.0001$). Serum fetuin-A levels were positively associated with serum albumin ($r = 0.651$, $P < 0.0001$) and negatively associated with serum CRP concentrations ($r = –0.656$, $P < 0.0001$). The significance level of correlation between serum fetuin-A and CRP levels also was increased by log transform of serum CRP levels (fetuin-A vs. log CRP, $r = –0.698$; $P < 0.0001$). A strong negative correlation was detected between age and fetuin-A ($r = –0.615$, $P < 0.0001$).

Associations between serum fetuin-A level and coronary artery calcification

Serum fetuin-A levels correlated inversely with coronary artery calcium scoring parameters. The correlations of serum fetuin-A versus CACS ($r = –0.881$, $P < 0.0001$), and mass and volume of plaques in the left main coronary artery ($r = –0.638$, $P < 0.0001$ and $r = –0.636$, $P < 0.0001$, respectively), left anterior descending artery ($r = –0.793$, $P < 0.0001$ and $r = –0.809$, $P < 0.0001$, respectively), right coronary artery ($r = –0.769$, $P < 0.0001$ and $r = –0.772$, $P < 0.0001$, respectively), and circumflex coronary artery ($r = –0.736$, $P < 0.0001$ and $r = –0.853$, $P < 0.0001$, respectively) were significant.

By univariate analysis, serum fetuin-A showed a highly significant association with coronary artery calcification ($P < 0.001$). Other factors relating to coronary artery calcification on univariate analysis are detailed in Table 3. In the multivariate Cox regression models for CACS, serum fetuin-A was significant when adjusting for age, HD vintage, and diabetes (Table 4), and retained its significance when serum CRP and serum albumin were also controlled for in a stepwise fashion (Table 4).

Association between serum fetuin-A level and bone mineral densitometry

Significant positive correlations were observed between serum fetuin-A levels and bone mineral densities of proximal radius ($r = 0.437$, $P < 0.0001$, Fig. 1a), neck of the femur ($r = 0.294$, $P = 0.023$, Fig. 1b), trochanter of the femur ($r = 0.413$, $P < 0.0001$, Fig. 1c), but not with that of lumbar vertebrate ($r = 0.158$, $P = 0.223$). Moreover, significant correlations were shown between serum fetuin-A levels and T-scores of the proximal radius ($r = 0.560$, $P < 0.0001$, Fig. 2a), neck of the femur ($r = 0.329$, $P = 0.011$, Fig. 2b), trochanter of the femur ($r = 0.31$, $P = 0.001$, Fig. 2c), but not with lumbar vertebrate ($r = 0.175$, $P = 0.178$).

Possible risk factors for and serum biochemical markers were entered as independent variables in a stepwise linear multiple regression analysis. These included age, gender (male vs. female), body mass index, diabetes, HD vintage, serum PTH levels, calcitriol therapy, fetuin-A, serum calcium, and phosphate levels. Independent of other influencing factors, HD vintage and serum PTH levels were significant determinants of low bone mass and T-scores in all anatomical sites whereas fetuin-A was an independent predictor in proximal radius, femoral neck, and trochanter (Table 5).

TABLE 3. *Univariate Cox regression model for CACS in maintenance HD patients*

Factors	Hazard ratio (95% confidence interval)	*P* value
Age	1.19 (0.92–1.44)	<0.001
Gender	0.87 (0.56–0.91)	0.870
Positive smoking history	0.76 (0.62–0.78)	0.108
HD vintage	1.14 (0.52–0.80)	0.003
Body mass index	0.59 (0.49–0.67)	0.884
Hypertension	0.67 (0.52–0.84)	0.914
Dyslipidemia	0.69 (0.59–0.81)	0.872
Presence of diabetes	1.08 (0.96–1.20)	0.005
Serum calcium	0.77 (0.63–0.84)	0.118
Serum phosphorus	0.76 (0.70–0.83)	0.134
Calcium–phosphorus product	0.80 (0.70–0.90)	0.174
Calcitriol therapy	0.65 (0.60–0.74)	0.240
Serum albumin level	1.02 (0.89–1.23)	<0.001
C-reactive protein level	1.32 (1.10–1.68)	<0.001
Fetuin-A level	1.55 (1.34–2.07)	<0.001

DISCUSSION

The main findings of this cross-sectional study are as follows:

1. In patients with ESRD, the extent of coronary artery calcification detected by electron beam computed tomography is associated with T-scores of proximal radius, anterior-posterior lumbar spine, neck, and trochanter regions of femur.
2. Serum fetuin-A levels correlated inversely with CACS, mass, and volume of plaques in coronary arteries.
3. Significant positive correlations were shown between serum fetuin-A levels and bone mineral densitometry parameters in patients with ESRD, except for the lumbar spine.

TABLE 4. *Multivariate Cox regression model for CACS in maintenance HD patients (expressed as 95% Confidence Intervals)*

Parameter	Unit increase	Model 1	Model 2 adding serum CRP levels	Model 3 adding serum albumin levels
Age	1 year	1.06 (0.89–1.14) $P = 0.096$	1.06 (0.87–1.13) $P = 0.120$	1.06 (0.87–1.13) $P = 0.420$
HD vintage	1 year	0.85 (0.58–0.95) $P = 0.048$	0.84 (0.58–0.90) $P = 0.098$	0.84 (0.60–0.90) $P = 0.144$
Presence of diabetes	—	0.88 (0.77–1.08) $P = 0.066$	0.80 (0.76–0.98) $P = 0.126$	0.76 (0.70–0.91) $P = 0.242$
Fetuin-A level	0.01 g/L	1.66 (1.46–2.23) $P = 0.004$	1.27 (1.14–1.60) $P = 0.010$	1.09 (1.01–1.16) $P = 0.014$
C-reactive protein level	0.1 mg/dL		1.05 (0.96–1.10) $P = 0.03$	1.05 (0.96–1.10) $P = 0.040$
Serum albumin level	0.1 g/dL			0.92 (0.89–0.96) $P = 0.044$

The relation between bone mineral densitometry and coronary artery calcification in the present study is consistent with inverse relationships found between bone density and both cardiac calcification and intima-media carotid thickness in uremic patients (2,25). Recent studies also demonstrated that vascular calcifications in some localizations were associated with both increased risk for having vertebral fractures and an increase in the incidence and the prevalence of any kind of nontraumatic fractures in both the general and dialysis population (26,27). More recently, Price et al. reported that the bisphosphonate ibandronate prevents the development of vascular calcification in rats with adenine-induced chronic renal failure maintained on a low-protein diet (28). That study represented the first experimental evidence implicating bone resorption in the pathogenesis of uremia-related vascular calcification and further confirmed animal studies suggesting a relationship between bone metabolism and vascular calcification in chronic kidney disease models (29).

Significant association between serum CRP levels and CACS found in the present work suggests that inflammation also contributes to the vascular calcification process and adds substantially to the previous evidence (30,31). Moreover, significant relations found between serum fetuin-A levels and serum CRP levels as well as between serum fetuin-A levels and serum albumin levels in the present work confirm previous findings (11,32). However, in contrast to previous studies and our findings, Hermans et al. did not demonstrate such relations between serum fetuin-A concentrations and serum levels of CRP and albumin (33). The authors thought that the lower level of inflammation in their study compared with earlier studies might play a role in different findings (33).

An association between circulating high sensitive CRP levels and BMD has been observed in several immune and inflammatory diseases (34). However, no significant relation was seen between serum CRP levels and BMD parameters in the present work, suggesting that other factors may be involved. In the present work, HD vintage, serum intact PTH, and fetuin-A levels were independent predictors of BMD. Furthermore, bone histology is abnormal to some extent in all dialysis patients (35), and these abnormalities may influence BMD. We can have patients with high bone turnover with low or normal/high BMD, but also low bone turnover can be associated with low, normal, or high BMD (26). Thus, despite not being performed in the present work, the particular histological pattern of renal osteodystrophy may be important determinants of BMD, along with other established osteopenic factors.

The positive correlation found between intravenous calcitriol administration and CACS suggests a possible risk effect of intravenous calcitriol therapy. However, the effect of intravenous calcitriol therapy appears to be of little importance in this study, which confirms previous findings (36). Moreover, intravenous calcitriol use also correlated significantly with bone mineral densities in the present work. This is inconsistent with the data suggesting that vitamin D supplements have been proven repeatedly to improve BMD and decrease fracture risks (37).

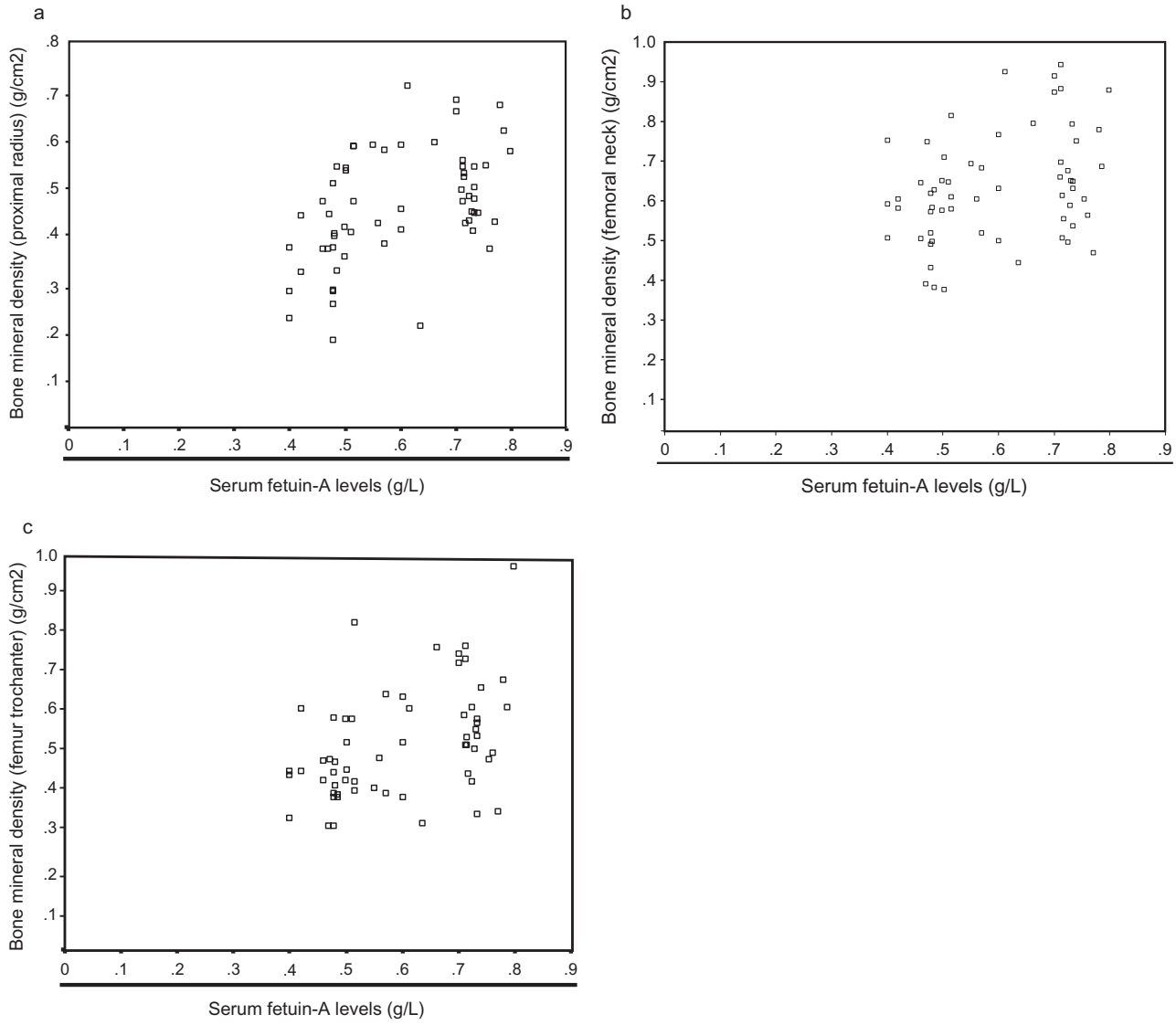

FIG. 1. Positive correlations between serum fetuin-A levels and bone mineral densities of (a) proximal radius ($r = 0.437$, $P < 0.0001$), (b) neck of the femur ($r = 0.294$, $P = 0.023$), and (c) trochanter of the femur ($r = 0.413$, $P < 0.0001$).

The association of fetuin-A levels with coronary artery calcification in this study is in agreement with previous studies (38,39). However, recent studies have failed to show a convincing association between low fetuin-A levels and high vascular calcification scores in predialysis or HD patients (38,40). Thus, the role of serum fetuin-A levels in vascular calcification may be far more complex than previously thought. The differences may be consistent with a different pathogenetic role of fetuin-A in different stages of chronic renal disease (40). Additionally, to relate vascular calcification with serum fetuin-A levels in a cross-sectional manner may be problematic, as vascular calcification is a slowly progressive process that develops over time with an unknown starting point, whereas serum fetuin-A levels may fluctuate, possibly dependent on flares of inflammation.

In the present study, we found an association between serum fetuin-A levels and bone mineral densitometry parameters. Our findings seem to be parallel to the results of a series of elegant studies by Schäfer et al. (7). Schäfer et al. showed that fetuin-A deficient mice developed severe calcification of various organs on a mineral and vitamin D-rich diet and on a normal diet when the fetuin-A deficiency was combined with a DBA/2 genetic background (7). Fetuin-A has some functions that may influence bone

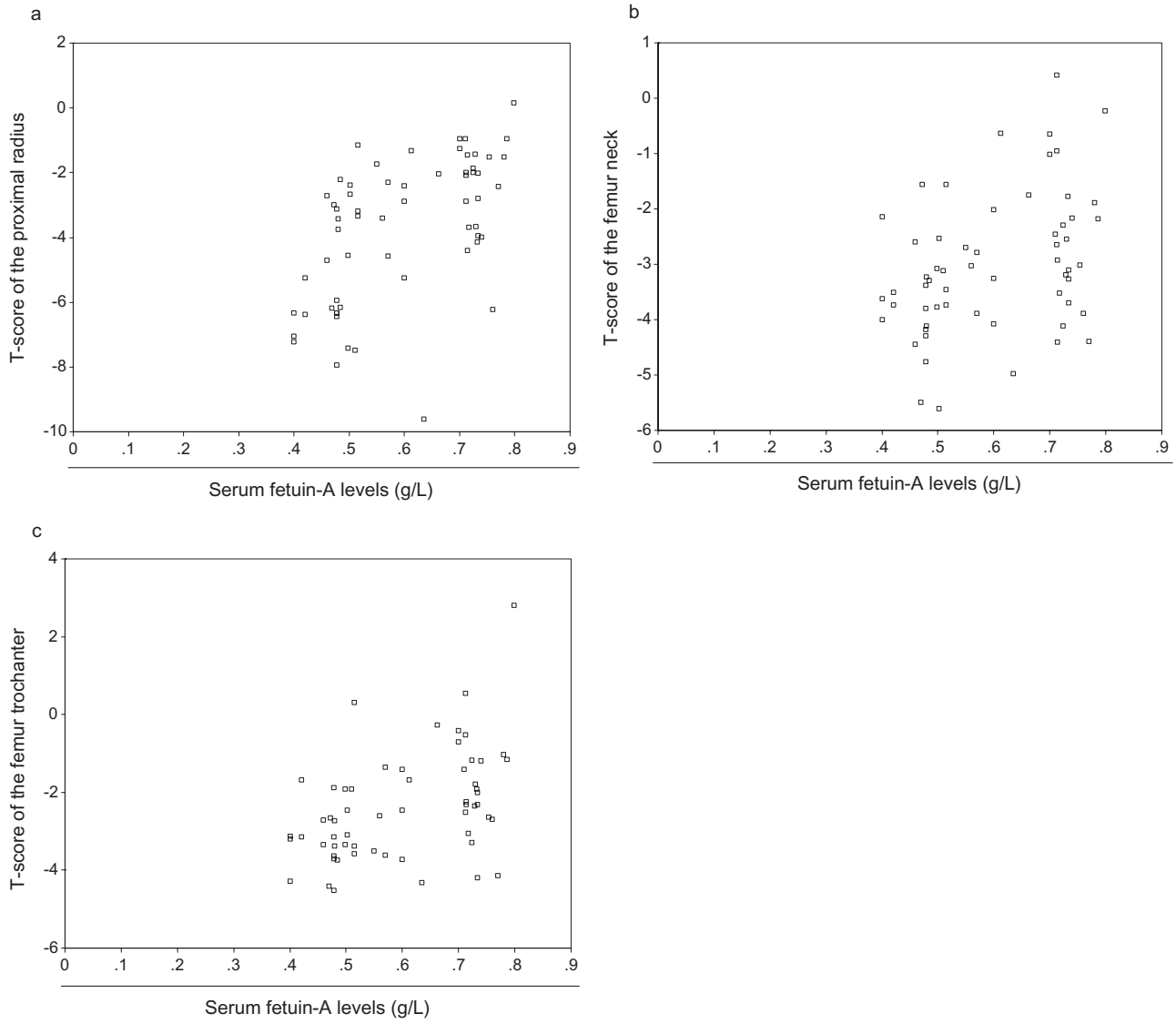

FIG. 2. Significant correlations between serum fetuin-A levels and T-scores of the (a) proximal radius ($r = 0.560$, $P < 0.0001$), (b) neck of the femur ($r = 0.329$, $P = 0.011$), and (c) trochanter of the femur ($r = 0.31$, $P = 0.001$).

biology as well as vascular smooth muscle. Fetuin-A has been shown to bind to bone morphogenetic protein-2 and transforming growth factor-β, inhibit mineralization, and suppress the expression of bone matrix proteins in bone marrow stromal cells (16,41). Studies on the effects of fetuin-A on mineralization of both Saos2 osteoblasts and 293 kidney epithelial cells in vitro also support a general role for fetuin-A in mineral metabolism (42). Moreover, fetuin-A null mice also have a disorder in mineralization of the bone and fetuin-A is a major protein component of the bone (7,12,43). Finally, results of a more recent study showed that serum fetuin-A serum levels are associated with bone cell activity and serum fetuin-A levels correlated inversely with several histomorphometric parameters connected with bone formation and bone resorption (44).

The absence of association between serum fetuin-A levels and bone mineral densitometry parameters at lumbar spine might arise from the DEXA method. DEXA was used to assess BMD in this study because DEXA measures the mineral content or density of the bone in a rapid, accurate, and a noninvasive way to identify osteopenia or osteoporosis in general use (45). Despite its frequent use, however, previous studies have suggested that unreliable results (46) or conflicting findings related to different sites used (47) could be observed for

TABLE 5. *Multiple regression analysis of predictors of the bone mineral densities and T-scores of radial, neck and trochanter of the femur, and lumbar spine*

Independent variable	Standard regression coefficient	*P* value
Radial BMD		
HD vintage	–0.43	0.0020
Intact parathormone levels	–0.21	0.0240
Serum fetuin-A levels	–0.38	0.0175
Femur neck BMD		
HD vintage	–0.59	0.0029
Intact parathormone levels	–0.24	0.0200
Serum fetuin-A levels	–0.32	0.0150
Femur trochanter BMD		
HD vintage	–0.63	0.0020
Intact parathormone levels	–0.25	0.0300
Serum fetuin-A levels	–0.29	0.0180
Lumbar spine BMD		
HD vintage	–0.61	0.0025
Intact parathormone levels	–0.33	0.0050
Radial T-score		
HD vintage	–0.52	0.0030
Intact parathormone levels	–0.23	0.0350
Serum fetuin-A levels	–0.40	0.0240
T-score (femur neck)		
HD vintage	–0.60	0.0027
Intact parathormone levels	–0.21	0.0380
Serum fetuin-A levels	–0.35	0.0200
T-score (femur trochanter)		
HD vintage	–0.55	0.0030
Intact parathormone levels	–0.27	0.0260
Serum fetuin-A levels	–0.41	0.0040
T-score (lumbar spine)		
HD vintage	–0.61	0.0023
Intact parathormone levels	–0.22	0.0200

The stepwise regression model included age, gender (male vs. female), body mass index, diabetes, HD vintage, serum PTH levels, calcitriol therapy, fetuin-A levels, serum calcium, and phosphate levels.

assessment of BMD with DEXA method in patients with ESRD. Moreover, dense vascular calcification in the aorta, rather than the spine, may absorb dual-energy X-ray beams projected blindly through the body and lead to falsely elevated BMD readings (48), whereas secondary hyperparathyroidism may cause trabecular bone sclerosis that results in increased spinal BMD (49). Thus, measured lumbar spine BMD values might not be related to serum fetuin-A levels as there is the possibility that BMD assessments by DEXA method might be affected by above mentioned factors and might not reflect the true mineral content.

The results of the present work should be interpreted with caution because of several limitations. First, subjects were on long-term HD at a single center, making it difficult to draw general conclusions. Second, the sample size was small. Third, the cross-sectional nature of the study may also influence the results. Fetuin-A levels were only assessed at a single point in time instead of having time-averaged values and these values are related to bone mineral densitometry parameters and coronary artery calcification that develop over a long time. Fourth, the accuracy of diagnosis of osteopenia and osteoporosis based on DEXA measurements in renal osteodystrophy remains uncertain (50). Fifth, bone biopsy, the gold standard method for the diagnosis of renal osteodystrophy, together with histomorphometric analysis has been underused in the evaluation of osteoporosis in patients with chronic kidney disease (51,52). We also could not perform such an analysis in the present work because all of the participants refused to undergo bone biopsy. Finally, the relationship among serum fetuin-A levels, BMD, and CACS was not studied in the control group, therefore correlations between serum fetuin-A levels, CACS, and bone mineral densitometry findings should be interpreted with caution and they may not be unique for HD patients.

Lower radial T-scores compared with femoral T-scores may reflect a preferential influence of fetuin-A deficiency on the femur (contains both cortical and trabecular bone) over the radius (contains cortical bone). Experimental studies of fetuin-A knockout mice showed accelerated trabecular bone remodeling and increased activity of the transforming growth factor-β/bone morphogenetic protein system, a protein system with osteoinductive properties (17). Furthermore, although the mean values of intact PTH of 6 months' duration were used in the present study, we can not easily rule out the possible effect of hyperparathyroidism due to the cross-sectional design. Hyperparathyroidism may result in sclerotic thickening of trabecular bone (proximal femur) with increased BMD and simultaneously stimulating resorption in cortical bone (radius) with reductions in BMD (45).

CONCLUSION

This study confirms the relation between coronary artery calcification and bone mineralization in maintenance HD patients as suggested previously. Moreover, significant associations between serum fetuin-A levels, coronary artery calcification, and bone mineral densitometry would suggest that fetuin-A might play a common role in both coronary artery calcification and bone mineralization in this patient group. However, the results should be interpreted with caution because of the cross-sectional design of the study and larger studies are needed to confirm our findings.

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