Evaluation of coronary artery calcification using multislice computed tomography in patients on dialysis: association with fetuin-A and osteoprotegerin

Sherif Aziz Zaki[a], Osama Hassan Shehata[a], Akram A. Deghedi[b], Shehab Mohamed Sami[c], Rehab H. M. Mersal[a]

Departments of [a]Internal Medicine, [b]Clinical Pathology, [c]Radiodiagnosis, Faculty of Medicine, Alexandria University, Alexandria, Egypt

Correspondence to Rehab H. M. Mersal, MSc, Department of Internal Medicine, Faculty of Medicine, Alexandria Smouha District 7 Kamal Eldin Salah, Alexandria, Egypt
Tel: +20 122 344 1494;
e-mail: mersal95@yahoo.com

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Introduction
Cardiovascular disease is the leading cause of mortality in patients with end-stage renal disease (ESRD) and is attributed to a combination of traditional and nontraditional risk factors. In recent years, there has been an increasing recognition of a very high prevalence of cardiovascular calcification in the ESRD population, including patients receiving hemodialysis (HD) and peritoneal dialysis. The mechanism is multifactorial, including structural and functional abnormalities in the large vessels, disorders in calcium (Ca^{2+}) and phosphate (P) metabolism, vascular smooth muscle cells changes, and regulatory markers such as fetuin-A and osteoprotegerin (OPG).

Aim of the work
The aim of the present study was to determine the utility of multislice computed tomography (MSCT) for the assessment of coronary artery calcifications (CACs) and to identify the potential risks for CAC, including calcification regulating proteins such as fetuin-A and OPG, among patients with ESRD on maintenance dialysis (HD and peritoneal dialysis).

Patients and methods
This study included 70 patients who were divided into four groups: 20 patients on continuous ambulatory peritoneal dialysis (CAPD), 10 patients with ESRD stage 4 and 5, 30 patients on HD (subdivided into three subgroups according to the duration of HD: for 1–5 years, for 5–10 years, and for more than 10 years), and 10 healthy controls. They were subjected to complete history-taking, thorough clinical examination, investigations including serum level of fetuin-A, serum level of OPG by using the enzyme-linked immunosorbent assay technique, as well as MSCT imaging using 128-detector scanners for the quantification of CAC (calcium scoring) by using the Agatston method.

Results
There was a significant decrease in the serum level of fetuin-A in patients on HD compared with patients on CAPD, as well as in healthy controls. Moreover, there was a significant increase in the serum level of OPG in patients on HD compared with its level in CAPD patients as well as in healthy controls. The calcium scoring was significantly high in the HD group of patients (group IIa) ($P = 0.032$), with a low calcium score in CAPD patients group (group I) ($P = 0.036$) compared with healthy controls in group IV. CAC scoring was correlated positively and significantly with serum level of OPG in the total samples ($r = 0.345^*$ and $P = 0.0270$). On the other hand, it was negatively and significantly correlated with the serum level of fetuin-A in the total samples ($r = -0.411^*$ and $P = 0.002$).

Conclusion
Fetuin-A and OPG can be early and important markers of vascular calcifications in patients on dialysis; in addition, calcium scoring using MSCT provides a noninvasive method of assessment of the vascular calcification in these patients. Vascular calcification is more evident in patients on HD than in patients treated using CAPD; this can help in the selection of the modality of treatment of patients with ESRD, as well as early detection and prevention of cardiovascular (CVS) diseases in patients with ESRD treated with dialysis either HD or CAPD.

Keywords:
Agatston calcium scoring, fetuin-A, osteoprotegerin, vascular calcifications

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Introduction

Patients with chronic kidney disease (CKD) experience up to 30-fold higher cardiovascular disease mortality than does the general population [1]. This

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staggering outcome is only incompletely explained by such traditional risk factors as aging, smoking, diabetes, dyslipidemia, or hypertension [2,3]. These traditional risk factors cannot completely account for the excessive prevalence of CKD [4], suggesting dialysis-specific risk factors that may contribute to hyperphosphatemia and elevations in the calcium × phosphorus product in patients with CKD-5 with increased mortality [5]. In addition, studies have linked these disorders of mineral metabolism with vascular calcification in-vitro, in-vivo animal models, and in humans [6].

Vascular calcification is a common complication in uremia, due in part to disturbed mineral metabolism and the therapies used to control it [7], as well as due to a complex, active process of osteogenesis in vascular smooth muscle cells [1,2,6]. Furthermore, cardiovascular calcifications in patients with CKD are more prevalent, progressive, extensive, and severe compared with the non-CKD population. The prevalence of coronary artery calcification (CAC) has been reported to range from 40% to nearly 100% in patients on dialysis [8].

Vascular calcifications can result in a range of pathologies including calcemic uremic arteriolopathy (formerly termed calciphylaxis) and extraosseous soft tissue calcification; however, valvular and vascular calcifications are the most common and perhaps the most clinically significant manifestations [9].

Research efforts have expanded because of the contribution of vascular pathologies to understand the nature of extraosseous calcification that occurs in patients with CKD.

There are two major types of calcification affecting different layers of the artery [10]:

(1) *Atherosclerotic plaque*, which occurs within the intimal layer of the arteries; arterial intimal calcification is seen in advanced atherosclerosis. Arterial intimal calcification has been shown to develop in older individuals and those with prolonged history of diabetes, hypertension, smoking, and other traditional risk factors [11].
(2) *Monckeberg's sclerosis*, which occurs in the medial wall (or tunica media).

Medial calcification is noninflammatory and increases vascular stiffness and reduces vascular compliance. This type of calcification is common in uremic patients, especially in young age.

Multiple inhibitors of vascular calcification are known. Local tissue inhibitors include matrix gla protein, pyrophosphates, osteopontin, fetuin-A, and osteoprotegerin (OPG) [9,12].

Fetuin-A [Heremans-Schmid glycoprotein (AHSG)] is an inhibitor of calcification. It is synthesized predominantly in the liver and is abundant in serum at levels of 0.5–1.0 g/l. The transcription and synthesis of fetuin-A are downregulated during inflammation; therefore, fetuin-A represents a reverse acute phase reactant. Low levels of fetuin-A in patients with CKD are associated with arterial calcification, calciphylaxis, vascular smooth muscle cells apoptosis, and mortality. Fetuin-A binds to both calcium and phosphate in the serum, forming small 'calciparticles', which are presumably removed through the reticuloendothelial system; therefore, fetuin-A acts in host defense to clean the blood of unwanted calcium and phosphate [13,14].

OPG is a regulatory factor produced by bone marrow-derived stromal cells. OPG has a pivotal role in the regulation of the bone turnover inhibiting osteoclast differentiation and acts like a decoy receptor for the receptor activator of nuclear factor-kB ligand (RANKL system). OPG-deficient mice have been shown to develop both severe aortic calcifications and osteoporosis [15].

On the one hand, OPG is considered to prevent vascular calcification as it blocks the bone remodeling process in the vascular tissue following the interaction between RANK (expressed by osteoclast-like cells) and RANKL (expressed by osteoblast-like cells). It is also a neutralizer of the proapoptotic actions of TRAIL (tumor necrosis factor-related apoptosis-inducing ligand), which strongly activates vascular cell apoptosis [16].

On the basis of these experimental considerations, OPG deficiency should be associated with an increased risk of extraosseous calcification; however, things appear to be quite different in humans, especially during the uremic condition [17].

Patients on dialysis have increased OPG levels when compared with the normal population. Moreover, elevated OPG levels have been found to be independently associated with aortic calcification [18].

A similar observation was already made in the nondialysis population in which higher OPG levels pointed to an increased cardiovascular risk – this phenomenon was termed a 'seeming paradox' [19]. It currently remains an unanswered question whether OPG upregulation is predominantly a marker of cardiovascular disease [20], an important but

incomplete self-defense mechanism trying to counteract excessive atherosclerosis and calcification, or possesses potentially damaging properties in humans that have not yet been identified [21].

The multidetector computed tomography scan system enables noninvasive detection of coronary artery disease, with an early detection of rupture-prone soft plaques [22]. Since 2001, multislice computed tomography (MSCT) has been able to display a promising capability in detecting not only coronary calcium but also the noncalcified component of plaques [23].

Thus, these advantages make MSCT a powerful tool that could be used in the early detection of vascular and valvular calcifications in patients on dialysis and even in patients with end-stage renal disease (ESRD).

Therefore, the aim of this study was to investigate the role of the serum inhibitors of calcifications – fetuin-A and OPG with MSCT – in early detection of CACs in patients with ESRD and in patients on dialysis [hemodialysis (HD) and peritoneal dialysis (PD)] with comparing the risk of CAC between the two modalities of dialysis.

Patients and methods

Patients

We studied 60 patients with kidney disease; 20 of them with ESRD were on maintenance PD for more than 6 months, with a mean age of 28.95 ± 7.94 years (group I). Group IIa comprised 10 patients with ESRD on maintenance HD for 1–5 years by bicarbonate HD (4 h per session, three times weekly) using polysulfone dialyzer with a surface area of 1.3 m^2, with a mean age of 25.50 ± 6.62 years; group IIb comprised 10 patients with ESRD on maintenance HD for 5–10 years, with a mean age of 28.80 ± 5.87 years; and group IIc comprised 10 patients on maintenance HD for more than 10 years, with a mean age of 27.50 ± 6.31years. Ten patients with ESRD, with creatinine clearance of average 20 ml/min, and a mean age of 26.10 ± 5.22 years, were included in group III. Exclusion criteria for the present study included diabetes mellitus, chronic inflammatory diseases, active infections, smoking, hyperlipidemia, and coronary heart diseases.

Control group

As the control group we selected 10 healthy volunteer subjects accurately age and sex matched to patients with ESRD and those on dialysis, with a mean age of 25.10 ± 4.36 years. To be selected, healthy subjects had to have no alteration at an extensive clinical and biochemical work-up, and had to have normal urine analysis and estimated glomerular filtration rate equal or greater than 90 ml/min/1.73 m^2, which is recommended threshold for the diagnosis of CKD.

Methods

The study was conducted in accordance with the ethical guidelines of the 1975 Declaration of Helsinki. An informed consent was obtained from all participants in the study.

All patients and controls were subjected to thorough history-taking, which included the history of phosphate binders with or without calcium content. In addition, full clinical examination was carried out with special observation for signs of calcemic uremic arteriolopathy (calciphylaxis).

Laboratory parameters

Laboratory investigations included complete blood count [24], renal function tests (blood urea, serum creatinine, and creatinine clearance) [25], serum ionized calcium, phosphorus with calculation of calcium-phosphorus product, alkaline phosphatase, and serum intact parathyroid hormone level [26]. Lipid profile [27], erythrocyte sedimentation rate, high-sensitive C-reactive protein (hs-CRP) [28], and plasma fibrinogen were also estimated [29].

Estimation of dialysis adequacy by measuring total weekly K_t/V for both HD and peritoneal patients was carried out [30,31]. Furthermore, patients on PD were subjected to total weekly test of peritoneal permeability by using the peritoneal equilibration test [32].

Serum fetuin-A measurement [33]

Serum fetuin-A and OPG levels were measured using the quantitative sandwich enzyme-linked immunosorbent assay technique. Quantizing kit, as described by Axelsson, was used for the study.

The Quantikine Human Fetuin-A Immunoassay contains NS0-expressed recombinant human fetuin-A and was performed according to the manufacturers' protocol provided by R&D Systems Inc. (Minneapolis, Minnesota, USA).

Serum osteoprotegerin measurement [34]

Detection of the serum level of OPG was carried out using the sandwich enzyme-linked immunosorbent assay kit using streptavidin-HRP, as described by Morena and colleagues. OPG was provided by DRG International Inc. (USA).

Radiological study

CACs were assessed by using MSCT with 128-detector scanner for quantification of CAC (calcium scoring) by using the Agatston method [35].

Quantification of calcifications is based on a score published in a study by Agatston *et al.* [35]. The 'calcium score' was determined by multiplying the area of calcification by a weighted density score: 1 = 130–199 HU, 2 = 200–299 HU, 3 = 300–399 HU, and 4>400 HU. Individual scores were calculated for the left main coronary artery, the descending branch of the left coronary artery, the circumflex branch of the left coronary artery, and the right coronary artery. The total coronary score is the sum of these individual scores [37].

Statistical analysis of the data [38]

All calculations were carried out on a personal computer with IBM SPSS software package (version 20.0) for Windows [39].

Qualitative data were presented as numbers (*n*) and percentages (%). Quantitative data were presented as mean and SD. Comparison between the means of quantitative variables was performed using the one-way analysis of variance (*F*-test). The correlations between different variables were evaluated by using Pearson's or Spearman's correlation coefficients according to the distribution of variables (continuous or discontinuous quantitative variables, respectively). *P* value less than or equal to 0.05 was accepted as statistically significant.

Results

Clinical and biochemical characteristics of the study participants are presented in Table 1.

Blood urea, serum albumin, serum ionized calcium, serum phosphorus, calcium×phosphorous product, serum alkaline phosphatase, serum intact parathormone level, serum fibrinogen, hs-CRP had a significant difference all through the three groups ($P < 0.001$); their levels were significantly higher in patients on HD for more than 10 years than in patients on PD and controls. There was no significant difference between the groups as regards other clinical parameters such as age.

Circulating serum fetuin-A levels were significantly higher in patients on PD and controls than in all patients on HD (1.26 ± 0.28 and 1.49 ± 0.23 g/l, respectively, vs. 0.56 ± 0.32/0.42 ± 0.14/0.30 ± 0.07 g/l in group IIa, IIb, and IIc, respectively). Furthermore, it was significantly higher in patients on PD than in patients with ESRD in group III (1.26 ± 0.28 vs. 0.69 ± 0.70 g/l).

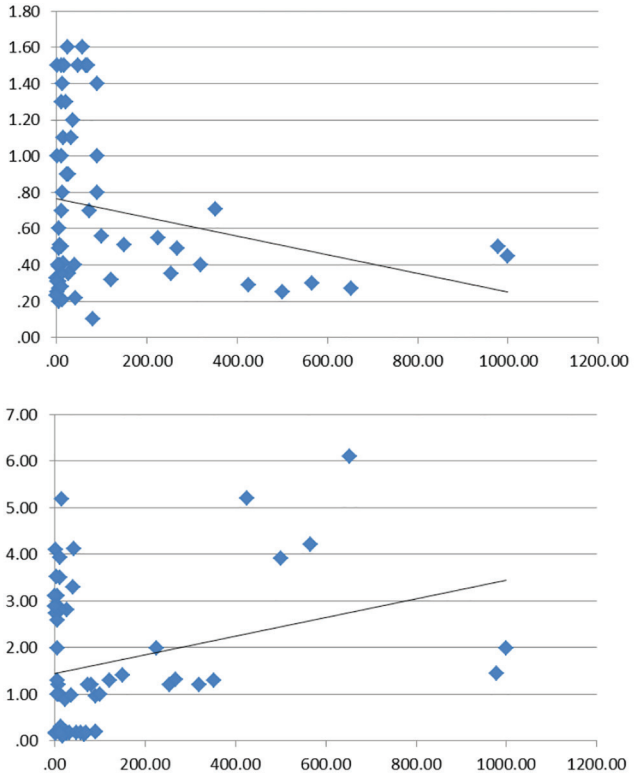
Serum OPG levels were significantly higher in patients on HD, especially group IIc (more than 10 years on HD), than in patients on PD and controls (10.65 ± 3.05 vs. 3.25 ± 0.98 ng/ml in PD and 2.84 ± 0.89 ng/ml in controls).

The mean value of CAC scoring using MSCT in patients on HD, especially in patients for more than 10 years on HD, was significantly higher than in patients on PD (321.6 ± 166.2 vs. 89.6 ± 52.3). In addition, using MSCT we found that 30% of the patients on HD for more than 10 years had increased calcifications (101–400 vs. only 10% of the patients on PD); meanwhile extensive calcifications (>400) were observed in 30% of the patients on HD compared with 0% in patients on PD.

Correlations between CAC scoring and different parameters in the different groups are shown in Table 2 and Fig. 1.

CAC scoring was correlated positively and significantly with the serum level of ionized calcium, phosphorus,

Figure 1



Correlation between coronary artery calcium score with osteoprotegerin (OPG) and fetuin-A in each group and total sample.

Table 1 Clinical and biochemical characteristics of the study

Parameters	Group I	Group IIa	Group IIb	Group IIc	Group III	Group IV	*P*
Age (years)							
Range	18.0–47.0	17.0–35.0	19.0–35.0	19.0–39.0	19.0–35.0	19.0–31.0	0.528 (NS)
Mean ± SD	28.95 ± 7.94	25.50 ± 6.26	28.80 ± 5.87	27.50 ± 6.31	26.10 ± 5.22	25.10 ± 4.36	
Hb (g/dl)	*P* = 0.006						
Range	9.8–12.7	4.3–10.0	9.5–11.0	9.9–12.1	7.5–10.5	11.5–13.5	0.006 (NS)
Mean ± SD	12.35 ± 0.76	7.19 ± 2.24	9.78 ± 0.59	11.58 ± 0.92	8.98 ± 1.07	12.11 ± 0.98	
Urea (mg/dl)	*P* = 0.002						
Range	87.5–150.3	115.2–182.1	101.8–170.1	120–160	100–200	20.0–24.1	0.002*
Mean ± SD	113.48 ± 16.18	140.14 ± 24.23	159.09 ± 23.74	139.66 ± 18.96	140.11 ± 35.2	22.15 ± 1.59	
Serum creatinine (mg/dl)	*P* = 0.007						
Range	7.9–11.3	10.5–12.3	9.7–12.5	9.5–11.5	4.0–6.5	0.6–0.9	0.007 (NS)
Mean ± SD	9.45 ± 1.21	11.90 ± 0.73	10.21 ± 0.95	11.00 ± 0.85	5.69 ± 1.07	0.75 ± 0.12	
Serum albumin (mg/dl)	*P* = 0.001						
Range	3.1–5.1	3.1–4.5	3.1–4.5	3.2–5.1	3.9–5.9	4.7–5.5	0.001*
Mean ± SD	4.00 ± 0.54	3.95 ± 0.49	3.80 ± 0.48	4.05 ± 0.68	4.87 ± 0.65	5.02 ± 0.23	
Serum uric acid (mg/dl)	*P* = 0.324 (NS)						
Range	3.5–6.8	3.2–6.5	3.2–6.5	4.3–6.3	3.7–5.6	3.5–5.2	0.324 (NS)
Mean ± SD	4.76 ± 0.98	4.83 ± 1.21	5.02 ± 1.04	5.18 ± 0.71	4.66 ± 0.72	4.30 ± 0.57	
Kt/V	*P* = 0.001						
Range	1.5–1.7	1.0–1.2	1.0–1.2	1.1–1.2	–	–	0.001*
Mean ± SD	1.66 ± 0.05	1.11 ± 0.07	1.13 ± 0.06	1.15 ± 0.05	–	–	
HDL	*P* = 0.013 (NS)						
Range	40.0–50.0	42.0–50.0	40.0–50.0	41.0–50.0	40.0–50.0	43.0–50.0	0.013
Mean ± SD	42.95 ± 4.03	48.00 ± 2.98	45.90 ± 4.07	46.80 ± 3.33	46.40 ± 3.57	46.40 ± 2.8	
LDL	*P* = 0.056 (NS)						
Range	101.0–123.0	103.0–124.0	106.0–129.0	109.0–129.0	103.0–129.0	100.0–24.0	0.056
Mean ± SD	106.10 ± 7.70	108.70 ± 8.01	117.70 ± 9.38	122.60 ± 7.92	111.80 ± 7.51	111.00 ± 6.51	
TG	*P* = 0.034 (NS)						
Range	132.0–194.0	135.0–198.0	138.0–198.0	138.0–198.0	153.0–191.0	135.0–200.0	0.034
Mean ± SD	158.00 ± 20.84	174.50 ± 21.10	172.00 ± 20.27	170.10 ± 16.45	174.90 ± 14.03	177.30 ± 27.86	
Serum ionized Ca^{2+} level (mg/dl)							
Range	4.5–5.6	4.0–5.7	5.5–6.4	5.9–6.9	4.2–5.5	4.4–5.5	0.047 (NS)
Mean ± SD	5.0 ± 0.4	4.9 ± 0.5	5.9 ± 0.29	6.42 ± 0.32	4.9 ± 0.63	4.95 ± 0.39	
Serum Ph level (mg/dl)	*P* = 0.001						
Range	3.6–6.2	3.5–6.2	3.5–6.5	6.5–9.0	5.3–7.1	2.1–3.8	0.001
Mean ± SD	4.71 ± 0.74	4.75 ± 0.91	5.19 ± 0.97	7.92 ± 1.09	6.74 ± 0.88	3.37 ± 0.55	
Total calcium × phosphorus product	*P* = 0.003						
Range	30.6–78.74	40–86.25	39–91.26	46.75–92	39.06–58.83	19.95–46.74	0.003*
Mean ± SD	51.43 ± 8.25	56.30 ± 11.25	60.26 ± 19.80	67.05 ± 18.30	49.82 ± 11.90	37.27 ± 15.10	
Serum iPTH (pg/ml)	*P* = 0.001						
Range	179.6–980.5	350.1–760.2	410.9–930.7	870.5–1325.0	153.7–335.1	68.2–99.2	0.001*
Mean ± SD	541.35 ± 215.63	561.37 ± 71.76	645.41 ± 141.0	109.77 ± 161.34	260.66 ± 68.44	81.25 ± 10.68	
Serum alkaline phosphatase (IU/l)	*P* = 0.0001						
Range	25–130	52–150	70–165	85–185	22–135	26–140	0.528 (NS)
Mean ± SD	64.3 ± 31.3	85.6 ± 28.9	125.6 ± 38.2	144.6 ± 29.8	65.5 ± 32.9	65.9 ± 30.8	
Serum hs-CRP (mg/l)	*P* = 0.001						
Range	2.3–6.2	3.9–6.1	4.9–6.5	3.5–6.3	3.7–6.1	2.1–5.0	0.001*
Mean ± SD	4.05 ± 1.13	5.33 ± 0.74	5.76 ± 0.64	5.20 ± 0.94	5.07 ± 0.83	3.56 ± 0.87	
Serum fibrinogen (g/l)	*P* = 0.0023						
Range	180–420	200–420	230–450	290–750	190–400	170–330	0.0023*
Mean ± SD	289.3 ± 65.6	310.2 ± 85.6	322.6 ± 67.8	520.6 ± 102.6	289.5 ± 115.6	210.3 ± 52.6	

Contd...

Table 1 Contd...

Parameters	Group I	Group IIa	Group IIb	Group IIc	Group III	Group IV	*P*
OPG	*P* = 0.015						
Range	1.3–5.6	2.0–9.3	2.5–10.5	5.3–19.8	0.9–5.5	1.0–4.1	0.015*
Mean ± SD	3.25 ± 0.98	5.69 ± 2.36	6.11 ± 3.65	10.65 ± 3.05	2.658 ± 1.02	2.84 ± 0.89	
Fetuin A (g/l)	*P* = 0.006						
Range	0.7–1.6	0.1–1.2	0.3–0.7	0.2–0.4	0.2–1.7	1.0–1.7	0.006*
Mean ± SD	1.26 ± 0.28	0.56 ± 0.32	0.42 ± 0.14	0.30 ± 0.07	0.69 ± 0.70	1.49 ± 0.23	
Calcium scoring	*P* = 0.001						
Range	0–235	3–400	21–560	95–750	5–82		0.001*
Mean ± SD	89.6 ± 52.3	122.6 ± 1010.3	228.6 ± 106.5	321.6 ± 166.2	54.6 ± 31.2		

Group IV, control; Group I, peritoneal dialysis patients; Group IIa, hemodialysis patients less than 5 years dialysis; Group IIb, hemodialysis patients from 5–10 years; Group IIc, hemodialysis patients more than 10 years; Group III, end-stage renal disease, HDL, high-density lipoprotein; hs-CRP, high-sensitive C-reactive protein; iPTH, intact parathyroid hormone; LDL, low-density lipoprotein; OPG, osteoprotegerin; TG, triglyceride; **P* value of Phosphorus is significant.

calcium×phosphorus product, alkaline phosphatase, intact parathyroid hormone, hs-CRP, and fibrinogen.

CAC scoring was correlated positively and significantly with the serum level of OPG in the total samples (r = 0.345* and P = 0.0270).

CAC scoring was correlated negatively and significantly with the serum level of fetuin-A in the total samples (r = –0.411* and P = 0.002).

Discussion

In the present study, serum OPG (P = 0.015) was significantly higher in patients on HD for more than 10 years (group IIc) than in all other participants; meanwhile its level was significantly lower in patients on PD (group I) and controls. In this study, there was a positive correlation between increased OPG serum level and elevated calcium scoring (P = 0.027, r = 0.345*).

In agreement with our study, Lee *et al.* [40] showed in their study that increasing serum levels of OPG in patients on HD was independent of age, HD duration, systolic blood pressure, pulse wave velocity, cholesterol, and triglycerides. In addition, the new cardiovascular events were higher among those with higher serum levels of OPG. A study by Pateinakis *et al.* [41] found out that OPG was the only variable that was independently associated with carotid intimal medial thickness and that the OPG level had a positive correlation with vascular calcifications.

Furthermore, the study conducted by Ozkok *et al.* [42] showed that both baseline and first-year serum OPG levels were significantly higher in the group of patients with progressive coronary calcification (OPG: 0.26 pmol/l, CACs: 495) compared with the nonprogressive group (OPG: 0.18 pmol/l, CACs: 2.15).

In the current study, the serum levels of fetuin-A in patients on HD for more than 10 years (group IIc) were significantly lower than that of participants in all other groups. Meanwhile, patients on PD, as well as the controls, showed significantly higher serum levels of fetuin-A than did patients on HD and those with ESRD. There was a negative correlation between the serum level of fetuin-A and CAC score (r = –0.411* and P = 0.002).

In agreement with the current work, Turkmen *et al.* [43] conducted a study on 78 patients on HD, all patients on dialysis for more than 6 months. CAC scores were determined. Serum C-reactive protein, interleukin-1b (IL-1b), IL-6, tumor necrosis factor-a, and serum fetuin-A levels were measured. They found out that there was a negative relationship between fetuin-A levels and total CAC scores.

In another study conducted by Stenvinkel *et al.* [44] on 258 patients with ESRD starting renal replacement therapy, hs-CRP, fetuin-A, S-albumin, and IL-6 were measured. Their study showed that a low fetuin-A level was associated with malnutrition, inflammation, and increased cardiovascular and all-cause mortality.

In their cross-sectional study on 81 stable chronic HD patients, Pateinakis *et al.* [41] measured carotid-to-femoral pulse wave velocity reflecting arterial stiffness, and common carotid intima–media thickness, a surrogate of early atherosclerosis, as well as the serum levels of fetuin-A and OPG. They concluded that common carotid intima–media thickness was negatively associated with fetuin-A and positively with OPG; they also found that both fetuin-A and OPG were independently associated with carotid-to-femoral pulse wave velocity.

In contrast to the findings of our study, a study by Hermans *et al.* [45] found no association between

Table 2 Correlation between coronary artery calcification, fetuin-A, and osteoprotegerin with different parameters in each group and total sample

Parameter	Ca score	Fetuin-A	OPG
OPG			
r	0.345*	−0.746	
P	0.0270	0.000	
Fetuin-A			
r	−0.411*		−0.746**
P	0.002		0.000
Age			
r	0.123	0.125	0.118
P	0.413	0.367	0.398
Hb			
r	−0.126	0.216	−0.018
P	0.269	0.072	0.880
Urea			
r	0.124	−0.260	0.075
P	0.321	0.030	0.535
Creatinine			
r	0.198	−0.193	0.048
P	0.2325	0.110	0.696
Uric acid			
r	0.045	−0.173	0.114
P	0.742	0.169	0.367
Kt/V			
r	−0.146	0.805	−0.605**
P	0.421	0.000	0.0001
Ionized Ca			
r	0.531**	−0.806	0.594**
P	0.0001	0.000	0.0001
Ph			
r	0.394*	−0.335	0.260*
P	0.018	0.005	0.029
PTH			
r	0.341*	−0.358	0.441**
P	0.009	0.002	0.0001
Ca × Ph			
r	0.466**	0.835	0.768**
P	0.002	0.000	0.0001
LDL			
r	−0.058	−0.177	0.152
P	0.659	0.143	0.210
HDL			
r	−0.225	0.411	−0.363**
P	0.084	0.000	0.002
TG			
r	0.093	−0.093	0.348**
P	0.477	0.477	0.003
CRP			
r	0.347**	−0.462	0.428**
P	0.009	0.000	0.000
Filinogen			
r	0.783**	−0.123	0.517
P	0.0001	0.351	0.001
PET			
r	0.189	0.212	0.247
P	0.426	0.324	0.295

*Significant. **Highly Significant. CRP, C-reactive protein; HDL, high-density lipoprotein; LDL, low-density lipoprotein; OPG, osteoprotegerin; PET, peritoneal equilibration test; PTH, parathyroid hormone; TG, triglyceride.

circulating fetuin-A and pulse wave velocity and cardiovascular mortality.

Another study, conducted by Reslerova *et al.* [46], showed that the serum levels of fetuin-A were negatively correlated with the CAC score, although the fetuin-A levels in subjects with no calcification did not differ from those with mild calcification (score 1–200) or with severe calcification (score>200), although a trend was present.

In the current study, CAC was assessed using the calcium score calculated by using MSCT; it was found that coronary calcification in patients on HD patients, specially patients on HD for more than 10 years, was significantly higher and more extensive than in patients on PD.

In agreement with the current work, Rubin [47] and Bazeeda *et al.* [48] in their respective studies assessed the role of computed tomography in diagnosing CAC in patients with ESRD patients and those on HD; they demonstrated that CAC progressively increased with the decline in the estimated glomerular filtration rate, and that measurement using noncontrast computed tomography has greater reliability and substantially with lower radiation exposure as it provides risk stratification of asymptomatic individuals with the intent of targeting therapy to prevent coronary heart disease and acts as a gatekeeper to cardiac catheterization to minimize unnecessary invasive diagnostic coronary procedures.

Studies conducted by Hernandes *et al.* [49] and Barreto *et al.* [50] reported results comparable to those of the present work. The first study, conducted on 23 adult patients on dialysis, demonstrated no CAC in 30% of the patients, moderate calcification in 12%, and severe calcification in 36% of the patients; and the second study, conducted on 101 patients on HD, demonstrated that the range of the calcium score was 0.5547; a total of 52 patients showed moderate and severe CAC, and 20% of them had calcium scores greater than 1000.

Conclusion

Serum levels of OPG were significantly lower in patients on PD than in patients on HD and there was significant positive correlation between the serum level of OPG and CAC assessed by using the calcium scoring calculated using MSCT. Serum levels of fetuin-A were significantly higher in patients on PD than in patients on HD, with significant negative correlation with CACs. Thus, serum OPG and fetuin-A can be regulatory markers for CAC, conjunction with

CAC scoring using MSCT contribute noninvasive assessment of CACs in ESRD patients as well a dialysis patients. In addition, we conclude that the low incidence and level of calcification in patients on PD make it an important modality of treatment of patients with ESRD.

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Conflicts of interest
There are no conflicts of interest.

References

1 Schoppet M, Shroff RC, Hofbauer LC, Shanahan CM. Exploring the biology of vascular calcification in chronic kidney disease: what's circulating? Kidney Int 2008; 73:384–390.

2 Mizobuchi M, Towler D, Slatopolsky E. Vascular calcification: the killer of patients with chronic kidney disease. J Am Soc Nephrol 2009; 20: 1453–1464.

3 Chertow GM, Raggi P, Chasan-Taber S, Bommer J, Holzer H, Burke SK. Determinants of progressive vascular calcification in haemodialysis patients. Nephrol Dial Transplant 2004; 19:1489–1496.

4 Cheung AK, Sarnak MJ, Yan G, Dwyer JT, Heyka RJ, Rocco MV, *et al.* Atherosclerotic cardiovascular disease risks in chronic hemodialysis patients. Kidney Int 2000; 58:353–362.

5 Block GA, Klassen PS, Lazarus JM, Ofsthun N, Lowrie EG, Chertow GM Mineral metabolism, mortality, and morbidity in maintenance hemodialysis. J Am Soc Nephrol 2004; 15:2208–2218.

6 Moe SM, Chen NX. Pathophysiology of vascular calcification in chronic kidney disease. Circ Res 2004; 95:560–567.

7 Goodman WG, London G, Amann K, Block GA, Giachelli C, Hruska KA, *et al.*, Vascular Calcification Work Group Vascular calcification in chronic kidney disease. Am J Kidney Dis 2004; 43:572–579.

8 Kidney Disease: Improving Global Outcomes (KDIGO) CKD-MBD Work Group. KDIGO clinical practice guideline for the diagnosis, evaluation, prevention, and treatment of Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD). Kidney Int Suppl 2009; 113:S1–S130.

9 Floege J, *et al.* Arteriopathy and extra-osseous calcification in dialysis patients. Kidney Int 2004; 65:2447–2462.

10 London GM, Guérin AP, Marchais SJ, Métivier F, Pannier B, Adda H. Arterial media calcification in end-stage renal disease: impact on all-cause and cardiovascular mortality. Nephrol Dial Transplant 2003; 18: 1731–1740.

11 Ishimura E, Okuno S, Kitatani K, Maekawa K, Izumotani T, Yamakawa T, *et al*. C-reactive protein is a significant predictor of vascular calcification of both aorta and hand arteries. Semin Nephrol 2004; 24:408–412.

12 Sage AP, Lu J, Tintut Y, Demer LL. Hyperphosphatemia-induced nanocrystals upregulate the expression of bone morphogenetic protein-2 and osteopontin genes in mouse smoothmuscle cells in vitro. Kid Int 2011; 79:414–422.

13 Ketteler M, Bongartz P, Westenfeld R, Wildberger JE, Mahnken AH, Böhm R, *et al.* Association of low fetuin-A (AHSG) concentrations in serum with cardiovascular mortality in patients on dialysis: a cross-sectional study. Lancet 2003; 361:827–833.

14 Yuce M, Davutoglu V, Cakici M. Fetuin-A, osteoporosis and inflammation – proposal of possible mechanisms for vascular and valvular calcification in chronic kidney disease. Nephrol Dial Transplant 2010; 25:2801–15.

15 Nishiura R, Fujimoto S, Sato Y, *et al.* Elevated osteoprotegerin levels predict cardiovascualr events in new hemodialysis patients. Am J Nephrol 2009; 9:257–263.

16 Scatena M *et al.* Osteoprotegrin: a novel secreted protein involved in the regulation of bone density. Arterioscler Thromb Vasc Biol 2007; 27: 2302–2309.

17 Bucay N, Sarosi I, Dunstan CR, Morony S, Tarpley J, Capparelli C, *et al.* Osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification. Genes Dev 1998; 12:1260–1268.

18 Nitta K, Akiba T, Uchida K, Otsubo S, Takei T, Yumura W, *et al.* Serum osteoprotegerin levels and the extent of vascular calcification in haemodialysis patients. Nephrol Dial Transplant 2004; 19:1886–1889.

19 Kiechl S, Schett G, Wenning G, Redlich K, Oberhollenzer M, Mayr A, *et al.* Osteoprotegerin is a risk factor for progressive atherosclerosis and cardiovascular disease. Circulation 2004; 109:2175–2180.

20 Shlipak MG, Fried LF, Cushman M, Manolio TA, Peterson D, Stehman-Breen C, *et al.* Cardiovascular mortality risk in chronic kidney disease: comparison of traditional and novel risk factors. JAMA 2005; 293: 1737–1745.

21 Stenvinkel P, Carrero JJ, Axelsson J, Lindholm B, Heimbürger O, Massy Z. Emerging biomarkers for evaluating cardiovascular risk in the chronic kidney disease patient: how do new pieces fit into the uremic puzzle? Clin J Am Soc Nephrol 2008; 3:505–521.

22 Deiter R. Multisclice computed tomography for the detection of coronary artery disease. Interven Cardiol 2006; 19:574–584.

23 Schmid M, Pflederer T, Jang I, Ropers D, Sei K, Daniel WG. Relationship between degree of remodeling and CT attenuation of plaque in coronary atherosclerotic lesions: an in-vivo analysis by multi-detector computed tomography. Atherosclerosis 2008; 197:457–464.

24 Bain BJ, Bates I. Basic haematological technique. In: Lewis SM, Bain BJ, Batyes I. *Dacie and Lewis practical haematology*. 9th ed. London: Licensing Agency; 2001. 19-27.

25 Newman DJ, Price CP. Renal function. In Burtis CA, Ashwood ER, editors. *Titetz fundamentals of clinical chemistry*. 5th ed. Philadelphia: W.B. Saunders Company; 2001. 705–721.

26 Norbert W, Ronald J, J Aldrich. *Fundamentals of clinical chemistry*. 6th ed. Philadelphia: W.B. Saunders Company; 2007. 706–725.

27 Sidhu D, Naugler C. Fasting time and lipid levels. Arch Intern Med 2012; 172:1–4.

28 Pearson TA, *et al.* High sensitive C-reactive protein measurement and cardiovascular risk. Circulation 2003; 107:499–511.

29 Mackie *et al.* Guidelines on fibrinogen assays. Br J Haematol 2003; 121:396-404.

30 National Kidney Foundation. KDOQI clinical practice guidelines in peritoneal dialysis: update 2000. Am J Kidney Dis 2001; 37:S65–S163.

31 Kramer H, Berns J, Choi M, Martin K, Rocco M. National kidney foundation. KDOQI guidelines in haemodialysis adequacy guidelines, 2006 update. Am J Kidney Dis 2006; 64:499–509.

32 La Milia V. Peritoneal equilibration test: a simple and fast method to assess free water and small solutes transport across peritoneal membrane. Kidney Int 2005; 68:840–846.

33 Reynolds JL, Skepper JN, McNair R, Kasama T, Gupta K, Weissberg PL, *et al.* Multifunctional roles for serum protein fetuin-a in inhibition of human vascular smooth muscle cell calcification. J Am Soc Nephrol 2005; 16:2920–2930.

34 Jono S, Ikari Y, Shioi A, Mori K, Miki T, Hara K, Nishizawa Y Serum osteoprotegerin levels are associated with the presence and severity of coronary artery disease. Circulation 2002; 106:1192–1194.

35 Agatston AS *et al.* Assessment of Agatston coronary artery calcium score using contrast-enhanced CT coronary angiography. J Am Coll Cardiol 1990;15:827–32.

36 George Y, MJ Budoff. Coronary artery calcium scoring, what is answered and what questions remain. Cardiovasc Diagn Ther 2012; 2:94–105.

37 Qian Z, Anderson H, Marvasty I, Akram K, Vazquez G, Rinehart S, Voros S. Lesion- and vessel-specific coronary artery calcium scores are superior to whole-heart Agatston and volume scores in the diagnosis of obstructive coronary artery disease. J Cardiovasc Comput Tomogr 2010; 4:391–399.

38 Kotz S, Balakrishnan N, Read CB, Vidakovic B. *Encyclopedia of statistical sciences*. 2nd ed. Hoboken, New Jersey: Wiley-Interscience; 2012.

39 Kirkpatrick LA, Feeney BC. *A simple guide to IBM SPSS statistics for version 20.0, Student ed.* Belmont, California: Wadsworth, Cengage Learning; 2013.

40 Lee JE, Kim HJ, Moon SJ, Nam JS, Kim JK, Kim SK, *et al.* Serum osteoprotegerin is associated with vascular stiffness and the onset of new cardiovascular events in hemodialysis patients. Korean J Intern Med 2013; 28:668–677.

41 Pateinakis P, Papagianni A, Douma S, Efstratiadis G, Memmos D. Associations of fetuin-A and osteoprotegerin with arterial stiffness and early atherosclerosis in chronic hemodialysis patients. BMC Nephrol 2013; 14:122.

42 Ozkok A, Caliskan Y, Sakaci T, Erten G, Karahan G, Ozel A, *et al*. Osteoprotegerin/RANKL axis and progression of coronary artery calcification in hemodialysis patients. Clin J Am Soc Nephrol 2012; 7: 965–973.

43 Turkmen K, Gorgulu N, Uysal, M, Ozkok A, Sakaci T, Unsal A, Yildiz A. Fetuin-A, inflammation, and coronary artery calcification in hemodialysis patients. Indian J Nephrol 2011; 21:90–94.

44 Stenvinkel P, Wang K, Qureshi AR, Axelsson J, Pecoits-Filho R, Gao P, *et al*. Low fetuin-A levels are associated with cardiovascular death: impact of variations in the gene encoding fetuin. Kidney Int 2005; 67:2383–2392.

45 Hermans MM, Brandenburg V, Ketteler M, Kooman JP, van der Sande FM, Gladziwa U, *et al*. Study on the relationship of serum fetuin-A concentration with aortic stiffness in patients on dialysis. Nephrol Dial Transplant 2006; 21:1293–1299.

46 Moe SM, Reslerova M, Ketteler M, O'neill K, Duan D, Koczman J, *et al*. Role of calcification inhibitors in the pathogenesis of vascular calcification in chronic kidney disease (CKD). Kidney Int 2005; 67:2295–2304.

47 Rubin GD. Emerging and evolving roles for CT in screening for coronary heart disease. J Am Coll Radiol 2013; 10:943–948.

48 Bazeeda MF, Moselheyb MS, Al Masswary AI. Value of multislice CT coronary arteries calcification scoring in the prediction of coronary arteries pathology in chronic kidney disease patients. Egy J Radiol Nuclear Med 2011; 42:275–280.

49 Hernandes FR, Barreto FC, Rocha LA, Draibe SA, Canziani ME, Carvalho AB. Evaluation of the role of severe hyperparathyroidism on coronary artery calcification in dialysis patients. Clin Nephrol 2007; 6:89–95.

50 Barreto DV, Barreto FC, Carvalho AB, Cuppari L, Cendoroglo M, Draibe SA, *et al*. Coronary calcification in hemodialysis patients: the contribution of traditional and uremia-related risk factors. Kidney Int 2005; 67:1576–1582.