Association of Serum Fetuin-A with Vascular Calcification in Hemodialysis Patients and Its' Impact on 3-year Mortality

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Introduction. Atherosclerosis is associated with increased intima- media thickness (IMT) and vascular calcification (VC) in maintenance hemodialysis (MHD) patients. Fetuin-A is a serum protein, which inhibits vascular calcification. The aim of this study was to investigate the association between fetuin-A and VC, in a group of MHD patients.

Methods. One hundred and forty-three MHD patients were included and followed for 3 years. Blood samples were studied for calcification and inflammation markers and fetuin-A was checked 3 times at the start, middle and the end of the study. We used common carotid doppler sonography for assessment of indices of VC, which were performed at baseline and at the end of the study. Vascular calcification was defined as a common carotid intima media thickness ≥ 0.8 mm on either side or the existence of any plaque or stenosis ≥ 50% on either side.

Results. From 143 patients (mean age 57.5 ± 15.9, 60.1% male), 104 patients (75.4%) had VC at baseline. The mean age and the prevalence of DM were significantly higher in patients with VC ($P < .001$ for both). There was no significant difference in the levels of Pi, PTH, and fetuin-A between the two groups. In a multiple logistic regression model at baseline only age (OR = 1.09, $P < .001$), and diabetes mellitus (OR = 4.59, $P < .05$) were associated with VC and dialysis vintage had a marginal association (OR = 1.20, $P = .09$). At the end of the study only age (OR = 1.12, $P < .001$), and CRP (OR = 1.14, $P < .05$) were associated with VC. The mean survival of patients with VC was significantly lower than the patients without VC (31.87 ± 0.95 vs. 33.73 ± 1.29, $P < .05$), however the mortality was not affected by fetuin-A level.

Conclusion. Survival rate of patients without VC was higher than the patients with VC. We didn't find any correlation between the level of fetuin-A and VC. It seems that the traditional risk factors of VC, including age and diabetes mellitus are the main predictors of VC in MHD patients.

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INTRODUCTION

Cardiovascular disease is the main cause of morbidity and mortality in chronic kidney disease (CKD) and maintenance hemodialysis (MHD) patients.[1] The numbers of atherosclerotic coronary plaques are increased in CKD patients and there are differences in plaque morphology in these patients in comparison with non-renal matched cases.[2,3] Other

than atherosclerosis, vascular medial calcification is also seen with increased frequency in CKD patients, which is especially due to increased oxidative stress and local and systemic inflammatory factors.[3-5] Intima media thickness (IMT) of the common carotid arteries, measured by B mode ultrasonography has been introduced as a marker of generalized atherosclerosis.[6,7] An association between IMT and coronary heart disease has been shown in a number of studies.[8-11] Traditional risk factors such as older age, hypertension, diabetes mellitus and dyslipidemia are prevalent in hemodialysis patients and involved in pathogenesis of their vasculopathy. Also risk factors related to uremia such as inflammation, oxidative stress and mineral bone disease are involved in this process either.[12] Studies suggest that vascular calcification (VC) is a highly regulated active process and the phenotypic trans-differentiation of vascular smooth muscle cells (VSMCs) into chondrocyte and osteoblast-like cells is a key pathogenic event which leads to deposition of collagen and non- collagenous proteins in the arteries.[13,14] Multiple factors including dysregulated mineral homeostasis and bone turnover and imbalance between promoters and inhibitors of extra-osseous bone formation affect this process.[11,12] Fetuin-A is a potent systemic inhibitor of calcification, and a negative acute phase reactant, derived from liver.[15] It acts as an inhibitor of ectopic calcification by solubilization of calcium-phosphorus complex as a colloid by forming "calciprotein particles".[16,17] Apart from direct inhibition of calcification, fetuin-A could also indirectly influence calcification by regulating energy and bone metabolism.[16] In particular, fetuin-A antagonizes transforming growth factors (TGFs) and bone morphogenetic proteins, which are potent osteogenic growth and differentiation factors, possibly involved in atherosclerotic and medial calcification.[16,18,19] In MHD patients, low fetuin-A levels have been associated with severe and extensive vascular calcification, as well as with increased all-cause and cardiovascular mortality.[20-22] The precise role of fetuin-A in the development of vascular calcification is not fully elucidated. The aim of this study was to investigate the association between serum fetuin-A and common carotid IMT, stenosis and plaque as markers of early atherosclerosis in a cohort of MHD patients and effect of VC on patient survival.

MATERIALS AND METHODS

Patients

One hundred and forty-three adult patients, who were on MHD between 2011 and 2014 in hemodialysis unit of Hasheminejad Kidney Center (HKC), and consented to participate in the study, were included. Demographic data including age, sex, dialysis vintage, history of diabetes mellitus and hypertension, were collected from Hemodialysis Data Processor Soft-ware (HDPS), a software designed for clinical use and investigational studies in our hemodialysis center in 2010 (AIP company, Tehran, Iran). Serum levels of hemoglobin (Hgb), creatinine, calcium, inorganic phosphorus (Pi), parathyroid hormone (PTH), cholesterol, triglyceride, high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), and quantitative C-reactive protein (CRP) were measured every 6 months for 3 years. Fetuin-A was measured yearly for 3 times, at the beginning, middle and at end of the study, with two-site enzyme-linked immunoassays [ZB-1386-H9648 kit, Padgin Teb Co (PTC), Tehran, Iran].

One nurse followed the patients for three years and registered all cardiovascular events, including myocardial infarction (MI), acute coronary syndrome (ACS) and cardiac death, and cerebrovascular events based on the relevant physician's diagnosis according to discharge summary note.

Vascular Calcification

Common carotid intima media thickness (ccIMT) was measured in all participants by one radiologist with Esaote Mylab 70 XVision machine and linear La 523 probe. The common carotid artery was assessed in the supine position with semi-extended neck, in a semi-dark room during a mid-week non-dialysis day.

Longitudinal two-dimensional images of the vessel were acquired, frozen in diastole and analyzed offline. Carotid intima media thickness was calculated as the distance between the leading edge of the lumen-intima interface and the media–adventitia interface on the far wall of the artery. Measurements were performed at 0.5, 1, and 2 cm below the carotid bifurcation (six measurements, three on each side) in a plaque-free arterial segment. The average measurement of the obtained values was defined as ccIMT of each side and used in analyses.

Plaques were scored as: 0 (no plaque), 1

(hypoechoic plaque), 2 (calcified plaque), and 3 (both types). Stenosis was scored as 0 (without stenosis), 1 (< 50%), 2 (50 to 70%), 3 (> 70%), and 4 (complete occlusion). Vascular calcification was defined as a common carotid IMT ≥ 0.8 mm on either side or the existence of any plaque or stenosis ≥ 50% on either side.

Statistical Analyses

Continuous data were expressed as mean ± standard deviation (SD). The associations between fetuin-A and each of the parameters of VC, ccIMT, stenosis, plaque and also with demographic, clinical and laboratory parameters were assessed by univariate analysis. Parameters with a significant association with fetuin-A were entered into a multivariate logistic regression model. Multivariate regression analysis, with simultaneous inclusion of values into the model, was used to assess the adjusted combined influence of variables with $P < 0.2$. Quantitative values were compared between the groups with independent samples t-test or Mann-Whitney U test. Correlations between continuous variables were tested by Pearson's or Spearman's correlation analyses. Survival rate was evaluated with Kaplan-Meier analysis. Log-rank test was used for comparison of the survival rates between the groups. Cardiovascular events or death considered as outcome measures. All statistical data analyses were performed using SPSS version 23.0 (IBM Corp, 2015).

RESULTS

One hundred and thirty-eight patients, including 55 women (39.9%) and 83 men (60.1%), were enrolled in the study. The mean age of the patients was 58.1 ± 15.9 (15.3 to 86.3). The mean duration of dialysis was 66.5 ± 72.9 months (range: 1 to 390 months). Most common causes of end-stage kidney disease were diabetes mellitus (37.0%), hypertension (9.4%), adult polycystic kidney diseases (5.1%), glomerulonephritis (4.3%), reflux nephropathy (3.6%), renal stone (2.2%), obstructive nephropathy (1.4%), and unknown in the rest (30.4%). Forty-six patients (33.3%) had history of hypertension and 19 patients (13.8%) had history of cardiovascular diseases before the start of MHD. The baseline and final laboratory data and ccIMT results of the patients are summarized in Tables 1. Final laboratory measurements were defined as the mean of repeated measurements during follow-up for each patient.

One hundred and thirty-eight patients underwent common carotid ultrasonography at baseline:

ccIMT: Fifty-six patients (40.6%) had ccIMT ≥ 0.8 mm.

Plaque: Forty-three patients (31.2%) had no plaques and 95 (68.8%) patients had carotid artery plaques including 9 (6.5%) with hypoechoic plaques, 85 (61.6%) with calcified plaques, and 1 patient (0.7%) with both types of plaques.

Stenosis: Forty-two patients (30.4%) had no stenosis, 93 patients (67.4%) had stenosis ≤ 50%,

Table 1. Laboratory Data and ccIMT Values in 143 MHD Patients

Parameter	Baseline		Final†	
	Mean ± SD	Range	Mean ± SD	Range
Fetuin-A, mg/dL	35.13 ± 8.3	19.8 to 60.5	36.82 ± 6.87	20.8 to 58.1
Calcium, mg/dL	8.86 ± 0.81	7 to 11	8.82 ± 0.6	7.27 to 10.5
Phosphorus, mg/dL	6.01 ± 1.47	3 to 11.3	5.57 ± 1.07	3.3 to 8.63
iPTH, pg/dL	399.58 ± 313.17	22 to 1395	408.72 ± 262.96	26.5 to 1334.29
Creatinine, mg/dL	10.42 ± 3.88	3.5 to 24.3	9.51 ± 2.49	3.96 to 18
Albumin, g/dL	4.08 ± 0.39	2.3 to 4.9	3.92 ± 0.29	3.16 to 4.8
Cholesterol, mg/dL	167.41 ± 38.15	93 to 297	151.9 ± 32.03	87.33 to 295
LDL, mg/dL	89.43 ± 27.38	27.8 to 155	80.53 ± 21.01	39.34 to 133
HDL, mg/dL	45.67 ± 13.3	20 to 104	40.46 ± 9.23	23.14 to 72
Triglycerides, mg/dL	165.67 ± 118.64	45 to 787	159.02 ± 102.8	49 to 875.86
Hgb, g/dL	10.74 ± 1.79	5.3 to 15.6	10.92 ± 1.17	6.47 to 13.9
Ferritin, ng/mL	503.29 ± 339.69	15 to 1232	509.71 ± 282.65	53.37 to 1252.5
CRP, mg/L	13.51 ± 20.03	0.1 to 99.5	16.58 ± 13.8	0.8 to 88.05
ccIMT, mm	0.74 ± 0.18	0.4 to 1.2	0.84 ± 0.22	0.5 to 1.5

iPTH, parathyroid hormone; LDL, low density lipoprotein cholesterol; HDL, high density lipoprotein cholesterol; Hgb, hemoglobin; CRP, C-reactive protein; ccIMT, common carotid intima-media thickness;
†The mean of repeated measurements during follow-up for each patient has been used.

1 patient (0.7%) had stenosis between 50 to 70%, 1 patient (0.7%) had stenosis ≥ 70%, and 1 patient had complete occlusion (0.7%). Overall 2.1% of the patients had significant stenosis, i.e. ≥ 50%.

Considering the definitions, at baseline 104 patients (75.4%) had and 34 patients (24.6%) did not have VC.

After 36 months 68 patients remained in the study. We lost 53 patients due to death, 9 patients due to kidney transplantation, 2 patients due to shift to peritoneal dialysis and 8 patients due to transfer to another center.

So, at the end of the study 68 patients underwent common carotid ultrasonography and of these 51 patients (75%) had and 17 patients (25%) did not have VC.

All patients with VC at the end of the study were those with VC at baseline, except for 1 patient who developed VC during the 3-year follow- up.

Difference Between VC with Laboratory and Clinical Parameters

At baseline, the mean age and the prevalence of DM were significantly higher in patients with VC compared to those without VC ($P < .001$ for both) (Table 2). The mean serum creatinine and albumin were significantly and marginally lower in patients with VC both at baseline and at the end of the study ($P = .02$ and $P = .09$, respectively) (Table 2). The mean serum Hgb levels of patients with VC were marginally higher than patients without VC at baseline ($P = .06$) (Table 2). There were no significant differences between the levels of Pi, PTH, and fetuin-A between the 2 groups (Table 2).

There was no difference in fetuin-A between diabetic and non- diabetic MHD patients (35.01 ± 7.29 vs. 35.20 ± 8.89, $P > .05$).

In a multiple logistic regression model including diabetes mellitus, history of CVD, age (year), dialysis vintage (year), serum calcium, creatinine, iPTH, Hgb and fetuin-A, only age (OR = 1.09, $P < .001$), and diabetes mellitus (OR = 4.59, $P < .05$) were associated with VC and dialysis vintage was marginally associated with VC (OR = 1.20, $P = .09$) (Table 3).

Table 2. Comparison of Baseline and Final Laboratory and Clinical Values in Patients With and Without VC

	Baseline			Final†		
	Patients With VC (n = 104)	Patients Without VC (n = 34)	*P*	Patients With VC (n = 51)	Patients Without VC (n = 17)	*P*
Fetuin-A, mg/dL	35.73 ± 8.63	33.16 ± 6.83	> .05*	37.87 ± 7.18	36.69 ± 5.54	> .05*
Calcium, mg/dL	8.92 ± 0.79	8.67 ± 0.84	> .05*	8.91 ± 0.56	8.72 ± 0.38	> .05&
Phosphorus, mg/dL	5.95 ± 1.45	6.19 ± 1.51	> .05*	5.38 ± 1.13	5.64 ± 0.87	> .05*
iPTH, pg/dL	374.81 ± 277.65	475.35 ± 398.351	> .05&	345.12 ± 221.34	381.75 ± 240.27	> .05&
Creatinine, mg/dL	9.98 ± 3.67	11.75 ± 4.26	< .05*	9.00 ± 2.31	10.93 ± 3.12	< .01*
Albumin, g/dL	4.06 ± 0.36	4.13 ± 0.45	> .05*	3.87 ± 0.24	4.01 ± 0.28	> .05*@
Cholesterol, mg/dL	166.35 ± 39.27	170.65 ± 34.86	> .05*	151.22 ± 31.05	150.93 ± 24.03	> .05*
LDL, mg/dL	89.88 ± 26.79	88.05 ± 29.49	> .05*	80.03 ± 20.41	78.32 ± 22.31	> .05&
HDL, mg/dL	44.94 ± 13.03	47.91 ± 14.05	> .05&	39.30 ± 8.65	41.48 ± 8.95	> .05&
Triglycerides, mg/dL	166.75 ± 125.24	162.38 ± 97.29	> .05&	166.48 ± 124.59	157.17 ± 51.87	> .05&
Hgb, g/dL	10.89 ± 1.71	10.25 ± 1.91	> .05*	11.08 ± 1.06	10.90 ± 0.78	> .05*
Ferritin, ng/mL	516.30 ± 346.52	463.47 ± 319.52	> .05&	507.95 ± 276.47	509.67 ± 312.08	> .05*
CRP, mg/L	13.97 ± 19.91	12.08 ± 20.63	> .05&$	16.78 ± 11.76	8.49 ± 4.48	< .05&
Age, y	62.89 ± 12.10	43.27 ± 17.10	< .001*	62.93 ± 11.48	42.09 ± 15.38	< .001*
Hypertension	34 (32.7)	12 (35.3)	> .05#	13 (25.5)	6 (35.3)	> .05#
Diabetes Mellitus	46 (45.2)	4 (11.8)	< .001#	21 (41.2)	3 (17.6)	> .05#**
Cardiovascular Disease	17 (16.3)	2 (5.9)	> .05#	11 (21.6)	0 (0.0)	> .05#
Dialysis Vintage, y&	3.05 ± 2.32	2.34 ± 2.31	> .05&	2.54 ± 2.34	2.76 ± 2.57	> .05&

Data are presented as mean ± SD or n (%).
†For laboratory data, of the mean of repeated measurements during follow-up for each patient has been used.
*T-test
&Mann-Whitney U test
#Fisher Exact test
+$P = .09$, $P = .06$, @$P = .05$, **$P = .07$
iPTH, parathyroid hormone; LDL, low density lipoprotein cholesterol; HDL, high density lipoprotein cholesterol; Hgb, hemoglobin; CRP, C-reactive protein; ccIMT, common carotid intima-media thickness

Table 3. Variables Associated with VC, in Univariate and Multiple Logistic Regression Analysis at Baseline

	Univariate	Multiple	
	P	*P*	OR (95% CI for OR)
Fetuin-A, mg/dL	> .05		
Calcium, mg/dL	> .05		
Phosphorus, mg/dL	> .05		
iPTH, pg/dL	> .05		
Creatinine, mg/dL	< .05		
Albumin, g/dL	> .05		
Cholesterol, mg/dL	> .05		
LDL, mg/dL	> .05		
HDL, mg/dL	> .05		
Triglycerides, mg/dL	> .05		
Hgb, g/dL	> .05		
Ferritin, ng/mL	> .05		
CRP, mg/L	> .05		
Age, y	< .001	< .001	1.087 (1.05 to 1.13)
Hypertension	> .05		
Diabetes Mellitus	< .001	< .05	4.589 (1.31 to 16.14)
Cardiovascular Disease	> .05		
Dialysis Duration, y*	> .05	> .05[+]	1.196 (0.98 to 1.47)

OR, odds raito; CI, confidence interval; iPTH, parathyroid hormone; LDL, low density lipoprotein cholesterol; HDL. high density lipoprotein cholesterol; Hgb, hemoglobin; CRP, C-reactive protein; ccIMT, common carotid intima-media thickness
*Non- significant in final multiple model, [+]*P* = .09

Table 4. Variables Associated with VC, in Univariate and Multiple Logistic Regression at Final Follow- up Analysis

	Univariate	Multiple	
	P	*P*	OR (95% CI for OR)
Fetuin-A, mg/dL	> .05		
Calcium, mg/dL	> .05		
Phosphorus, mg/dL	> .05		
iPTH, pg/dL	> .05		
Creatinine, mg/dL	< .05		
Albumin, g/dL	> .05		
Cholesterol, mg/dL	> .05		
LDL, mg/dL	> .05		
HDL, mg/dL	> .05		
Triglycerides, mg/dL	> .05		
Hgb, g/dL	> .05		
Ferritin, ng/mL	> .05		
CRP, mg/L	< .05	< .05	1.14 (1.00 to 1.28)
Age, y	< .001	< .001	1.12 (1.05 to 1.19)
Hypertension	> .05		
Diabetes Mellitus	> .05		
Cardiovascular Disease	> .05		
Dialysis Duration, y	> .05		

OR, odds raito; CI, confidence interval; iPTH, parathyroid hormone; LDL, low density lipoprotein cholesterol; HDL, high density lipoprotein cholesterol; Hgb, hemoglobin; CRP, C-reactive protein; ccIMT, common carotid intima-media thickness

At the end of the study, in a multiple logistic regression model including diabetes mellitus, age, CRP, creatinine, albumin, and other laboratory values, only age (OR = 1.12, $P < .001$), and CRP (OR = 1.14, $P < .05$) were associated with VC (Table 4).

Correlations Between ccIMT and Clinical and Laboratory Parameters

At baseline ccIMT value correlated positively with age (r = 0.64, $P < .001$), serum LDL (r = 0.18, $P < .05$), hemoglobin (r = 0.255, $P < .05$), CRP (r = 0.16, $P = .05$), negatively with serum creatinine (r = -0.19, P < .05), and serum HDL (r = -0.16, $P = .06$) (Table 5). At the end of the study serum creatinine, albumin and CRP had a significant correlation with ccIMT (Table 5). The value of ccIMT was significantly higher in men compared with women (0.76 ± 0.19 vs. 0.69 ± 0.18, $P < .05$) and in patients with DM compared with those did not have a history of DM (0.80 ± 0.17 vs. 0.70 ± 0.19, $P < .01$). There was no association between ccIMT value and dialysis vintage (R = 0.00, $P > .05$). There was no significant difference between the ccIMT value of patients with and without hypertension (0.73 ± 0.19 vs. 0.74 ± 0.18, $P > .05$) or with and without cardiovascular diseases (0.78 ± 0.21 vs. 0.73 ± 0.18, $P > .05$). The value of ccIMT in the 68 patients who remained in the study after 3

Table 5. Correlations Between Laboratory Values and ccIMT

	Baseline		Final[†]	
	R	*P*	R	*P*
Fetuin-A, mg/dL	0.04*	> .05	0.03[&]	> .05
Calcium, mg/dL	0.14*	> .05	0.10[&]	> .05
Phosphorus, mg/dL	-0.08*	> .05	-0.15[&]	> .05
iPTH, pg/dL	-0.03[&]	> .05	0.02[&]	> .05
Creatinine, mg/dL	-0.19*	< .05	-0.26[&]	< .05
Albumin, g/dL	-0.11[&]	> .05	-0.33[&]	< .01
Cholesterol, mg/dL	0.08*	> .05	-0.02[&]	> .05
LDL, mg/dL	0.18*	< .05	0.06[&]	> .05
HDL, mg/dL	-0.16[&]	> .05[$]	-0.10[&]	> .05
Triglycerides, mg/dL	0.06[&]	> .05	-0.11[&]	> .05
Hgb, g/dL	0.26*	< .01	0.06[&]	> .05
Ferritin, ng/mL	-0.08[&]	> .05	0.00[&]	> .05
CRP, mg/L	0.16[&]	> .05[+]	0.30[&]	< .05
Age, y	0.64*	< .001	0.55[&]	< .001
Dialysis Duration, y	0.06[&]	> .05	-0.10[&]	> .05

*Pearson Correlation
[&]Spearman Correlation, [$]*P* = .06, [+]*P* = .05
iPTH, parathyroid hormone; LDL, low density lipoprotein cholesterol; HDL, high density lipoprotein cholesterol; Hgb, hemoglobin; CRP, C-reactive protein; ccIMT, common carotid intima-media thickness

years, significantly increased from 0.73 ± 0.18 to 0.84 ± 0.22 ($P < .001$). But yet there was no correlation between ccIMT and fetuin-A values (R = 0.03, $P > .05$) (Table 5).

Correlations Between Stenosis with Laboratory and Clinical Parameters

At baseline the mean serum calcium and Hgb were significantly higher in patients with stenosis compared with patients who did not show any stenosis ($P < .05$ and $P = .06$, respectively) and at the end of the study the final creatinine and albumin levels were lower ($P < .05$ and $< .05$, respectively). Patients with stenosis were older and had higher frequency of diabetes mellitus and cardiovascular diseases (Table 6).

Correlation Between Existence of Carotid Plaques and Laboratory and Clinical Parameters

The baseline serum calcium, creatinine and CRP and the mean of serum calcium, creatinine and CRP measurements during the 3-year follow-up were significantly higher in patients with plaques compared with patients without plaques (Table 7).

The mean survival of patients with VC was significantly lower than patients without VC (31.87 ± 0.95 vs. 33.73 ± 1.29, $P < .05$) however the mortality was not affected by fetuin-A level (Figure).

DISCUSSION

In this 3- year prospective cohort study on 138 maintenance hemodialysis patients, we used carotid intima media thickness and carotid stenosis and plaques as surrogate markers for vascular calcification and studied the correlation of each and all of the mentioned indices with the presumed risk factors of VC and especially fetuin-A, which has been introduced as a predictor of VC.

Fetuin-A has been presented as a potent systemic inhibitor of calcium phosphate precipitation and calcification.[16,17] However different studies have found contradictory results regarding the correlation between VC and fetuin-A levels in CKD and dialysis patients. A number of previous studies have shown a strong correlation between serum fetuin-A levels and VC. Shroff showed that

Table 6. Comparison of Mean Laboratory Values Between Patients With and Without Stenosis

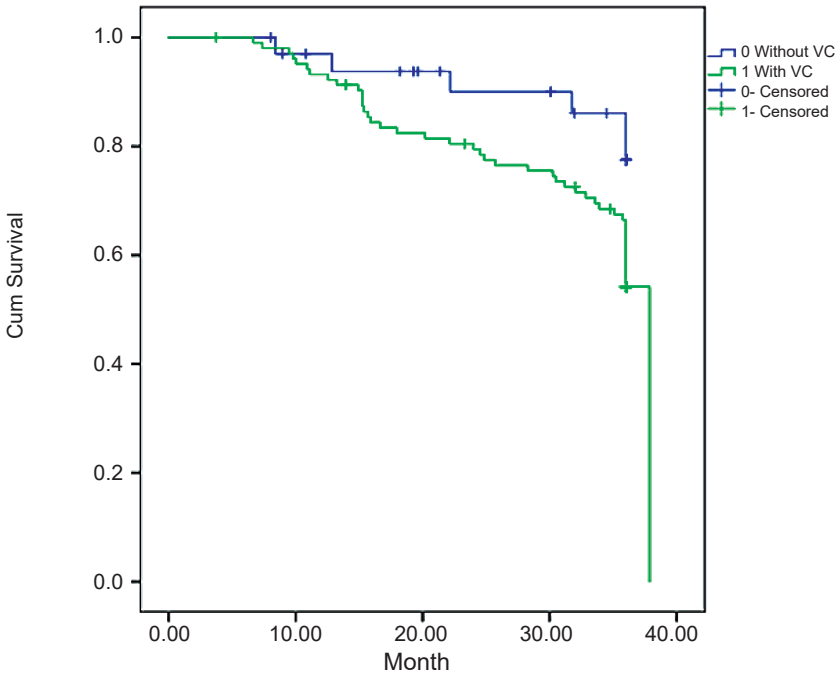
	Baseline			Final†		
	Patients With Stenosis (n = 96)	Patients Without Stenosis (n = 42)	*P*	Patients With Stenosis (n = 46)	Patients Without Stenosis (n = 22)	*P*
Fetuin-A, mg/dL	35.42 ± 8.38	34.44 ± 8.15	> .05*	37.62 ± 7.31	37.51 ± 5.7	> .05*
Calcium, mg/dL	8.97 ± 0.74	8.6 ± 0.9	< .05*	8.93 ± 0.56	8.72 ± 0.43	> .05&
Phosphorus, mg/dL	5.96 ± 1.48	6.12 ± 1.45	> .05*	5.39 ± 1.14	5.56 ± 0.95	> .05*+
iPTH, pg/dL	379.52 ± 282.96	445.43 ± 372.96	> .05&	353.5 ± 226.8	355.92 ± 226.31	> .05&
Creatinine, mg/dL	10.15 ± 3.5	11.02 ± 4.63	> .05*	8.97 ± 2.24	10.57 ± 3.13	< .05*
Albumin, g/dL	4.07 ± 0.36	4.08 ± 0.44	> .05&	3.86 ± 0.25	3.99 ± 0.25	< .05*
Cholesterol, mg/dL	168.09 ± 39.58	165.83 ± 35.08	> .05*	153.19 ± 31.53	146.88 ± 24.01	> .05*
LDL, mg/dL	91.12 ± 26.81	85.55 ± 28.58	> .05*	81.53 ± 20.1	75.57 ± 21.95	> .05&
HDL, mg/dL	44.73 ± 12.79	47.83 ± 14.31	> .05&	38.94 ± 7.57	41.74 ± 10.67	> .05&
Triglycerides, mg/dL	171.09 ± 128.77	153.29 ± 91.58	> .05&	171.36 ± 129.77	149.07 ± 51.21	> .05&
Hgb, g/dL	10.92 ± 1.73	10.32 ± 1.85	> .05*	11.08 ± 1.02	10.95 ± 0.95	> .05*
Ferritin, ng/mL	513.02 ± 342.99	481.05 ± 335.03	> .05&	536.8 ± 271.71	448.94 ± 304.17	> .05*
CRP, mg/L	13.66 ± 18.63	13.15 ± 23.16	> .05&$	17.66 ± 11.96	8.53 ± 4.54	< .001&
Age, y	62.27 ± 12.73	48.42 ± 18.2	< .001*	62.75 ± 11.41	47.19 ± 17.51	< .001*
Hypertension	32 (33.3)	14 (33.3)	> .05#	12 (26.1)	7 (31.8)	> .05#
Diabetes mellitus	45 (46.9)	6 (14.3)	< .001#	20 (43.5)	4 (18.2)	< .05#
Cardiovascular disease	16 (16.7)	3 (7.1)	> .05#	11 (23.9)	0 (0.0)	< .05#
Dialysis duration, y&	2.97 ± 2.25	2.68 ± 2.52	> .05&	2.58 ± 2.35	2.61 ± 2.52	> .05&

Data are presented as mean ± SD or n (%).
*T-test
&Mann-Whitney U test, $P = .06, +P = .09
#Fisher Exact test
iPTH, parathyroid hormone; LDL, low density lipoprotein cholesterol; HDL, high density lipoprotein cholesterol; Hgb, hemoglobin; CRP, C-reactive protein; ccIMT, common carotid intima-media thickness
†Final Laboratory Measurement of the Mean of Repeated Measurements During Follow-up for Each Patient

Table 7. Comparison of Mean Laboratory and Clinical Values in Patients With and Without Carotid Plaques

	Baseline			Final†		
	Patients With Carotid Plaques (n = 95)	Patients Without Carotid Plaques (n = 43)	*P*	Patients With Carotid Plaques (n = 46)	Patients Without Carotid Plaques (n = 22)	*P*
Fetuin-A, mg/dL	35.26 ± 8.42	34.83 ± 8.09	> .05*	37.62 ± 7.31	37.51 ± 5.7	> .05*
Calcium, mg/dL	8.98 ± 0.76	8.58 ± 0.87	< .01*	8.93 ± 0.56	8.72 ± 0.43	> .05&
Phosphorus, mg/dL	5.91 ± 1.46	6.23 ± 1.47	> .05*	5.39 ± 1.14	5.56 ± 0.95	> .05*+
iPTH, pg/dL	373.65 ± 282.61	456.86 ± 369.15	> .05&	353.5 ± 226.8	355.92 ± 226.31	> .05&
Creatinine, mg/dL	9.76 ± 3.6	11.87 ± 4.12	< .01*	8.97 ± 2.24	10.57 ± 3.13	< .05*
Albumin, g/dL	4.04 ± 0.36	4.17 ± 0.43	< .05&	3.86 ± 0.25	3.99 ± 0.25	< .05*
Cholesterol, mg/dL	168.45 ± 39.82	165.09 ± 34.5	> .05*	153.19 ± 31.53	146.88 ± 24.01	> .05*
LDL, mg/dL	91.22 ± 27.33	85.46 ± 27.39	> .05*	81.53 ± 20.1	75.57 ± 21.95	> .05&
HDL, mg/dL	44.98 ± 13.16	47.21 ± 13.63	> .05&	38.94 ± 7.57	41.74 ± 10.67	> .05&
Triglycerides, mg/dL	171.23 ± 129.54	153.4 ± 90.18	> .05&	171.36 ± 129.77	149.07 ± 51.21	> .05&
Hgb, g/dL	10.89 ± 1.77	10.39 ± 1.8	> .05*	11.08 ± 1.02	10.95 ± 0.95	> .05*
Ferritin, ng/mL	516.79 ± 351.94	473.47 ± 312.82	> .05&	536.8 ± 271.71	448.94 ± 304.17	> .05*
CRP, mg/L	14.55 ± 20.62	11.19 ± 18.68	> .05&	17.66 ± 11.96	8.53 ± 4.54	< .001&
Age, y	63.13 ± 12.11	46.84 ± 17.56	< .001*	62.75 ± 11.41	47.19 ± 17.51	< .001*
Hypertension	33 (34.7)	13 (30.2)	> .05#	12 (26.1)	7 (31.8)	> .05#
Diabetes Mellitus	45 (47.4)	6 (14.0)	< .001#	20 (43.5)	4 (18.2)	< .05#
Cardiovascular Disease	15 (15.8)	4 (9.3)	> .05#	11 (23.9)	0 (0.0)	< .05#
Dialysis duration, y&	3.04 ± 2.29	2.53 ± 2.4	> .05&	2.58 ± 2.35	2.61 ± 2.52	> .05&

Data are presented as mean ± SD or n (%).
*T-test
&Mann-Whitney U test, +*P* = .09
#Fisher Exact test
iPTH, parathyroid hormone; LDL, low density lipoprotein cholesterol; HDL, high density lipoprotein cholesterol; Hgb, hemoglobin; CRP, C-reactive protein; ccIMT, common carotid intima-media thickness



Comparison of Survival in Patients With and Without Vascular Calcification (VC)

serum fetuin-A and osteoprotegerin (OPG) level increased with age and fetuin-A had an inverse correlation with dialysis vintage, time-averaged serum phosphate, and hs-CRP and a negative correlation with aortic pulse wave velocity (PWV) and could independently predict both aortic PWV

and cardiac calcification.[23] The correlation between fetuin-A with CIMT was weak (P = .08).

Lee and colleagues also showed a lower level of serum fetuin-A level in patients with VC, presented by plain radiographs and echocardiography, among 81 adult hemodialysis patients.[24] Porazko found a negative correlation between fetuin-A and VC, presented by aortic pulse wave velocity in 155 CKD patients, 77 on hemodialysis, and Cozzolino found the same result in 115 hemodialysis patients.[25,26] Interestingly age was a strong predictor in most of the studies.[24-26] Also Ziolkowska reported a negative correlation between fetuin-A and IMT in ESKD patients.[27] Of note is that these studies were cross sectional and based on single measurements. Pertosa found an inverse and independent association between fetuin-A and IMT in 174 dialysis patients.[28] In this study 2 measurements of fetuin-A (baseline and after 24 months) were made and T0 serum fetuin-A level was associated with T24 cIMT in multiple regression analysis (P < .01). Also cardiovascular mortality was independently associated with lower T0 serum fetuin-A level.

However the results of other studies are inconsistent. Pencak and colleagues failed to show any correlation between fetuin-A levels and coronary artery and abdominal aortic calcification scores in 104 adult hemodialysis patients evaluated by CT scans.[29] In their study age was the only significant risk factor for development of VC. Kim found that fetuin-A level was an independent risk factor for VC detected by plain radiography in PD patients, but not in hemodialysis patients.[30] Lee and colleagues evaluated VC by heart-femoral pulse wave velocity and vascular stiffness in 91 MHD patients and showed that serum OPG, but not fetuin-A, levels was closely associated with increased vascular stiffness.[31] They suggested OPG as an independent predictors of new CV events in HD patients.

Schlieper found no correlation between fetuin-A and IMT in 97 hemodialysis patients and Hermans and Pateinakis found a correlation that lost statistical significance after adjustment for age.[32-4] Caglar showed a negative correlation between fetuin-A with carotid IMT in non-diabetic patients with CKD stage 1 to 5 but it lost significance in multivariate analysis.[35] Also Mann and colleagues failed to show any relationship between fetuin-A and aortic calcification.[36] They showed that calcium deposition in L1 to L4 vertebra, seen on multi-slice CT scan, increases with age and duration of dialysis but is not related to fetuin-A levels. Toussaint and colleagues found a high prevalence of 90% VC in predialysis CKD patients, worse with increasing age, triglycerides and reducing renal function.[37] Also there are other studies including ours, which have found a correlation between the traditional risk factors of atherosclerosis and IMT. Preston and Brzosko found that CC-IMT increases with age and low-density lipoprotein level.[38,39] In the study by Pateinakis the correlation of fetuin-A with ccIMT were significant only in the non-diabetic patients, although a trend in the same direction was also observed in the diabetics.[34] We found no correlation between IMT and fetuin-A and found a positive correlation between IMT and age, LDL and creatinine and also a higher IMT in diabetic patients. We also could not find any correlation between fetuin-A and ccIMT in diabetic or non-diabetic patients, neither the mean value of Fetuin-A was different between the two groups of patients. However it should be noted that the number of diabetic patients was smaller than non-diabetics and studies on larger number of patients are needed to confirm these findings.

The above discrepant results may, at least partially, reflect the differences in methodology and patient population however they throw a doubt on the correlation between VC and circulating fetuin-A levels.

Fetuin-A has also been introduced as a risk factor for cardiovascular mortality in a number of studies. Scialla showed that fetuin-A may be a risk factor for all-cause and cardiovascular mortality in patients undergoing dialysis (602 dialysis patients over up 13 years follow-up), but does not improve risk prediction.[40] Ketteler and Stenvinkel showed that low fetuin-A level is associated with increased cardiovascular and all-cause mortality.[20,22] These studies demonstrated an effect of variations in the AHSG gene on both circulating fetuin-A levels and outcome, which indicates that ESRD patients with the AHSG 256Ser allele are at risk of accelerated vascular calcification. We found that the mean survival of patients with VC was approximately lower than patients without VC but we could not show an effect of serum fetuin-A level on patient survival rates.

Considering the controversial results of the studies on the relationship between fetuin-A

level and valvular or vascular calcification in hemodialysis patients, results of our study, which showed no significant relationship between VC and fetuin-A level, are not much unexpected. The differences in methodology and study populations with different characteristics can partially explain the observed controversies and differences. The presence of different coding genes of fetuin-A can affect its level in different populations.[41] It has also been stated that serum fetuin-A in CKD patients is more observed as fetuin-mineral complex (FMC), and not as free fetuin-A.[42] FMC is a combination of fetuin-A, fibrinogen, fibronectin 1, and calcium. In fact, with reducing GFR, the FMC level, but not the free fetuin-A, increases. This is associated with higher levels of coronary artery calcification score in dialysis patients.[42] Hence based on the current knowledge, circulating serum fetuin-A has a poor relationship with VC.[43] In addition, some other factors such as obesity, insulin resistance, metabolic syndrome, nonalcoholic fatty liver, lifestyle, smoking, adynamic bone diseases, and the use of vitamin D metabolites can also affect the Fetuin-A level.[44] To our knowledge this is one of the few and longest prospective studies with a 3- year follow up of MHD patients, and repeated measurement of serum fetuin-A as well as other potential predictors of vascular calcification, in which the correlation between these markers and evolution of vascular calcification has been studied. Most of the previous studies have relied on single cross sectional fetuin-A measurement.

CONCLUSION

Various biomarkers are shown to be associated with VC, ccIMT and cardiovascular mortality in different studies with conflicting results. In the present study we found no correlation between fetuin-A level and VC.

Higher survival rate was found in patients without VC in comparison with patients with VC but there was no effect of fetuin-A on cardiovascular survival and we believe that despite anti-calcifying effect of fetuin-A, the association of its serum level with vascular calcification in end stage kidney patients still remains unclear and needs to be further evaluated.

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