Associations of fetuin-A level with vascular disease in hemodialysis patients with or without type II diabetes mellitus

Amani K. Mohamed[a], Amany M. Abdallah[a], Maha A. Hassan[a], Nagwa A. Mohammed[b], Solaf A. Kamel[b]

[a]Department of Internal Medicine, Al-Azhar University, Cairo and [b]Department of Clinical and Chemical Pathology, National Research Center, Cairo, Egypt

Correspondence to Amani K. Mohamed, Department of Internal Medicine, Al-Azhar University, Mokattam, zone C, 7781, app 301, Cairo, Egypt
Tel: +20 100 876 1110;
e-mail: amani_kamal@hotmail.com

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Introduction
Fetuin-A is a circulating inhibitor of calcium deposition in the vasculature and may be involved in the pathogenesis of cardiovascular disease. Low plasma fetuin-A level is independently associated with increased risk for cardiovascular disease mortality among men and women without diabetes; in addition, low level of fetuin-A is linked to mortality in patients on dialysis.

Aim of the study
The aim of the study was to investigate the role of fetuin-A as a marker for microvascular and macrovascular diseases in a high-risk population of end-stage renal disease patients on dialysis, with and without diabetes mellitus.

Patients and methods
This study included 30 end-stage renal disease patients on regular hemodialysis, with and without diabetes and 10 age-matched and sex-matched apparently healthy controls. All patients were subjected to careful history-taking, including history of strokes and acute myocardial infarction and thorough physical examinations, and cardiac assessment was performed using ECG and ECHO. Routine laboratory tests were performed, such as hemoglobin, fasting blood glucose, serum creatinine, serum urea, serum Na, serum K, uric acid, serum cholesterol, serum triglycerides, serum aspartate aminotransferase, serum alanine aminotransferase, serum albumin, serum calcium, serum phosphorus, intact parathyroid hormone (iPTH), serum iron, total iron binding capacity (TIBC), and serum fetuin-A.

Results
The study showed significant statistical decrease in serum fetuin-A level in chronic renal failure (CRF) and diabetes patients with vascular strokes when compared with CRF patients and CRF patients with diabetes without history of vascular strokes. There was significant positive correlation between fetuin-A and hemoglobin, serum Ca, serum albumin, TIBC, and total protein (TP), whereas there was significant negative correlation between fetuin-A and serum cholesterol, serum triglyceride (TG), fasting blood glucose (FBG), serum urea, serum creatinine, serum uric acis (UA), iPTH, serum Na, and serum K. No correlation was found between fetuin-A and age or BMI.

Conclusion
Our findings suggest a unique role for fetuin-A deficiency as a biomarker of vascular diseases in the setting of CRF and type 2 diabetes mellitus.

Keywords:
chronic renal failure on hemodialysis, diabetes mellitus, serum fetuin-A

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Introduction

Fetuin-A, also known as α-Schmid Heremans glycoprotein, is a potent systemic calcification inhibitor [1]. Low level of fetuin-A is associated with cardiovascular mortality in patients on dialysis [2]. In addition, low fetuin-A level has been linked to vascular calcification [3] and flow-limiting aortic stenosis [4]. Fetuin-A interacts with the insulin receptor tyrosine kinase and induces insulin resistance in rodents [5]. Stefan *et al*. [6] demonstrated in a prospective case cohort study that elevated fetuin-A level is an dependent risk factor for developing diabetes. In addition to renal (dialysis) patients, several studies showed that high level of fetuin-A is associated with atherosclerosis and its manifestations in nonrenal patients [7]. Fetuin-A is synthesized by the liver and secreted into the blood stream, where its concentration in adult mammals ranges 0.5–1.0 g/l. Fetuin-A occurs in high serum concentrations during fetal life and is involved in protease inhibitory activities and development-associated regulation of calcium metabolism and osteogenesis. It accumulates in bones and teeth as a major fraction of noncollagenous bone proteins. Biologically, studies have demonstrated that fetuin-A is the major calcification inhibitor found in circulation, where it interferes with calcium salt precipitation. Recent study has indicated that fetuin-A level decreases in uremic patients on hemodialysis in comparison with normal healthy controls. Low

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fetuin-A level in patients with chronic kidney failure is strongly associated with a higher cardiovascular mortality. In contrast, it was demonstrated that higher than normal level of serum fetuin-A in older populations is associated with incident diabetes, which is independent from other markers of insulin resistance. Furthermore, a higher fetuin-A level may be an independent risk marker in patients with cardiovascular disease (CVD) [8].

Patients and methods

This study was conducted in the Internal Medicine Department, Faculty of Medicine for Girls, Al-Azhar University. This cross-sectional study was conducted from January 2012 to January 2013. This study included 30 end-stage renal disease patients on regular hemodialysis [chronic renal failure (CRF)] with and without diabetes and 10 age-matched and sex-matched apparently healthy controls, collected from Al Zahra Hospital Medical Department staff. All participants gave informed consent to participate in this study.

Exclusion criteria

We excluded patients with type 1 diabetes mellitus, severe infectious disease, and acute kidney injury. Furthermore, none of our patients had received antibiotics and anti-inflammatory or corticosteroid medications during the study period.

Written informed consent was obtained from both patients and controls before participation in this study.

All patients were subjected to complete clinical assessment including history-taking and thorough clinical examination including blood pressure measurements (systolic and diastolic) and assessment of BMI. In all individuals, cardiac assessment was carried out using ECG and ECHO. Hemodialysis machines with volumetric control (Fresenius Medical Care 4008B and 4008S; Homburg, Germany) were used. The standard dialysis bath consisted of sodium, 140 mEq/l; potassium, 2 mEq/l; calcium, 3 mEq/l; and bicarbonate, 35 mEq/l. The ultrafiltration rate was programmed to reach the patient's optimal dry weight defined as the postdialysis body weight below which the patients developed symptomatic hypotension or muscle cramps in the absence of edema. Heparin was used for anticoagulation.

Patients were divided into three groups, and the fourth group for the control.

Group I included 10 CRF patients on dialysis, who did not have diabetes.

Group II included 10 CRF patients on dialysis, who had diabetes without diabetic complications.

Group III included 10 CRF patients on dialysis, who had diabetes with diabetic complications such as history of strokes, acute MI, and amputation.

Group IV included 10 age-matched and sex-matched apparently healthy controls, collected from Al Zahraa hospital.

All patients and the control group were subjected to the following investigations.

(1) History-taking and clinical examination.
(2) Fasting blood glucose.
(3) Serum creatinine, blood urea, serum Na, serum K, and uric acid.
(4) Lipid profile, including serum cholesterol, serum triglycerides, serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), and serum albumin.
(5) Serum calcium, serum phosphorus, iPTH, total protein, serum iron, TIBC, and serum fetuin-A.

A volume of 5 ml of fasting (12–16 h) venous blood sample was drawn from each participant in the study; 2 ml of venous blood was added to a tube containing EDTA for CBC determination using Coulter Counter T890 (Coulter Counter, Harpenden, UK) and the remaining 3 ml of venous blood was transferred to a plain tube. Blood was left to clot, the serum was separated by centrifugation at 3000*g* for 5 min, and fasting blood glucose was determined immediately by colorimetric technique using Hitachi 912 autoanalyzer (Roche-Hitachi, Tokyo, Japan). The rest of the serum was stored at −20°C for determination of the following: Serum ALT, serum AST, serum albumin, serum total protein, serum total cholesterol, serum triglyceride, serum urea, serum creatinine, serum uric acid, serum sodium, serum potassium, and serum iron using Hitachi autoanalyzer 912 (Roche-Hitachi) by colorimetric techniques. TIBC was measured after saturation of transferrin by iron solution and adsorption of excess iron on magnesium hydroxycarbonate. Determination of iron bound to transferrin was performed using colorimetric kits on Hitachi 912 autoanalyzer. Serum sodium and serum potassium were determined using ion selective electrodes on Hitachi 912 autoanalyzer. The determination of serum iPTH was carried out using Immulite 2000 analyzer by solid-phase, two-site chemiluminescent enzyme-labeled immunometric assay (Siemens AG, Erlangen, Germany) [9]. The determination of serum fetuin-A level was carried out by the immunoturbidimetric method [10] using RA 50 (Bayer's Diagnostic, Pittsburgh, USA); the

kit was supplied by BioVendor Laboratory Medicine (BioVendor GmbH, Heidelberg, Germany).

Statistical analyses

Statistical analyses of the results were performed using SPSS software version 17.0 (SPSS Inc., Chicago, Illinois, USA). Continuous variables were expressed as mean ± SD. Comparison between two sets of patients was performed by the independent *t*-test, but more than two sets of patients were compared by one-way ANOVA. Pearson correlation coefficient '*r*' was used to describe the association between serum fetuin-A and the variables of interest. *P* values less than 0.05 were considered statistically significant. Scheffé's method is a single-step multiple comparison procedure, which applies to the set of estimates of all possible contrasts among the factor level means; it is significant if *P* value is less than 0.05.

Results

Laboratory characteristics of all CRF patients without and with diabetes mellitus, diabetic patients with complications, and healthy control participants and comparison between all groups are given in Table 1. There was no significant statistical difference between the groups with respect to age, BMI, AST, ALT, and serum iron ($P > 0.05$). There was significant statistical difference between the studied groups with respect to hemoglobin, serum FBG, serum urea, serum creatinine, uric acid, Na, K, Ca, phosphorous (PH), iPTH, serum cholesterol, serum TG, serum albumin, TP, TIBC, and fetuin-A ($P < 0.05$). Table 2 showed the comparison between the groups using post-hoc Scheffé's test. There was significant statistical decrease in fetuin-A level between the groups; in group I the level was 504.58 ± 45.64, in group II it was 385.62 ± 47.37, in group III it was 270.8 ± 42.45, and in group IV it was 613.03 ± 47.54 ($P_1 < 0.0001$, $P_2 < 0.0001$, $P_3 < 0.0001$, $P_4 < 0.0001$, $P_5 < 0.0001$, $P_6 < 0.0001$). All *P* values were significant; the highest level was in the control group and the lowest level was in group III patients with CRF, who had complicated diabetes mellitus. Other variances and their significance are shown in Table 2. Correlation study revealed that each of the parameters, hemoglobin, serum Ca (Fig. 3), serum albumin (Fig. 6), TP (Fig. 5), and TIBC, were significantly and positively correlated with fetuin-A ($r = 0.585$, 0.465, 0.611, 0.525, and 0.428, respectively and $P < 0.005$), whereas blood urea, creatinine, UA (Fig. 1), iPTH, serum Na, serum K, FBG (Fig. 2), serum TG, and serum cholesterol (Fig. 4) were significantly negatively correlated with fetuin-A ($r = -0.494$, −0.435, −0.451, −0.557, −0.299, −0.427, −0.717, −0.316, and −0.535, respectively and $P < 0.005$) as shown in Table 3 and Figs. 1–6.

Discussion

Fetuin-A is a multifunctional liver-derived protein found in high concentrations in human serum [11]. Two of the primary physiological functions of fetuin-A may be critically important to cardiovascular health. Fetuin-A acts as an inhibitor of calcification by increasing the blood solubility of calcium and

Table 1 Demographic, clinical, and laboratory characteristics of the studied groups

ANOVA	Group I (CRF)	Group II (CRF with DM, no complication)	Group III (CRF with complicated DM)	Group IV (control)	*P*	Significance
Age (years)	44.4±6.51	45.5±6.43	40.50±3.30	42.4±5.94792	0.231	NS
Sex (male : female) (%)	50 : 50	80 : 20	60 : 40	60 : 40	–	–
Duration of dialysis (years)	5.2±3.42	5.35±4.39	4.1±3.63	–	0.003*	HS
BMI (kg/m^2)	27.27±2.42	26.26±2.62	26.94±2.82	26.48±1.96	0.800	NS
HB	10.95±1.9	9.3±2.12	9.54±2.98	13.76±1.15	0.0001**	HS
FBG (g/dl)	84.7±9.11	135.30±24.24	122.30±13.67	78.40±7.22	0.0001**	HS
Urea (mg/dl)	149.4±24.54	131.8±45.30	119.60±22.11	23.3±5.01	0.0001**	HS
Creatinine (mg/dl)	11.29±1.72	7.64±2.24	7.47±2.71	0.49±0.202	0.0001**	HS
Uric acid (mg/dl)	6.93±1.49	7.05±1.07	6.57±1.67	4±0.60	0.001**	HS
Na (mmol/l)	139.5±3.85	137.8±3.52	137.8±6.17	133.6±4.27	0.039	S
K (mmol/l)	5.8±1.10	6.59±1.14	5.77±0.923	4.39±0.453	0.0001**	HS
Ca (mg/dl)	8.16±0.812	8.51±0.578	5.23±2.20	9.58±0.559	0.005	HS
PH (mg/dl)	5.32±1.41	5.32±1.95	5.23±2.196	3.48±0.385	0.041	S
iPTH (ng/dl)	176.36±256.2	489±229.56	417.9±368.22	43.6±5.1	0.001	HS
AST (mg/dl)	20.3±9.34	18.1±10.6	21.4±7.98	14.2±2.04	0.229	NS
ALT (mg/dl)	18.1±11.69	18.4±13.19	17.2±8.28	17.30±4.69	0.991	NS
Cholesterol (mg/dl)	156.6±44.3	160.4±44.52	182.2±42.27	99±17.54	0.0001**	HS
TG (mg/dl)	94.80±42.73	162.8±84.54	130.4±35.22	85.8±14.755	0.007*	HS
Albumin (g/dl)	3.95±0.143	3.62±0.441	3.55±0.49	4.56±0.35	0.0001**	HS
TP (g/dl)	7.24±0.60	6.96±0.568	6.79±0.492	7.68±0.379	0.002*	HS
Iron (μg/dl)	98.38±47.103	81.37±50.18	82.8±53.21	98.9±14.65	0.704	NS
TIBC (μg/dl)	200.2±28.125	210.8±37.03	238.2±56.9	336.8±54.94	0.0001**	HS
Fetuin-A (μg/ml)	504.58±45.64	385.62±47.37	270.8±42.45	613.03±47.54	0.0001*	HS

ALT, alanine aminotransferase; AST, aspartate aminotransferase; AVOVA, analysis of variance; CRF, chronic renal failure; DM, diabetes mellitus; HB, hemoglobin. *Significant (S). **Highly significant (HS). $P < 0.05$ is considered significant. $P < 0.01$ is considered highly significant.

Table 2 Comparison of fetuin-A and serum Creatinine,serum Uric acid,S. Ca, PH, and iPTH among the studied groups

Scheffé's test	Group I (CRF)	Group II (CRF + DM)	Group III (CRF + DM + vascular complication)	Control	*P* value	Significance
Fetuin-A (μg/ml)	504.58±45.64	385.62±47.37	270.8±42.45	613.03±47.54	$P_1 \leq 0.0001$***	HS
					$P_2 \leq 0.0001$***	HS
					$P_3 \leq 0.0001$***	HS
					$P_4 \leq 0.0001$***	HS
					$P_5 \leq 0.0001$***	HS
					$P_6 \leq 0.0001$***	HS
Creatinine (mg/dl)	11.29±1.72	7.64±2.24	7.47±2.71	0.49±0.202	$P_1 \leq 0.003$***	HS
					$P_2 \leq 0.001$***	HS
					$P_3 \leq 0.0001$***	HS
					$P_4 \leq 0.889$	NS
					$P_5 \leq 0.0001$***	HS
					$P_6 \leq 0.0001$***	HS
Uric acid (mg/dl)	6.93±1.49	7.05±1.07	6.57±1.67	4±0.60	$P_1 = 0.998$	NS
					$P_2 = 0.941$	NS
					$P_3 \leq 0.001$**	HS
					$P_4 = 0.873$	NS
					$P_5 \leq 0.001$**	HS
					$P_6 \leq 0.0001$***	HS
Serum Ca (mg/dl)	8.16±0.812	8.51±0.578	5.23±2.20	9.58±0.559	$P_1 = 0.940$	NS
					$P_2 = 0.685$	NS
					$P_3 = 0.108$	NS
					$P_4 = 0.344$	NS
					$P_5 \leq 0.007$**	HS
					$P_6 = 0.311$	NS
Serum PH (mg/dl)	5.32±1.41	5.32±1.95	5.23±2.196	3.48±0.385	$P_1 = 1$	NS
					$P_2 = 1$	NS
					$P_3 = 0.119$	NS
					$P_4 = 1$	NS
					$P_5 = 0.149$	NS
					$P_6 = 0.119$	NS
Serum iPTH (ng/dl)	176.36±256.2	489±229.56	417.9±368.22	43.6±5.1	$P_1 = 0.07$*	HS
					$P_2 = 0.223$	NS
					$P_3 = 0.710$	NS
					$P_4 = 0.940$	NS
					$P_5 = 0.020$*	S
					$P_6 = 0.004$**	HS

CRF, chronic renal failure; DM, diabetes mellitus. *Significant (S). **Highly significant (HS). $P > 0.5$ is statistically nonsignificant. $P < 0.05$ is statistically significant. P_1 = Group I vs. Group II. P_2 = Group I vs. Group III. P_3 = Group I vs. Group IV. P_4 = Group II vs. Group III. P_5 = Group III vs. Group IV. P_6 = Group II vs. Group IV. ***Highly significant (HS).

Table 3 Correlation between fetuin-A and other factors in the studied groups

Parameters	Fetuin-A	
	r	*P*
Age (years)	0.110	0.5
BMI	0.071	0.663
HB	0.585	0.0001**
FBG	−0.717	0.0001**
Urea	−0.494	0.001**
Creatinine	−0.435	0.005**
Na	−0.299	0.060*
K	−0.427	0.006**
Ca	0.465	0.002**
PH	−0.256	0.111
iPTH	−0.557	0.0001**
AST	−0.237	0.142
ALT	0.006	0.972
TP	0.525	0.001**
Albumin	0.611	0.0001**
UA	−0.451	0.003**
Cholesterol	−0.535	0.0001**
TG	−0.316	0.047*
TIBC	0.428	0.006**

ALT, alanine aminotransferase; AST, aspartate aminotransferase; HB, hemoglobin. *Correlation is significant at the 0.05 level (two-tailed). **Correlation is significant at the 0.01 level (two-tailed).

phosphorus and preventing spontaneous mineral precipitation in the vasculature [12]. It is considered an anti-inflammatory mediator that participates in macrophage deactivation [13]. It is also recognized that fetuin-A can actively regulate the cell-mediated process in the vessel wall, can inhibit mineralization in a concentration-dependent manner, and can enhance the phagocytosis of apoptotic bodies by vascular smooth muscle cells [14]. Some studies have demonstrated an association between serum fetuin-A level and all-cause mortality of dialysis patients [15,16]. Serum fetuin-A also showed important associations with atherosclerosis, malnutrition, and cardiovascular events in peritoneal dialysis patients [16]. In end-stage renal disease populations, lower plasma fetuin-A level is associated with greater prevalence and severity of vascular calcification [17] and increased risk for CVD events and mortality [16], independent of the traditional CVD and kidney disease risk factors. It is believed that low fetuin-A level is associated with atherosclerosis, inflammation, and cardiovascular risk. In the present study, the plasma concentration of fetuin-A was highly significantly decreased in the hemodialysis patients groups compared with healthy controls. Our results agreed with the findings of Mona *et al.* [18], who found lower fetuin-A level in hemodialysis (HD) patients compared with those without dialysis; however, it did not reach significance.

Figure 1

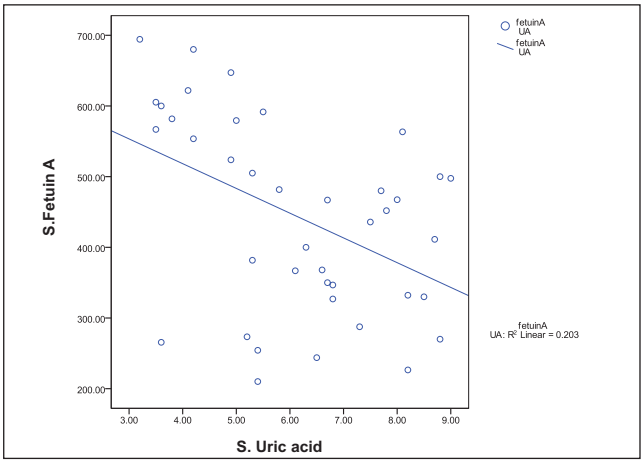


Figure 2

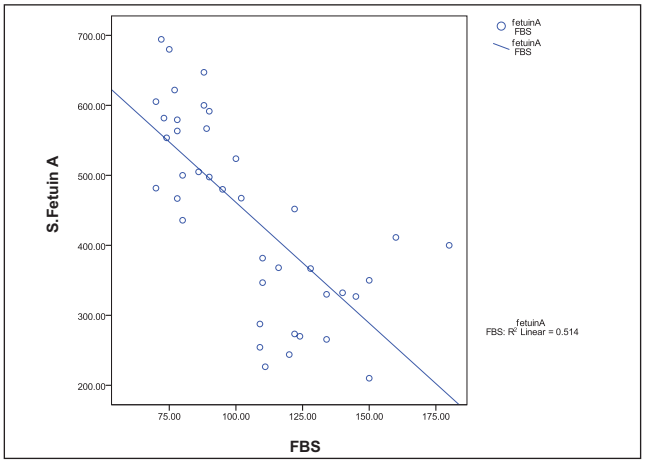


Figure 3

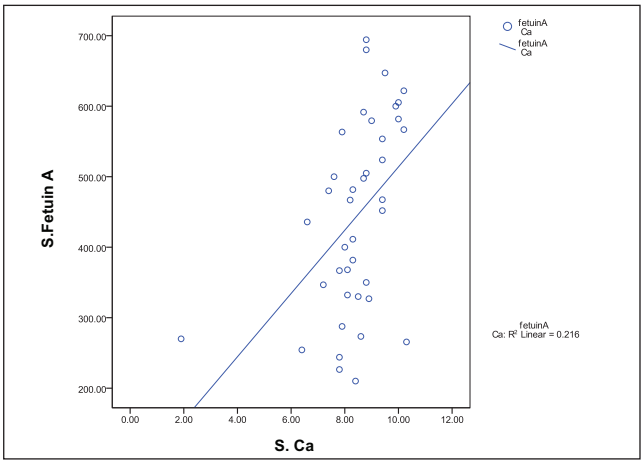
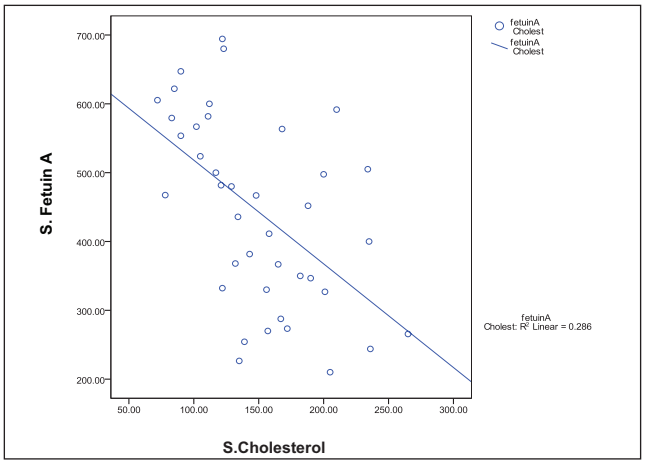


Figure 4



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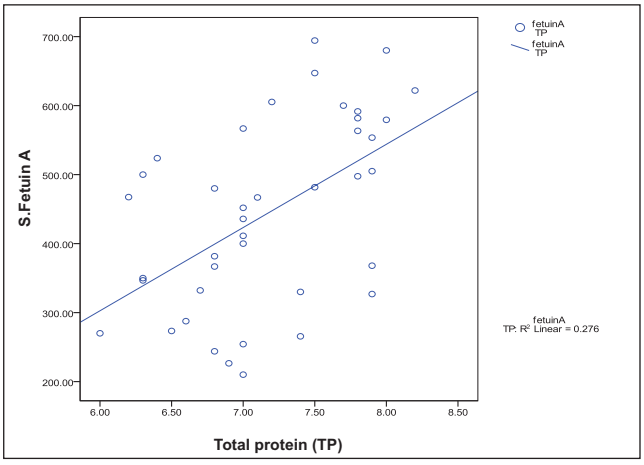
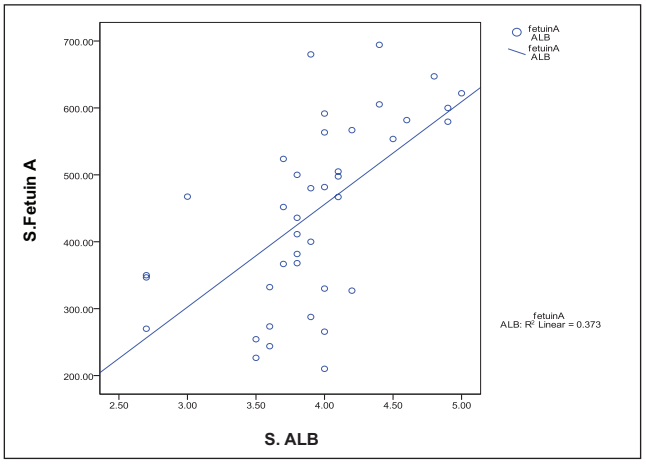


Figure 6



These findings are in agreement with the findings of Dervisoglu *et al.* [19]. The study by Cozzolino *et al.* [20] confirmed the lowering of serum fetuin-A level, due to chronic inflammation in HD patients and the significant association between reduced serum fetuin-A level and multisite cardiovascular complication in HD patients [21]. Cozzolino and colleagues also reported that a single HD session significantly lowers serum fetuin-A level, possibly secondary to the inflammatory processes induced by the HD therapy. However, Hermans *et al.* [22] and Mehrotra *et al.* [23] showed no difference between ESRD and healthy participants when compared according to their serum fetuin-A level. Schlieper *et al.* [24] showed that a high fetuin-A level is now considered to be a strong indicator of better CV outcomes in dialysis patients. In the present study, the plasma concentration of fetuin-A was highly significantly decreased in the diabetic hemodialysis patients groups compared with the nondiabetic hemodialysis patients group [25]. The relationship between fetuin-A and arterial stiffening was found to be different in diabetic and nondiabetic patients, which underlines the concept that, in diabetic chronic kidney disease (CKD) patients, arterial stiffening may be driven by different processes compared with that in the nondiabetic population. These may include deposition of advanced glycation products, calcification of more extensive atherosclerotic plaque, or development of additional de-novo atherosclerosis. Our results agreed with the study by Mehrotra *et al.* [23]; both coronary and aortic calcifications were found to be related to low fetuin-A level in patients with diabetic nephropathy. A positive association between fetuin-A level and the coronary calcification score was demonstrated by Ix *et al.* [26], who found that lower fetuin-A level is independently associated with greater coronary artery calcification severity but not with peripheral arterial disease or internal intima-media thickness. In the present study, the plasma concentration of fetuin-A was highly significantly decreased in the diabetic hemodialysis patients with macrovascular complication groups compared with the diabetic hemodialysis patients without macrovascular complication group. Our results agreed with the study by Marcel *et al.* [27]. This study demonstrates that lower fetuin-A level seems to be associated with prevalent macrovascular disease in patients with type 2 diabetes (The results of this cross-sectional study suggest that lower fetuin-A level is associated with macrovascular late complications in high-risk type 2 diabetes patients, whereas there are no associations of fetuin-A with metabolic status or microvascular complications). In addition, this study was in agreement with the study by Luis *et al.* [28], who found the association of low fetuin-A with peripheral arterial disease. In contrast, Mehrotra *et al.* [23] observed an association of increased fetuin-A level with coronary artery calcification. In our study, there was significant statistical increase between the patients groups and the control group with respect to creatinine ($P < 0.05$), but there was no significant difference between group II and group III ($P > 0.05$). There was significant statistical increase between group I and control with respect to serum uric acid ($P_3 < 0.001$) and also between group II, group III, and the control group (P_5 and $P_6 < 0.01$). Our study showed significant positive correlations between fetuin-A and hemoglobin, Ca, albumin, TIBC, and TP ($r = 0.585$, 0.465, 0.611, 0.428, and 0.525 and $P = 0.0001$, 0.002, 0.0001, 0.006, and 0.001, respectively) as shown in Table 3 and Figs. 3, 5 and 6. No correlation was found between fetuin-A and age or BMI. It is believed that low fetuin-A level is associated with atherosclerosis, inflammation, and cardiovascular risk. Stefan *et al.* [29] found that nonalcoholic liver diseases related to insulin resistance, including type 2 diabetes, are associated with higher level of fetuin-A. This is in contrast with our results, which showed that the lowest level of Fetuin-A was found in group II and group III (CRF and diabetic, CRF and diabetic with vascular strokes). Our study showed significant positive correlations between fetuin-A and Ca ($r = 0.465$ and $P < 0.002$). In our study, there were significant negative correlations between fetuin-A and cholesterol, TG, FBG, urea, creatinine, UA, iPTH, Na, and K ($r = -0.535$, -0.316, -0.717, -0.494, -0.435, -0.451, -0.557, -0.299, and -0.427 and $P = 0.0001$, 0.047, 0.0001, 0.001, 0.005, 0.003, 0.0001, 0.060, and 0.006, respectively) as shown in Table 3 and Figs. 1, 2 and 4. Our study showed other cardiovascular risk factors, such as anemia, atherogenic lipid profile, and hyperuricemia, which is in accordance with the study conducted by Çubukcuoğlu *et al.* [30]. In patients undergoing hemodialysis, lower fetuin-A level is associated with higher mortality. CKD patients have significantly lower level of fetuin-A than healthy controls. Short-term treatment with sevelamer increases serum fetuin-A concentration in stage 4 CKD patients [31]. In dialysis patients, calcimimetic therapy for secondary hyperparathyroidism significantly decreases fetuin-A level [32]. However, serum fetuin-A level increased obviously following parathyroidectomy in patients with refractory renal hyperparathyroidism [33].

Conclusion

Low plasma fetuin-A level is associated with vascular strokes in CRF patients with type 2 diabetes, independent of the traditional and contemporary risk factors. Our findings suggest a unique role for fetuin-A deficiency as a biomarker of vascular

diseases in the setting of CRF and type 2 diabetes. Future studies, with larger populations, are required to determine whether measurement of plasma fetuin-A will be useful as a CVD risk stratification tool and whether prediction criteria will differ for those with and without diabetes.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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