ORIGINAL ARTICLE

Serum Fetuin A, hs-CRP and Homocysteine as Biochemical Markers of Cardiovascular Complications in Chronic Dialysis Patients

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ABSTRACT

Introduction: Chronic kidney disease (CKD) patients are considered a high risk group of cardiovascular disease in which vascular calcification plays central role. A pivotal role in the inhibition of calcification is played by fetuin-A. The measurement of inflammatory markers such as high sensitivity C-reactive protein (hs-CRP) and homocysteine which promotes atherosclerosis is helpful in predicting cardiovascular disease in ESRD patients on regular dialysis**. Material and Method:** The study included 40 adult CKD patients divided into 30 ESRD patients on conventional hemodialysis, 15 with CVD and 15 without CVD, as well as 10 CKD patients on conservative treatment. Ten healthy subjects served as a control group. Enzyme-linked immunosorbent assays were used for fetuin-A, hs-CRP and homocysteine. **Results:** ESRD patients showed a significant increase in serum hs-CRP, homocysteine and decrease in fetuin-A compared to control group. In addition, ESRD patients with CVD and without CVD showed a significant increase in hs-CRP, homocysteine and only those with CVD had significantly decreased fetuin-A in relation to CKD patients. The study revealed increased levels of hs-CRP and decrease in fetuin-A in ESRD patients with CVD compared to ESRD patients without CVD. Fetuin-A showed a negative correlation with hs-CRP and homocysteine in ESRD patients with and without CVD. **Conclusion**: The combined use of hs-CRP at a cutoff of (10 mg/dL) with either fetuin-A at a cutoff value of (0.26 g/L) or alternatively with homocysteine at a cutoff value of (48.23 µmol/L) proved to be effective for discrimination of CVD patients from other ESRD or CKD patients.

Keywords: Chronic kidney disease, C-reactive protein, Fetuin-A, Homocysteine

INTRODUCTION

Chronic kidney disease (CKD) is a worldwide major public health problem, with increasing incidence, prevalence, high costs and poor outcomes.[1] More than 50 million people world-wide have CKD, and more than one million of them are receiving kidney replacement therapy. Additionally, because of the age-related decline in GFR that may largely be attributable to hypertension, atherosclerosis, or heart failure, the incidence of CKD increases with age, approximately 17 percent of persons older than 60 years have an estimated GFR of less than 60 mL per minute per 1.73 m^2. Early detection may help slow the progression of kidney disease and avoid kidney failure.[2]

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The incidence of CVD is seven to ten folds greater in patients with CKD than in non CKD age and sex matched controls.[3] By the time patients develop the need for renal replacement therapy, the risk of cardiovascular death is 10 to 20 times greater than age and sex matched individuals without kidney disease.[4]

Vascular calcification is common in ESRD and it is a central characteristic of the atherosclerotic cardiovascular disease frequently observed in hemodialysis patients.[5] Pathogenesis of vascular calcification seems to be multi-factorial, it is strongly related to bone and mineral metabolism disorder, particularly enhanced bone resorption and high phosphate blood levels commonly present in renal patients, but greater attention has been focused on in vivo circulating modulators, working as endogenous inhibitors of calcium and phosphate precipitation.[6]

Regarding the in vivo circulating modulators of calcification, fetuin-A, also known as α_2 – Heremans – Schmid glycoprotein (AHSG), is a circulating calcium – regulatory glycoprotein present throughout the

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extracellular space and acts as an inhibitor of calcium and phosphate precipitation.[7] Fetuin-A is taken up by vascular smooth muscle cells and loaded to intracellular vesicles leading to formation of soluble colloidal spheres, containing fetuin, and basic calcium phosphate (BCP) which is known as calciprotein particles. These soluble calciprotein particles are then cleared by the phagocytic cells of the reticuloendothelial system.[8] Fetuin-A seems to be involved in both inflammation and vascular calcification processes. Actually, fetuin-A is down regulated during systemic inflammation as a negative acute phase reactant. Chronic micro-inflammation state is frequently observed in ESRD and is related to vascular calcification that ultimately leads to increased cardiovascular risk. Moreover, activated acute phase response and fetuin-A deficiency might account for accelerated atherogenesis.[9]

Increased level of hs-CRP is found in a substantial proportion of ESRD patients and is associated with poor clinical outcomes, including all cause mortality. The presence of increased hs-CRP confirms the existence of a chronically activated acute phase response in the ESRD, with hs-CRP possibly acting as a measure of atherosclerosis. However, patients whose hs-CRP levels remain elevated overtime would be expected to have greater mortality than patients with occasionally elevated levels.[10]

Hyper-homocysteinemia is recognized as an independent risk factor for cardiovascular morbidity and mortality in ESRD patients. Elevated homocysteine is observed in more than 90% of uremic patients.[11] In fact, homocysteine has a major role in the pathogenesis of atherosclerosis. It has been incriminated in stimulating smooth muscle cell proliferation, increasing susceptibility of LDL oxidation, increasing platelet aggregation, activating the coagulation factors and finally directs damage of the endothelium.[12]

Thus, the aim of this study was to evaluate the clinical utility of serum fetuin-A, in addition to hs-CRP, homocysteine as biochemical markers of atherosclerotic CVD in ESRD patients subjected to regular hemodialysis.

MATERIALS AND METHODS

I- Subjects

This study was conducted at Ain Shams University Hospital on forty patients with CKD, of whom thirty patients had an ESRD and were subjected to conventional hemodialysis, fifteen of them had CVD, and fifteen without CVD as well as ten patients with CKD on conservative treatment. The study also included ten sex and age matched controls.

A-Patients' Groups (Group I, n=40)

This group included forty patients with CKD, 25 males and 15 females. Their ages ranged between 21 to 68 with a mean of 47±18.7 years. They were admitted to the Nephrology Department and Hemodialysis Unit of Ain Shams University Hospitals. They were further divided into the following subgroups according to the presence or absence of CVD and the type of treatment:

1-Subgroup Ia (n=15): This subgroup included fifteen patients, 12 males and 3 females. Their ages ranged from 22 to 64 years (mean 48.9±11.9 years). They were subjected to conventional hemodialysis and were diagnosed as having CVD, as evidenced by full history taking for previous ischemic heart disease, blood pressure measuring, echocardiography and electrocardiogram previously done for the patients.

2-Subgroup Ib (n=15): This subgroup included fifteen patients, 8 males and 7 females. Their ages ranged from 22 to 58 years (mean 44.2±9.87 years). They were subjected to conventional hemodialysis and had no history of CVD.

3-Subgroup Ic (n=10): This subgroup included ten patients, 5 males and 5 females. Their ages ranged from 21 to 68 years (mean 47.2±18.71 years). They had CKD, at different stages ranged from 1 to 4 and they are on conservative treatment.

Exclusion Criteria

Patients with clinical conditions known to be associated with decreased fetuin-A level were excluded. These conditions include: chronic liver disease, hepatitis B or hepatitis C virus infection, liver cell failure, Hypo/hyperthyroidism, infectious and immune-inflammatory diseases, patients with severe malnutrition with body mass index (BMI ≤20 Kg/m^2) and diabetics.

B-Control Group (Group II, n=10)

This group included ten age and sex matched healthy subjects. They were 4 males and 6 females. Their ages ranged from 18 to 54 years (mean 35.4±11.11 years).

II- Blood Samples

Five millimeters of venous blood were collected after 12-14 hours fasting and before the hemodialysis session in patients on hemodialysis and under complete aseptic precautions in plain test tubes without anticoagulant. After coagulation, samples were centrifuged (at 1500 g for 15 minutes). The separated serum was divided into three aliquots. One was designated for the immediate assay of routine renal function, serum calcium, phosphorus and lipid profile. The other two aliquots were stored at -20°C for subsequent assay of hs-CRP, homocysteine and fetuin-A. Hemolysed

samples were discarded. Repeated freezing and thawing was avoided.

III- Methods

A-Analytical Methods

1-Routine kidney function test, serum calcium (Ca), serum phosphorus (P) and Complete lipid profile: were measured on Synchron CX-9 autoanalyzer[1*].

2- High Sensitivity C-reactive Protein (hs- CRP)

High sensitivity C-reactive protein concentrations were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit supplied by Accu-Bind, Inc[2**]. Patients and controls sera were diluted 100 fold prior to use.

3- Homocysteine

Homocysteine concentrations were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Axis Homocysteine) supplied by IBL International, Inc[3*].

Axis Homocysteine is an enzyme immunoassay for the determination of total homocysteine in blood. Protein bound homocysteine is reduced to free homocysteine and enzymatically converted to S-adenosyl-L-homocysteine (SAH) in a separate procedure prior to the immunoassay. The enzyme is specific for the L-form of homocysteine, which is the only form present in the blood. The solid-phase enzyme immunoassay was based on competition between SAH in the sample and immobilized SAH bound to the walls of the micro-titre plate for binding sites on a monoclonal anti-SAH antibody. After removal of unbound anti-SAH antibody, a secondary rabbit anti-mouse antibody labeled with the enzyme horse radish peroxidase (HRP) was added. The peroxidase activity was measured spectrophotometrically at 450nm after addition of substrate, and the absorbance was inversely related to the concentration of total homocysteine in the sample.

4- Fetuin-A

Fetuin-A concentrations were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit supplied by Bio Vendor Laboratory Medicine, Inc[4**].

Surface of wells in microtitration plate is coated with polyclonal anti-human Fetuin-A specific antibody. Standards, Quality Controls (QC) and diluted samples were pipette into the wells (serum samples were diluted 1: 10000 with dilution buffer just prior to performance of the test).

Any human fetuin-A present was captured by immobilized antibody and unbound protein was washed away after the first incubation period. Then a horse radish peroxidase (HRP) conjugated polyclonal anti-human fetuin-A antibody was added to the wells and incubated. After another washing step, unbound antibody- conjugate was removed; a substrate solution was added to the wells. The enzymatic reaction yield a blue product that turned yellow when stop solution was added. The intensity of the color, was measured spectro-photometrically at 450 nm and was directly proportional to the amount of the human fetuin-A bound in the initial step. Concentration of the test samples were then read off the standard curve, multiplied by the dilution factor 10000 to obtain fetuin-A results in ng/mL, then results were divided by 1000000 to be changed into g/L.

Statistical Methods

Statistical analysis was done using SPSS software package, version 15.0, 2006, Ecosoft corporation, USA. Data was expressed descriptively as mean ± standard deviation (SD) for quantitative parametric data and median and inter-quartile range for quantitative skewed data. Comparison between groups was done using the student's t test for parametric data and Wilcoxon's rank sum test for skewed data. Correlation study between the different analyzed parameters was done using Spearman's rank correlation coefficient test for skewed data and Pearson's correlation coefficient for parametric data. Furthermore, the diagnostic performance of homoscystine, hs CRP and fetuin-A was evaluated using receiver operating characteristic curve (ROC) analysis. The best cutoff value (the point nearest to the left upper corner of the curve) was determined.

Ethics Statement

This study was approved by the Institutional Review Board of Faculty of Medicine, Ain Shams University, Cairo, Egypt. A written informed consent was obtained from each participant.

RESULTS

Results of the present study are shown in Tables from (1) to (5) and Figures from (1) to (5).

Descriptive statistics of various studied parameters in all studied groups are shown in Table (1).

In subgroup Ia (ESRD patients with CVD), there was statistical non significant difference in serum T-Chol and LDL-C (t=0.71; t=1.35 with $p > 0.05$, respectively) compared to subgroup Ib (ESRD patients without CVD). However,

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[2] Monobind Inc, Lake Forest, USA. Ca 92630.Tel:949 951 2665. Fax:949 951 3539. E-mail:info@monobind.com
[3] IBL International Gmbh, Flughafenstrasse 52, D-22335 Hamburg, Germany.
[4] Biovender GmbH, Im Neuenheimer Feld 583, D-69120 Heidelberg, Germany.

Table 1: Descriptive statistics of the different studied parameters in all patients group and the control group

Parameter	Mean±SD			
	n=15		n=10	
	Subgroup Ia	Subgroup Ib	Subgroup Ic	Control group II
Age (years)	48.9±11.9	44.2±9.87	47.2±18.7	35.4±11.11
BMI (Kg/m²)	29.8±8.3	27.9±5.58	29.6±4.90	25.4±2.36
SBP (mmHg)	128.6±11.87	118.9±33.9	131.0±14.4	119.0±8.75
DBP (mmHg)	82.6±7.9	80.0±8.41	83.0±8.23	80.0±8.16
Creat (mg/dL)	9.84±1.81	10.90±2.4	2.61±1.05	0.77±0.27
BUN (mg/dL)	132.1±29.7	130.5±39.2	93.2±69.4	24.2±6.4
Uric acid (mg/dL)	6.36±1.27	6.0±1.15	6.23±1.44	4.19±1.075
Ca (mg/dL)	8.26±1.6	7.50±0.88	8.99±0.511	9.09±0.59
P (mg/dL)	5.44±1.22	5.3±2.3	4.09±0.91	3.510±0.463
T- Chol (mg/dL)	200.8±32.73	195.5±31.4	178.1±31.64	169.8±42.5
TG (mg/dL)	139.4±44.5	132.9±23.1	110.5±89.6	103.8±46.19
HDL-C (mg/dL)	48.66±14.97	63.00±10.64	58.7±11.66	57.27±14.32
LDL-C (mg/dL)	105.6±37.74	89.46±26.9	107.7±30.8	121.4±35.4
hs-CRP (mg/dL)	42.5 (0.6-100)*	23.4 (0.5-100)*	3.24 (0.4-10.0)*	2.26 (0.4-3.7)*
Homocysteine (mmol/L)	34.68±9.36	36.13±9.68	20.09±5.48	10.7±3.37
Fetuin A (g/L)	0.125±0.075	0.245±0.077	0.275±0.068	0.313±0.072

Data are expressed as mean±SD or median (interquartile range)* in case of Skewed Data. P>0.05: Non significant difference. t: Student t test

Table 2: Statistical comparison between each two subgroups in patient's group (Group I) regarding the different studied parameters using the student t-test for parametric data and wilcoxcon's rank sum test for skewed data

Parameter	Subgroup Ia/Ib n=15/15		Subgroup Ia/Ic n=15/10		Subgroup Ib/Ic n=15/10	
	t/z*	p	t/z*	p	t/z*	p
hs-CRP (mg/L)	5.88*	<0.001	10.19*	<0.001	17.60*	<0.001
Homocysteine (µmol/L)	−0.415	>0.05	4.428	<0.001	4.733	<0.001
Fetuin-A (g/L)	−4.33	<0.001	−5.07	<0.001	−0.982	>0.05

p<0.05, Significant difference. z*:Wilcoxcon's rank sum test. p<0.01, <0.001: Highly significant difference

Table 3: Statistical comparison between each subgroups in patient's group (Group I) and control group (Group II) regarding the different studied parameters using the student t-test for parametric data and wilcoxcon's rank sum test for skewed data

Parameter	Subgroup Ia/II n=15/10		Subgroup Ib/II n=15/10		Subgroup Ic/II n=10/10	
	t/z*	p	t/z*	p	t/z*	p
hs-CRP (mg/L)	10.4*	<0.001	18.44*	<0.001	−0.04*	>0.05
Homocysteine (µmol/L)	7.724	<0.001	7.939	<0.01	4.641	>0.05
Fetuin-A (g/L)	−6.23	<0.001	-2.22	<0.05	−1.22	>0.05

p>0.05: Non significant difference. t: Student t test. p<0.05, Significant difference. z*:Wilcoxcon's Rank Sum Test. p<0.01, <0.001: Highly significant difference

Table 4: Correlation study between serum Fetuin-A and hs-CRP, homocysteine in patients' subgroups using pearson's correlation coefficient for parametric data (r)

Parameter	ESRD with CVD		ESRD without CVD		CKD	
	r	p	r	p	r	p
hs-CRP (mg/L)	−0.010	<0.05	−0.087	<0.05	0.164	>0.05
Homocysteine (µmol/L)	−0.045	<0.05	−0.450	<0.05	0.228	>0.05

p>0.05: Non significant correlation, p<0.05: Significant correlation

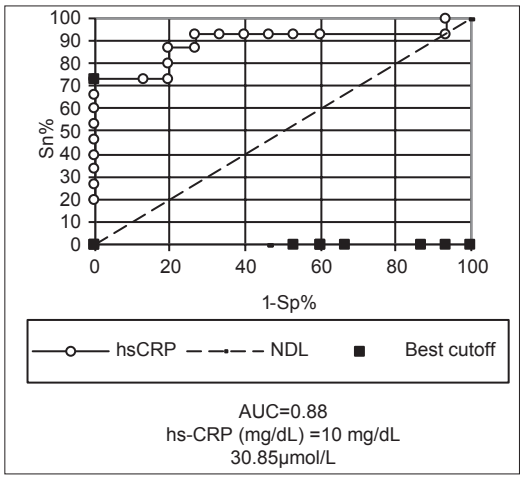

Figure 1: ROC curve analysis showing the diagnostic performance of hs-CRP for discriminating patients of subgroup Ia from those of subgroup Ib

there was a statistically significant increase in TG (t=2.75, p<0.05) and a statistically highly significant decrease in HDL-C (t=-3.02, p< 0.01), as well as a statistical highly significant increase in hs-CRP and decrease in fetuin-A (z=5.88; t=-4.33, respectively, p < 0.001) in subgroup Ia (Figure 5). On the other hand, a non significant difference in homocysteine was found between the two subgroups (t= -0.42 with p > 0.05) (Table 2).

In addition, statistical comparison between subgroup Ia and subgroup Ic (CKD patients on conservative treatment) revealed a statistical non-significant difference in T-Chol, TG, HDL-C and LDL-C between the two subgroups (t=-

Table 5: Diagnostic performance of serum hsCRP (mg/dl), homocysteine (mmol/L) and fetuin-A (g/L) in ESRD patients with CVD (subgroup Ia) versus ESRD patients without CVD (subgroup Ib)

Parameter	ROC cut off	Diagnostic sensitivity (%)	Diagnostic specificity (%)	Negative predictive value (%)	Positive predictive value (%)	Diagnostic efficacy (%)
hsCRP (mg/dL)	≥10	73.3	100.0	78.9	100.0	86.7
Homocysteine (µmol/L)	30.85	80.0	46.7	70.0	60.0	63.3
Fetuin-A (g/L)	≤0.17	80.0	46.7	70.0	60.0	63.3
hs-CRP (mg/dL)+ Fetuin A (g/L)	10.0 0.26	93.3	100.0	93.8	100.0	96.7
hs-CRP (mg/dL)+ Homocysteine (mmol/L)	10.0 48.23	93.3	100.0	93.8	100.0	96.7

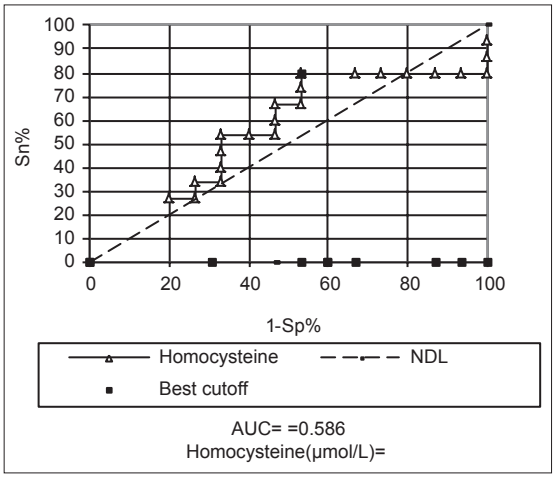

Figure 2: ROC curve analysis showing the diagnostic performance of homocysteine for discriminating patients of subgroup Ia from those of subgroup Ib

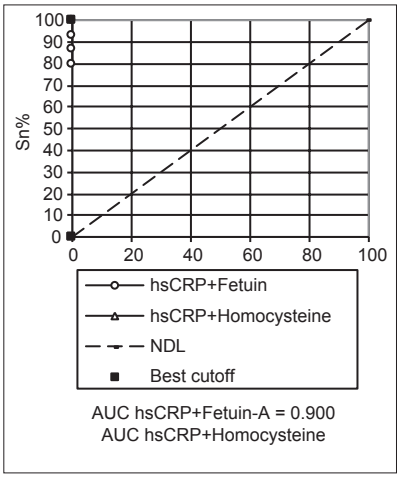

Figure 4: Multi-ROC curve analysis showing the diagnostic performance of hsCRP+fetuin-A (or homocysteine) for discriminating patients of subgroup Ia from those of subgroup Ib

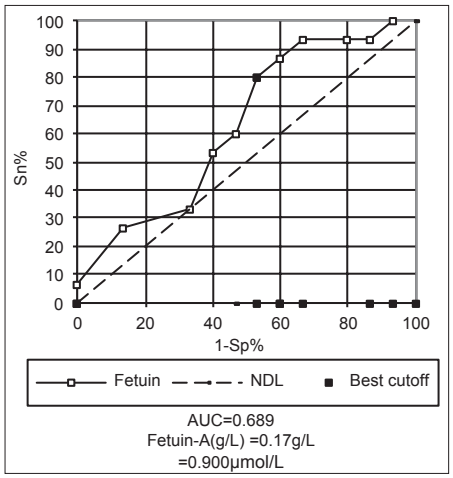

Figure 3: ROC curve analysis showing the diagnostic performance of serum fetuinA for discriminating patients of subgroup Ia from those of subgroup Ib

1.32; t=0.24; t=-1.78; t=-0.15 with p> 0.05, respectively). However, there was a statistically highly significant increase in hs-CRP, homocysteine and decrease in fetuin-A was found in subgroup Ia (z=10.19; t=4.43; t=-5.07 with p <0.001, respectively) (Table 2). Similarly, a statistical comparison between subgroup Ib and subgroup Ic revealed a non significant difference in T-Chol, TG, HDL-C and LDL-C (t=-1.99; t=-1.21; t=0.95; t=-1.56 with p > 0.05, respectively). Moreover, there was a statistically highly significant increase in hs-CRP and homocysteine found in subgroup Ib (z=17.60; t=4.73 with p < 0.001, respectively), However, a non significant difference in fetuin-A was found between the two subgroups (t=-0.99 with p > 0.05) (Table 2, Figure 5).

Furthermore, when subgroups Ia, Ib, and Ic were compared to the control group (group II) were compared, subgroups showed a non significant difference in T-Chol, TG, HDL-C and LDL-C. In subgroup IA, a highly statistically significant increase in serum hs-CRP, homocysteine and decrease in fetuin-A (z=10.44; t=7.72; t=-6.23, p < 0.001) were recorded in comparison to control group (Table 3). Moreover, when subgroup Ib was compared to the control group (group II), there was a statistically highly significant increase in hs-CRP and homocysteine (z=18.44; t=7.93, p< 0.001, <0.01, respectively), and a statistically significant decrease in fetuin-A (t=-2.22. p<0.05) (Table 3). Meanwhile when CKD patients (subgroup Ic) were compared to the control group, hs-CRP, homocysteine and fetuin-A were comparable among the two subgroups (z=-0.04; t=4.64; t=-1.22 with p > 0.05, respectively) (Table 3, Figure 5).

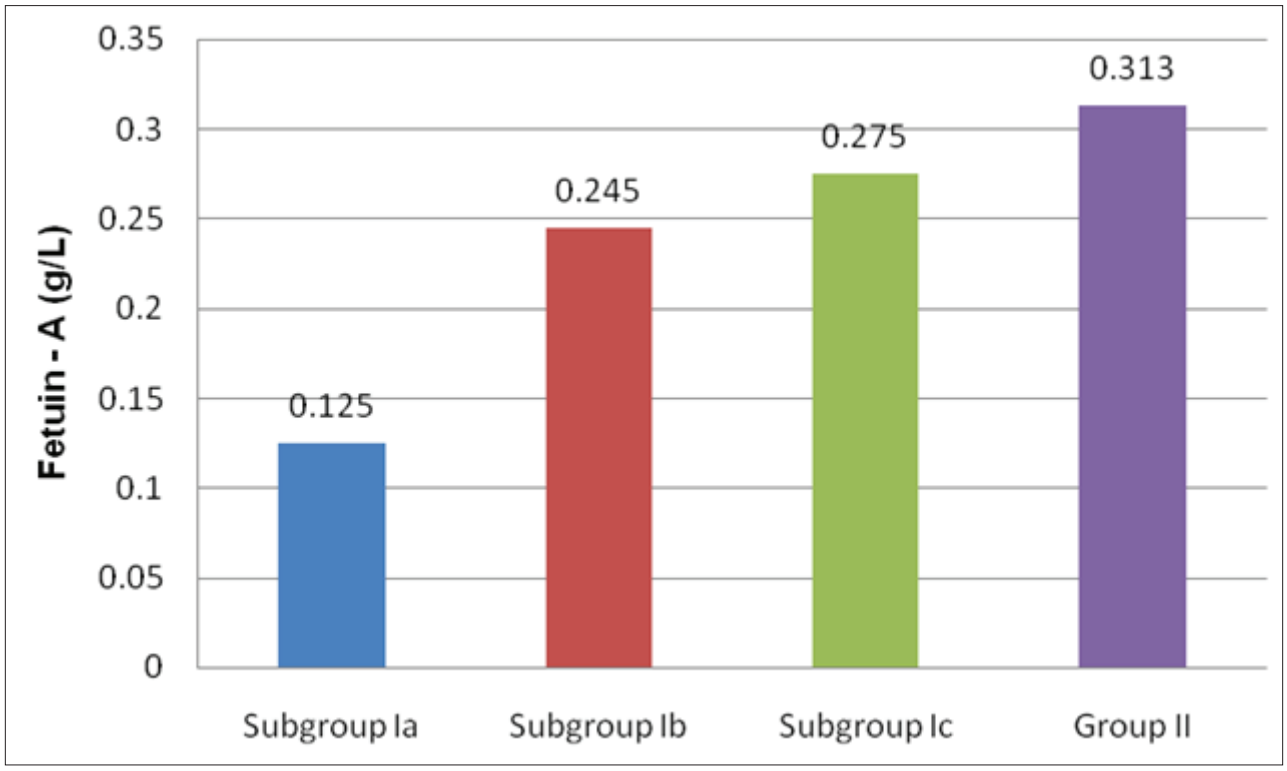

Figure 5: Comparison between various studied groups as regards mean values of fetuin-A

Table (4) shows the correlation study between serum fetuin-A and hs-CRP, homocysteine and with other studied parameters in ESRD patients with CVD (subgroup Ia). Fetuin-A showed statistically significant negative correlation with hs-CRP, and homocysteine (r=-0.272; r_s=-0.010; r=-0.045, p<0.05, respectively) and statistically significant positive correlation with diastolic blood pressure (DBP) (r=0.58, p < 0.05). Meanwhile, fetuin-A showed non statistically significant correlation with T-Chol, TG, HDL-C, LDL-C, SBP, (r=-0.27; r=0.40; r=0.32; r=-0.35; r=0.23, p >0.05, respectively). The correlation study between serum fetuin- A and hs-CRP, homocysteine and with other studied parameters in ESRD patients without CVD (subgroup Ib). Serum fetuin-A showed a statistically significant negative correlation with hs-CRP and homocysteine (rs=-0.087 and r=-0.450, respectively, p< 0.05). Fetuin-A shows a statistically non significant correlation with T-Chol, TG, HDL-C, LDL-C, DBP and SBP (r=-0.08; r=0.05; r=-0.51; r=-0.15; r=-0.03; r=0.02, p>0.05, respectively. The correlation between serum fetuin-A levels and hs-CRP, homocysteine and with other studied parameters in CKD patients on conservative treatment (subgroup Ic) shows non statistically significant correlation with T-Chol, TG, HDL-C, LDL-C, DBP, SBP, hs-CRP and homocysteine (r=0.05; r=0.34; r=0.56; r=-0.28; r=0.03; r=0.15; rs=0.16 and r=0.23; p > 0.05, respectively).

The receiver operating characteristic (ROC) curve analysis was applied to assess the diagnostic performance of serum hs-CRP, homocysteine and fetuin-A levels in ESRD patients with CVD (subgroup Ia) versus ESRD patients without CVD (subgroup Ib). The AUC was (0.88, 0.59 and 0.69, respectively), and the optimum cutoff level was (10 mg/dL, 30.85 mmol/L and 0.17 g/L, respectively) (Figures 1, 2 and 3). The analysis revealed that hs-CRP had highest diagnostic specificity of 100%, both negative and positive predictive values of (78.9% and 100%, respectively) and highest diagnostic efficacy of (86.7%), but lower diagnostic sensitivity of (73.3%). However, homocysteine and fetuin-A showed a corresponding performance with lower diagnostic efficacy of (63.3%) than hs-CRP. The markers showed similar diagnostic sensitivity and specificity of (80% and 46.7%, respectively). In addition, identical negative and positive predictive values (70.0 %, 60.0 % respectively) were observed for both markers (Table 5).

Moreover, multi-ROC curve analysis was applied to assess the diagnostic performance utilizing a combination of both serum hs-CRP and fetuin-A, as well as hs-CRP and homocysteine for discriminating ESRD patients with CVD (subgroup Ia) from ESRD patients without CVD (subgroup Ib). Each combination of markers revealed the same diagnostic performance with AUC (0.90) using a diagnostic cutoff value of (10 mg/dL) for hs-CRP applied first with cutoff value of (0.26 g/L) for fetuin-A, and in the other application with cutoff value of (48.23 mmol/L) for homocysteine (Figure 4). Both multi- ROC analyses had a diagnostic sensitivity, specificity, negative predictive value,

positive predictive value and diagnostic efficacy of (93.3%, 100%, 93.8%, 100.0%, and 96.7%, respectively) (Table 5).

DISCUSSION

Chronic kidney disease is a major public health problem throughout the world.[1] The risk of CVD morbidity and mortality is high in all stages of CKD. However, it becomes more evident upon the initiation of dialysis treatment.[4] The atherosclerotic cardiovascular disease is the most frequent complication in patients undergoing chronic hemodialysis treatment. The atherosclerotic lesions are more likely to be heavily calcified due to accelerated vascular calcification commonly observed in those patients.[3] A potential inhibitor of vascular calcification is fetuin-A.[6]

The aim of the present work was to evaluate the clinical utility of serum levels of fetuin-A, hs-CRP and homocysteine as biochemical markers of atherosclerotic cardiovascular disease in ESRD patients subjected to conventional hemodialysis.

Concerning hs-CRP, a highly significant increase was detected in ESRD patients collectively (subgroup Ia and Ib) compared to CKD patients on conservative treatment (subgroup Ic) and the healthy control group (group II). This result is in agreement with Apple et al.[10] and Stenvinkel et al.[13] who explained that renal insufficiency causes a prolonged acute phase inflammatory reaction that was accompanied with elevated inflammatory markers such as hs-CRP, IL-6, albumin and fibrinogen. These inflammatory markers are significantly associated with cardiovascular morbidity and mortality. Moreover, the significant increase in hs-CRP level among ESRD patients with CVD (subgroup Ia) compared to those of ESRD patients without CVD (subgroup Ib) confirms the existence of more vascular events with hs-CRP possibly acting as a measure of atherosclerosis.[14]

As regards homocysteine a highly significant increase was detected in ESRD patients collectively (subgroup Ia and Ib) compared to CKD patients on conservative treatment (subgroup Ic) and the healthy control group (group II). This finding agrees with Nolin et al.[15] who stated that hyper-homocysteinemia is a consistent finding in uremic patients due to reduction in nephron mass as tubular cells play an important role in homocysteine metabolism through re-methylation and trans-sulfuration. In fact no statistically significant difference was recorded regarding homocysteine between ESRD patients with CVD and those without CVD. This can be attributed to that both subgroups have folate or cobolamin deficiency that usually occurs in malnourished hemodialysis patients and leads to hyper-homocysteinemia.[16]

Interestingly, fetuin-A shows a significant decrease in ESRD patients with CVD compared to ESRD patients without CVD, CKD patients on conservative treatment and the healthy control group. This result was in accordance to *Stenvinkel et al.*[17]; *Ciaccio et al.*[9] *and Cottone et al.*[18] These researchers explained that ESRD patients who clinically manifest CVD have more evidence of inflammation, more vascular calcification and carotid plaques reflected by significant elevated levels of hs-CRP and other inflammatory mediators. These inflammatory mediators down regulate fetuin-A hepatic messanger RNA decreasing fetuin-A level that acts as a negative acute phase reactant.

Meanwhile fetuin-A shows a significant positive correlation with diastolic blood pressure in ESRD patients with CVD. This could be explained by arterial wall stiffness and loss of arterial compliance related to medial calcification (arteriosclerosis) attributed to low fetuin-A level. This results in increased systolic, decreased diastolic blood pressure and increased pulse pressure that have been found to be independent risk factors for morbidity in hemodialysis patients with CVD.[19]

Interstingly, negative correlation was found between fetuin-A and hs-CRP in ESRD patients collectively. These results were in accordance to *Oikawa et al.*[20] *and Roos et al.*[14] who reported that hs-CRP was negatively correlated with fetuin-A whose level goes down with inflammation as a negative acute phase reactant. Also a negative correlation between fetuin-A and homocysteine was recorded in ESRD patients collectively in which hyperhomocysteinemia were frequently present in association with progression of atherosclerotic CVD.[16]

In accordance with our results, are the studies done by *Ix et al.*[21]; *Jakub et al.*[12] *and Cottone et al.*[18] who found no correlation between fetuin-A, lipid profile in all patients' groups.

Concerning the diagnostic performance of serum hs-CRP (mg/dL), homocysteine and fetuin-A (g/L) (mmol/L) in ESRD patients with CVD versus the subgroup of ESRD patients without CVD, hs-CRP had a superior diagnostic performance in comparison to other two markers with a higher diagnostic specificity of 100.0%, and diagnostic efficacy of 86.7%, respectively, but lower diagnostic sensitivity of 73.3%.

However, an increment in performance was achieved through the combined use of homocysteine or fetuin-A alternatively with hs-CRP. The best ROC cutoffs was 48.23 (mmol/L) for homocysteine, 0.26 (g/L) for fetuin-A and 10 (mg/dL) for hs-CRP. These obviously had a better diagnostic sensitivity of 93.3%, specificity of 100%, and

diagnostic efficacy of 96.7% and AUC of 0.90. These results raise the possibility of combined use of different markers for better discrimination of hemodialysis patients with cardiovascular disease.

The result of this study confirm that serum fetuin-A is a biomarker for identification of ESRD patients who are at high risk of cardiovascular disease, the sustained decrease of which might account for accelerated atherogenesis and increased risk of mortality, and that the effectiveness of serum fetuin-A as a biomarker for cardiovascular disease in hemodialysis patients is increased when it is employed in combination with other proven biomarkers, such as hs-CRP and homocysteine.

Future research studies are needed to evaluate the potential clinical utility of fetuin-A as a novel therapeutic agent to prevent ectopic calcification accompanying various diseases.

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