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Research Article

Association between Low Serum Fetuin A, Ischaemic Heart and Early Chronic Kidney Disease

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Abstract

Introduction; Stiffness of vessel walls caused by calcification is very important in the pathogenesis of organ damage, overall morbidity and mortality. In coronary arteries, the presence of numerous calcium deposits in the vessel wall restricts the diastole and makes the probability of coronary incidents high. Vascular calcification (VC) was in the past considered to be a passive degenerative process. Recently, intense research is being conducted into intracellular interactions and molecular processes underlying arterial calcification. Fetuin-A is a circulating calcium-binding glycoprotein. Its serum concentration was found to be inversely associated with mitral annular calcification in coronary heart disease and inversely associated with aortic sclerosis. In the context of prior studies, these data are consistent with the hypothesis that Fetuin-A may function as an important inhibitor of dystrophic valvular calcification. Further studies were required to evaluate whether Fetuin-A is associated with dystrophic calcification among other vascular tissues including coronary calcifications. ***The aim of this work;*** was to determine the level of fetuin A in patients with ischemic heart disease and to find out any possible correlation between its level and evidence of coronary calcification. ***Patients & methods;*** The study was conducted on 30 non diabetic patients with normal serum creatinine (Group A) complaining of chronic stable angina randomly selected from patients attended the clinic for elective cardiac catheteralization, and a control group (B) matching group A in age, sex & body mass index. Group A patients were subjected to resting ECG, coronary angiography, kidney function test, serum calcium and phosphorus levels, Lipid profile, Liver function tests: AST, ALT, PT, serum albumin, bone alkaline phosphatase ALP, random blood sugar. Serum Fetuin-A level using ELISA was done for both groups. ***Results***; patients and control were matched in age, gender and BMI. Fetuin A level was lower among patients with CAD with significant difference. According to the coronary angiography, patients were subdivided into 3 subgroups i. patients with coronary calcification 19 (63.3%) , ii. patients with no coronary calcification yet diseased vessels 5 (16.7%) , iii. patients with no calcifications and normal coronaries 6 (20%). Fetuin A level was lower among patients with CAD than healthy people. Fetuin-A was significantly lower among patients with coronary calcifications in comparison to those without. Serum creatinine was highest among patients with coronary calcifications. ***Conclusion***; low serum Fetuin-A level may be considered as a risk factor for coronary artery disease, even before evident coronary calcification and in the absence of abnormal serum creatinine. Vascular calcification with low serum Fetuin-A starts in early stages of chronic kidney disease before the rise of serum creatinine which may indicate a causal relationship rather than uremia associated consumption of fetuin A.

Keywords: ischemic heart disease, CKD, CAD, vascular calcification, Fetuin A.

Introduction

Vascular calcification (VC) was in the past considered to be a passive degenerative process involving advanced atherosclerotic lesions. Vascular calcification risk factors are similar to those of atherosclerosis: increased low-density lipoproteins (LDL), decreased high-density lipoproteins (HDL), hypertriglyceridemia, obesity, and hypertension.[1] It has also been shown that diabetes and renal failure contribute significantly to higher risk of accumulation of calcium depositions in the vessel wall .[2]

In coronary vessels, calcification restricts the diastole and changes the physical properties of atherosclerotic plaques. Despite the reduction of classical risk factors, the presence of numerous calcium deposits in the vessel wall makes the probability of coronary incidents high. [3] Stiffness of vessel walls caused by calcification is very important in pathogenesis of organ damage, overall morbidity and mortality .[4]

Recently, intense research is being conducted into intracellular interactions and molecular processes underlying arterial calcification .[5] Initiation of the biomineralization process requires the presence of so-called crystallization nucleators, which trigger the formation of primary crystal nucleus and the removal of mineralization inhibitors such as multipass transmembrane protein transporter (ANK), nucleotide pyrophosphatase (NPPS), matrix Gla protein (MGP) or fetuin-A .[6]

Fetuin-A, a circulating calcium-regulatory glycoprotein that inhibits vascular calcification, is predominantly synthesized in the liver. It is secreted into the blood stream and deposited as a noncollagenous protein in mineralized bones and teeth.[7] Fetuin-A in bone accounts for 25% of the noncollagenous proteins. [8] Fetuin-A, one of the most abundant fetal plasma proteins, was found to be essential for the inhibition of the proinflammatory cytokine tumor necrosis factor production by spermine **&** have been associated with the tolerance of the fetus; "nature's transplant" .[9]

Fetuin-A is a member of type 3 cystatin family together with four structurally related plasma proteins containing cystatin-like protein domains; fetuin-A/α_2-HS glycoprotein, fetuin-B, histidine-rich glycoprotein, and kininogen. Cystatin domain 1 in fetuin-A is strongly negatively charged with a high affinity for calcium rich minerals . Its level declines following infection, acute or chronic inflammatory states and malignancy.[10]

Calcium phosphate crystals induce proinflammatory cytokine secretion through the NLRP3 gene inflammasome in monocytes/macrophages ,[11] cell death in human vascular smooth muscle cells ,[12] and cell activation in chondrocytes .[13] fetuin-A is a potent and specific crystal-bound inhibitor of neutrophil stimulation by hydroxyapatite crystals [14]

Fetuin-A stabilizes supersaturated mineral solutions by forming soluble colloidal nanospheres termed calciprotein particles in analogy to lipoprotein particles. The uptake of fetuin-A by cells and the vesicular recycling of fetuin–mineral complexes reduces both apoptosis and calcification in live cells subjected to elevated extracellular concentrations of mineral ions .[15]

Fetuin-A concentrations was found to be inversely associated with mitral annular calcification among ambulatory persons with coronary heart disease and without severe kidney disease. Fetuin-A concentrations also were inversely associated with AS among participants without diabetes mellitus. In the context of prior studies, these data are consistent with the hypothesis that Fetuin-A may function as an important inhibitor of dystrophic valvular calcification among persons with coronary heart disease and that this function does not require the presence of kidney disease or other traditional cardiovascular risk factors. **Joachim et al, (2011)** concluded that further studies were required to evaluate whether Fetuin-A is associated with dystrophic calcification among other vascular tissues including coronary calcifications.[16] Plasma levels of fetuin-A were independently and directly associated with eGFR in CKD patients, showing a progressive and significant decrease related to decreasing eGFR. A uraemia associated factor like inflammation or oxidative stress may contribute to accelerated atherogenesis.[17] A potential clinical example of fetuin-A consumption may be the calcific uremic arteriolopathy .[18]

The aim of this work

The aim of the present work was to determine the level of fetuin A in patients with ischemic heart disease and to find out any possible correlation between its level and evidence of coronary calcification.

Patients and methods

The study was conducted on 30 patients (Group A) complaining of chronic stable angina, and 15 healthy individuals as a control group (Group B) matching group A in age, sex & body mass index.

Exclusion criteria: Known diabetic patients, smokers, patients with advanced renal failure; chronic kidney disease stage 4 & 5, patients over 60 years.

Group A patients were subjected to full medical history, physical examination, resting ECG and coronary angiography for detection of coronary calcification, number of vessels affected. Laboratory investigations done were serum creatinine, blood urea, serum calcium and phosphorus levels, lipid profile: LDL, HDL, total cholesterol and triglycerides, Liver function tests: AST, ALT, PT, serum albumin, bone Alkaline phosphatise ALP, Random blood sugar, serum Fetuin-A level using ELISA, eGFR (estimated glomerular filtration rate) using Gault Cockroft formula and Modification of Diet in Renal Disease (MDRD) equation was calculated.[19] While only the serum Fetuin A level was done to the control group (Group B).

Gault Cockroft formula

Cr Cl = ((140 - Age) / (Serum Creat)) * (Weight / 72)* 0.85 in females

Modification of Diet in Renal Disease (MDRD) equation

GFR (mL/min/1.73m2) = 186 × (age/years) − 0.203 × (serum creatinine mg/dL) − 1.154 (× 0.742, if female), (×1.21, if African American).

Statistical analysis

Data were collected, revised, verified, edited on P.C., then analyzed statistically using SPSS statistical program for social science for windows version 12.

I- Descriptive analysis:

1. Quantitative variables were expressed as mean, SD and range
2. Qualitative variables were expressed as number and percentage

II- Comparative analysis

1. Chi-square test was used to compare qualitative variables
2. Fisher exact test was used instead of chi-square when one expected cell less than or equal 5.
3. Unpaired t-test was used to compare two groups as regard quantitative variable in parametric data (SD<50% mean)
4. Anova test was used to compare parametric variable among more than 2 groups.

III- **Correlation coefficient test** was used to study the correlation between two variables. Positive correlation means that as one variable increases, the other variable tends to increase and negative correlation means as one variable increases, the other variable tends to decreases.

Results

Table (1): Difference between patients with chronic stable angina (Group A) & the control group (Group B) as regards Age, sex, BMI & Fetuin A level:

	Group A (n=30)	**Control group**	**t**	**P**
Age (years)			0.8	0.4
Min-Max	42-60	30-60		
Mean±SD	52.9±4.65	49.6±20.6		
BMI (kg/m^2)			0.4	0.6
Min-Max	23.1-31.4	23-33		
Mean±SD	27.7±2.3	28±2.3		
Fetuin A			10.4	0.0001**
Min-Max	20-80	20-200		
Mean±SD	37.6±17	100±22.5		
Sex			X	p
Male N (%)	28 (93.3%)	12 (80%)	0.7	0.4
Female N (%)	2 (6.7%)	3 (20%)		

Table 1 shows that there were insignificant differences between the patients group A and the control group as regards the age, BMI and sex. Fetuin A level was significantly higher among the control group.

Table (2): Descriptive data of laboratory results of chronic stable angina patients (Group A) as regards Kidney function tests, Lipid profile, RBS & liver function tests:

	Min-Max	**Mean±SD**
Kidney Function Tests		
Serum creatinine (mg/dl)	0.8-1.3	1.1±0.1
Serum Urea(mg/dl)	18-41	29.7±6.2
eGFR by Cockcroft-Gault (ml/min)	68-115	89.8±2.1
eGFR by MDRD (ml/min)	59.9-94.5	73.2±8.8
Lipid profile		
Serum total Cholesterol (mg/dl)	165-211	193±12.1
TG (mg/dl)	85-122	101.9±9.4
LDL-Cholesterol (mg/dl)	102-166	142.2±14.8
HDL-Cholesterol (mg/dl)	22-41	32.6±5.02
RBS(mg/dl)	75-108	90.7±9.6
Liver function tests		
ALT (IU/l)	9-27	13.4±3.7
AST(IU/l)	10-25	13.8±4
Serum Albumin(mg/dl)	3.8-4.7	4.2±0.3
PT(seconds)	9-16	12.4±1.6
Calcium (mg/dL)	6.5-9.5	8.1±0.7
Phosphorus (mg/dL)	4.5-8.4	6.2±0.9
Bone ALP (IU/L)	25-175	66.7±32.8
Fetuin A (ηg/ml)	20-80	37.6±17

Table (3): History of Calcium intake, ECG, angiogram findings & history of Ca intake among group A

	Number	**%**
Calcium intake		
Yes	1	3.3
No	29	96.7
ECG		
Non specific	19	63.4
Evidence of old infarction	2	6.7
Non Q myocardial ischemia	9	30
Angiogram		
Normal	6	20
1 or more vessel disease	21	70
Mild CAD	1	3.3
Slow flow phenomenon	2	6.7
Calcification	19	63.4

Table (4): Correlations between Fetuin A with other laboratory investigations among group A patients

	r	p
Serum Creatinine	-0.46	0.009*
Urea	-0.28	0.1
eGFR by Cockcroft-Gault (ml/min)	0.46	0.009*
eGFR by MDRD (ml/min)	0.27	0.1
Serum total Cholesterol	-0.05	0.7
TG	0.2	0.2
LDL- Cholesterol	0.06	0.7
HDL- Cholesterol	0.14	0.4
RBS	-0.3	0.06
Serum Phosphorus	-0.12	0.5
Total serum Ca	-0.03	0.8
ALT	0.31	0.09
AST	0.1	0.5
Albumin	0.26	0.1
PT	0.23	0.2
Bone ALP	-0.004	0.9

Table 4 shows that there was positive correlation between Fetwin A & each of the following; creatinine clearance, GFR, TG, LDL, HDL, ALT, AST, Albumin & PT, and negative correlation with serum creatinine, urea, Cholesterol, RBS, phosphorus calcium & bone ALP; being significant only with serum creatinine and creatinine clearance.

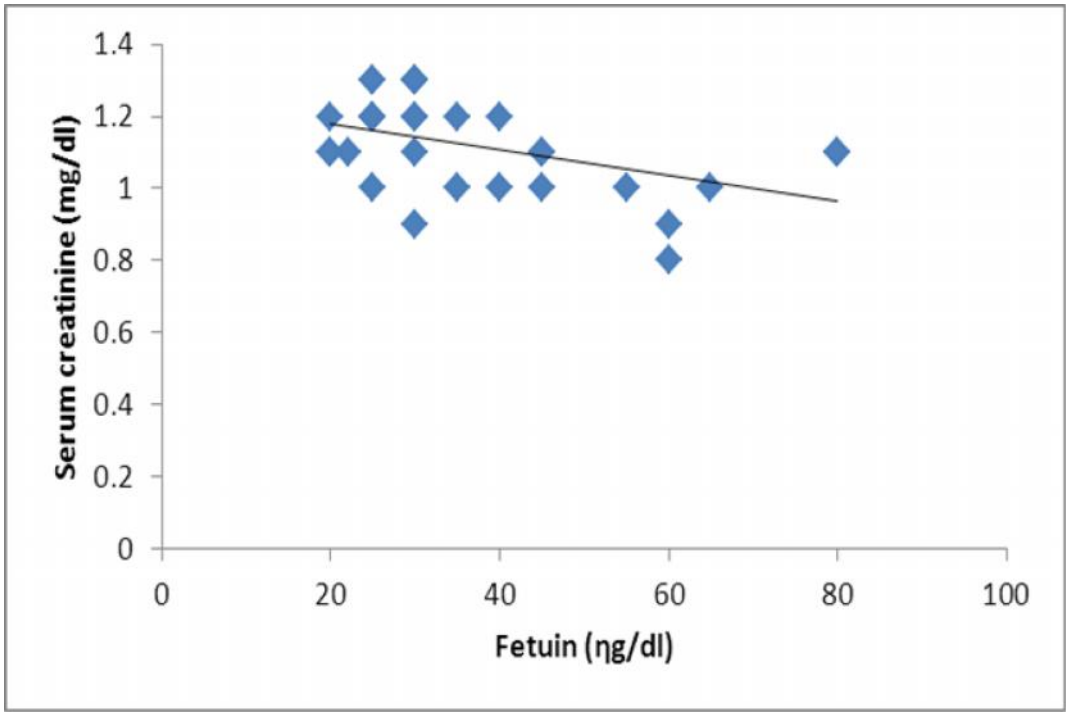

Figure (1): Significant Negative correlation between Fetuin A level and serum creatinine

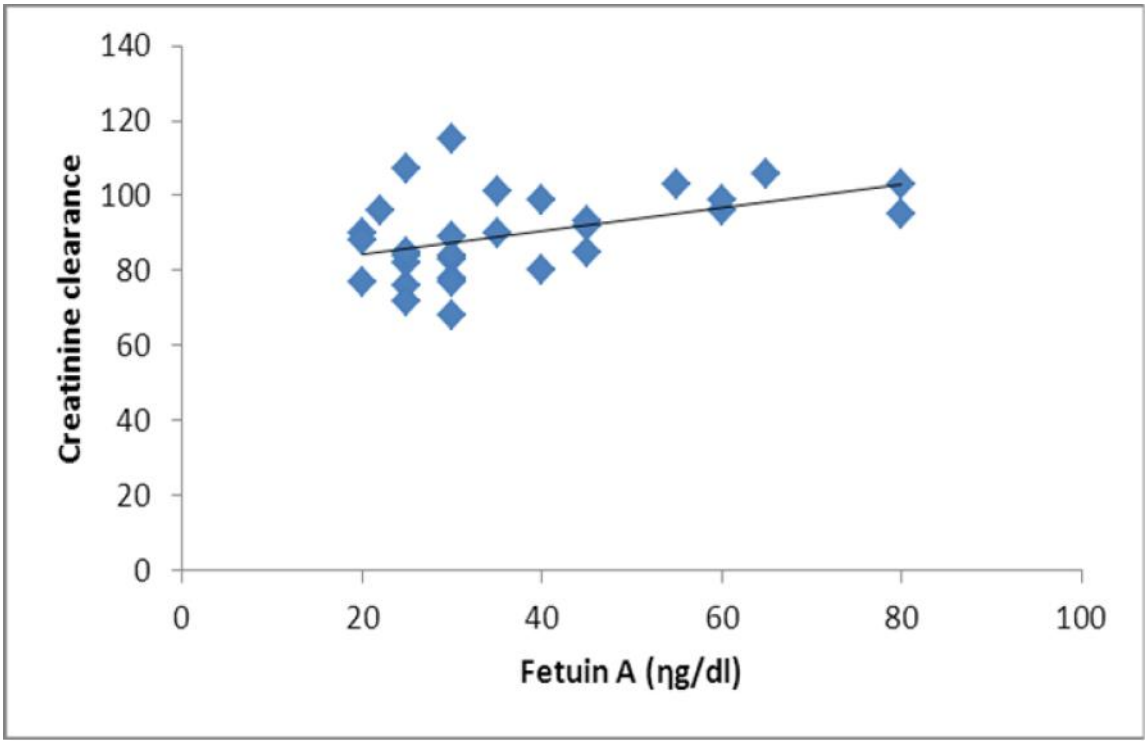

Figure (2): Significant Positive correlation between Fetwin A level and eGFR by Cockcroft-Gault (ml/min)
According to the presence of coronary calcification, group A patients were divided into three subgroups; 19 patients with evidence of calcification and non- calcification group with normal vessels (n=6) and non calcification group with diseased vessels (n=5) according to the angiogram findings.

Table (5): Comparison between patients subgroups with calcification, without Calcification with normal vessels and without calcification with diseased vessels as regards their age, sex and BMI:

	With calcification (n=19)	Non calcification group with normal vessels (n=6)	Non calcification group with diseased vessels (n=5)	F	P
Age (years) Min-Max Mean±SD	 42-60 52.7±4.6	 47-60 53.33±5.81	 48-59 53.4±4.27	0.066	0.9
BMI (kg/m^2) Min-Max Mean±SD	 23-31 27.7±2.3	 24.1-33.1 28.86±3.14	 27-30.4 28.36±1.29	0.602	0.5

Table 5 shows no significant difference between the three subgroups as regards age and BMI (p>0.05).

Table (6): Difference between chronic angina patients with calcification, without Calcification with normal vessels and without calcification with diseased vessels as regards Kidney function tests:

	With calcification (n=19)	with normal vessels (n=6)	Non calcified diseased vessels (n=5)	F	P
Serum creatinine (mg/dl) Min-Max Mean±SD	0.9-1.3 1.16±0.11	0.8-1.1 0.96±0.1	1-1.2 1.1±0.07	8.59	0.001*
Non calcification group with diseased vessels (n=5)	t=1.14 p=0.2	t=2.3 p=0.04*			
Non calcification group with normal vessels (n=6)	t=4.9 p=0.0001*				
Serum Urea (mg/dl) Min-Max Mean±SD	21-41 31.2±5.8	23-38 29.66±5.5	18-36 24.4±6.94	2.603	0.09
eGFR by CG(ml/min) Min-Max Mean±SD	68-115 86.4±11.9	93-106 100±4.89	80-99 90.2±7.66	3.92	0.03*
Non calcification group with diseased vessels (n=5)	t=0.6 p=0.5	t=2.69 p=0.01*			
Non calcification group with normal vessels(n=6)	t=2.57 p=0.02*				
eGFR by MDRD (ml/min) Min-Max Mean±SD	59.9-94.5 71±9.6	67.9-85.1 78.86±6.54	67.4-81.3 74.64±4.98	2.01	0.15

Table 6 shows significant differences between the three subgroups as regards the serum creatinine level and creatinine clearance; serum creatinine was highest among patients with calcification while eGFR by CG was highest among those with normal vessel ($p<0.05$). With a highly significant difference between patients with normal vessels and the calcification group, and was significant between those with normal vessels and those with diseased non calcified vessels.

Table (7): Difference between chronic angina patients with calcification, without Calcification with normal vessels and without calcification with diseased vessels as regards Lipid profile & RBS:

	With calcification (n=19)	with normal vessels (n=6)	Non calcified diseased vessels (n=5)	F	P
Cholesterol (mg/dl) Min-Max Mean±SD	175-211 195.7±10.8	185-205 194.83±7.78	165-196 180.2±14.34	4.09	0.02*
Non calcification group with diseased vessels (n=5)	t=2.6 p=0.01*	t=2.16 p=0.059			
Non calcification group with normal vessels (n=6)	t=0.18 p=0.8				
TG (mg/dl) Min-Max Mean±SD	85-122 99±8.9	124-161 145.16±13.19	102-145 129.2±17.19	43.63	0.00*

LDL- Cholesterol (mg/dl) Min-Max Mean±SD	118-166 144.6±13.6	93-115 106.16±8.4	98-121 107.8±9.23	33.24	0.00*
Non calcification group with diseased vessels (n=5)	t=5.66 p=0.0001*	t=6.48 p=0.0001*			
Non calcification group with normal vessels (n=6)	t=0.3 p=0.7				
HDL- Cholesterol (mg/dl) Min-Max Mean±SD	22-41 31.8±4.7	25-40 31.5±6.34	33-41 36.6±3.04	2.1	0.1
RBS (mg/dl) Min-Max Mean±SD	75-108 92.4±9.9	45-80 60.83±11.58	76-98 87.6±9.71	21.97	0.00*
Non calcification group with diseased vessels (n=5)	t=0.9 p=0.3	t=4.09 p=0.002*			
Non calcification group with normal vessels (n=6)	t=6.55 p=0.0001*				

Table 7 shows significant differences between the three subgroups as regards cholesterol level; being highest among patients with calcifications. With a highly significant difference between the calcification group and the non calcification group with diseased vessel. There were highly significant differences between the three groups as regards the triglyceride level, LDL-cholesterol and RBS levels; being significantly higher among those without calcification ($p<0.01$). Both LDL and FBS were highest among patients with calcifications, meanwhile TG was highest among non calcification group with normal vessels.

Table (8): Difference between chronic angina patients with calcification, without Calcification with normal vessels and without calcification with diseased vessels as regards Liver function tests:

	With calcification (n=19)	Non calcification group with normal vessels (n=6)	Non calcification group with diseased vessels (n=5)	F	P
ALT(IU/l) Min-Max Mean±SD	9-21 12.6±3	10-27 15.66±5.95	10-16 13.8±2.48	1.61	0.21
AST(IU/l) Min-Max Mean±SD	10-24 13.1±3.9	11-25 15.33±5.31	12-18 14.4±2.5	0.77	0.4
Albumin (mg/dl) Min-Max Mean±SD	3.8-4.7 4.2±0.3	4-4.6 4.2±0.27	3.8-4.5 4.18±0.28	0.008	0.9
PT(seconds) Min-Max Mean±SD	10-15 12.2±1.4	10-14 12.5±1.51	9-16 13±2.54	0.48	0.6

Table 7 shows insignificant differences between the three subgroups as regards all the liver function tests ($p>0.05$).

Table (8): Difference between patients with calcification, without Calcification with normal vessels and without calcification with diseased vessels as regards Serum Ca, P, bone ALP, Ca intake & Fetuin A level:

	With calcification (n=19)	Non calcification group with normal vessels (n=6)	Non calcification group with diseased vessels (n=5)	F	P
Calcium (mg/dl) Min-Max Mean±SD	 7-9.5 8±0.6	 6.5-8.8 8.1±0.86	 6.5-9 8.06±1.02	0.04	0.9
Phosphorus (mg/dl) Min-Max Mean±SD	 5.3-7.7 6.2±0.8	 4.5-8.4 5.88±1.33	 4.9-7.5 6.22±1.21	0.26	0.7
Bone ALP (IU/l) Min-Max Mean±SD	 25-175 70.5±36.9	 35-85 59.16±17.44	 25-100 61±33.05	0.34	0.7
Fetuin A (ηg/dl) Min-Max Mean±SD	 20-35 26.9±4.6	 45-80 60.83±11.58	 40-80 50±16.95	37.92	0.00**
Non calcification group with diseased vessels (n=5)	t=5.5 p=0.0001*	t=1.25 p=0.2			
Non calcification group with normal vessels (n=6)	t=10.7 p=0.0001*				
Calcium intake (n) Yes No	 0 19	 1 5	 0 5	χ^2 4.14	P 0.12

Table 8 shows insignificant differences between the three subgroups as regards calcium, phosphorus levels, bone ALP level and the history of calcium intake ($p>0.05$). Meanwhile there were significant difference between the three groups as regards the serum Fetuin A level; being highest among patients without calcification with normal vessels, and lowest among the calcification group with a higly significant difference. However there was no significant difference between the non calcification with normal vessels & with diseased vessels groups regarding the Fetuin A.

Table (9): Fetuin A level differences among all studied groups and subgroups:

	Group B (n=15)	With calcification (n=19)	Non calcification group with normal vessels (n=6)	Non calcification group with diseased vessels (n=5)	F	p
Fetuin A Min-Max Mean±SD	 20-200 100±22.5	 20-35 26.9±4.6	 45-80 60.83±11.58	 40-80 50±16.95	29.87	0.00**

This table shows highly significant differences among groups and subgroups as regards Fetuin A level, being highest among the control group and lowest among the calcification group

. Table (10): Correlations of Fetuin A among non calcification group

	r	p
Creatinine	-0.11	0.7
Urea	-0.1	0.7
Creatinine clearance	0.56	0.07
Cholesterol	0.4	0.2
TG	0.3	0.2
LDL	0.3	0.3
HDL	-0.2	0.4
RBS	-0.57	0.06
P	-0.4	0.9
Ca	-0.1	0.7
ALT	0.2	0.3
AST	0.03	0.9
Albumin	0.4	0.1
PT	0.16	0.6
Bone ALP	0.42	0.18

Among the non calcification group, there was a negative correlation between Fetuin A & each of serum creatinine, urea, HDL, Ca & P level, but all the correlations were insignificant ($p>0.05$).

Table (11): Correlations of Fetuin A among calcification group

	r	p
Creatinine	-0.003	0.9
Urea	0.03	0.8
Creatinine clearance	0.12	0.6
Cholesterol	0.47	0.04*
TG	-0.24	0.3
LDL	0.08	0.7
HDL	0.39	0.09
RBS	0.09	0.7
P	-0.16	0.5
Ca	-0.17	0.4
ALT	-0.26	0.2
AST	-0.56	0.013*
Albumin	0.47	0.03*
PT	0.26	0.2
Bone ALP	0.17	0.4

There were significant positive correlation between Fetuin A level and each of albumin and cholesterol levels and significant negative correlation between fetuin A and AST.

Table (12): ALP level differences among all studied groups and subgroups:

	Group B (n=15)	With calcification (n=19)	normal vessels (n=6)	Non calcified diseased vessels(n=5)	F	p
Bone ALP Min-Max Mean±SD	12.1-42.7 20.5±11.3	25-175 70.5±36.9	35-85 59.16±17.4	25-100 61±33.05	9.37	0.000
With calcification (n=19)	t=5.04 p=0.0001*					
normal vessels (n=6)	t=5.79 p=0.0001*					
Non calcified diseased vessels (n=5)	t=4.2 p=0.0005*					

This table shows highly significant differences among groups as regards bone ALP level, being highest among patients with calcification group and lowest among control group. There is significant difference was more noticed between the control group and the three subgroups.

Discussion

Coronary artery disease (CAD) due to atherosclerosis represents a major health care problem. Atherosclerosis is regarded as chronic inflammatory condition of the vascular wall that is converted to an acute clinical event by the induction of plaque rupture, which in turn leads to thrombosis. The early lesions of atherosclerosis consist of subendothelial accumulation of cholesterol-engorged macrophages, called 'foam cells' .[20]

Vascular calcification is an important manifestation of atherosclerosis. Cardiovascular calcification refers to pathological calcium phosphate deposition in the blood vessels, myocardium, and cardiac valves similar to the hydroxyapatite found in bone.[21]

Fetuin A is a member of the cystatin superfamily of cysteine protease inhibitors, originally discovered as the major component of fetal bovine serum. It is a carrier for growth factors, binds to and inactivates transforming growth factor (TGF)–β and bone morphogenic protein, and is a major component of mineralized bone[22]. Fetuin-A is also an acute-phase glycoprotein, produced in adults primarily in the liver, it interacts with calcium and phosphorus, increasing their solubility and inhibiting precipitation[23].

Several investigators have suggested that Fetuin-A exerts a protective effect against ischemia in cardiomyocytes [24], and a recent experimental study demonstrated that fetuin-A infusion may be used as a therapy to reduce ischemia-related lesions in a stroke model [25].

It's to be noted that studies concerning Fetuin-A were generally limited to patients with impaired renal function. The aim of this study was to determine the level of Fetuin-A in patients with ischaemic heart disease & to find out any possible correlation between its level & evidence of coronary calcification.

This study was conducted on 30 patients (Group A) complaining of chronic stable angina & 15 healthy age, gender & BMI matched individuals as a control group (Group B). Fetuin A level was lower among patients with CAD with highly significant difference.
Furthermore patients group were subdivided according to the coronary angiography into 3 subgroups i. patients with coronary calcification 19 (63.4%), ii. patients with no coronary calcification yet diseased vessels 5 (16.6%), iii. patients with no calcifications and normal coronaries 6 (20%). Our study was also concordant with ***Uz et al. (2009)*** who studied 64 consecutive patients who underwent MSCT for suspected coronary artery disease. They found that coronary calcification was detected in 32 patients (50%).[26]

Comparison between subgroups regarding the kidney function tests were significant regarding serum creatinine; being highest among patients with coronary calcifications (1.16±0.11 vs. 1.02±0.11 mg/dl, t=3.35 p<0.05), eGFR by CG; being higher among those without calcifications and normal coronaries than those with diseased vessels and both sub groups were higher than those patients with coronary calcification (100±4.89 vs. 90.2±7.66 vs. 86.4±11.9) ml/min, F = 3.92 p = 0.03), meanwhile insignificant differences were found between subgroups as regards serum urea level and eGFR by MDRD. No previous studies were done estimating the serum fetuin A level in coronary artery disease patient with normal serum creatinine in very early chronic kidney disease stages 1 & 2 where eGFR > 60 ml/min.

Significant differences were found as shown in table 7 between the three subgroups as regards cholesterol level; being highest among patients with calcifications. There were highly significant differences between the three groups as regards the triglyceride level, LDL and RBS levels; being significantly higher among those without calcification (p<0.01). Both LDL and FBS were highest among patients with calcifications, meanwhile TG was highest among non calcification group with normal vessels.

But as regard liver function tests table 8 shows insignificant differences between the three subgroups as regards all the liver function tests (p>0.05).

In our study, studying the correlations of Fetuin A level among group A showed significant negative correlation with the serum creatinine (r=-0.46, p<0.05) and significant positive correlation with eGFR by Cockcroft-Gault (r=0.46, p<0.05). Meanwhile there were insignificant correlations between Fetuin A level and each of urea, eGFR by MDRD (ml/min), serum cholesterol, TG, LDL-cholesterol, HDL-cholesterol, RBS, serum phosphorus, total serum calcium, ALT, AST, albumin, PT and Bone ALP. as shown in table 4.

Also in this study, the mean Fetuin A level among patients group was 37.6±17ηg/mL. This agrees with *Ix et al. (2012)* who studied the association of fetuin-A with coronary arterial calcification (CAC) among 2457 community-living individuals without cardiovascular disease (CVD) and evaluated the association of fetuin-A with CAC incidence and progression. They found that the mean fetuin-A concentration was 48±0.10 ηg /mL. Twelve hundred participants (49%) had prevalent CAC at the baseline examination.[27]

Also our results were similar to ***Roos et al. (2010)*** who conducted their study to define the role of Fetuin-A as marker for micro- and macrovascular disease in a high risk population of patients with type 2 diabetes mellitus and early diabetic nephropathy. They found insignificant correlations between Fetuin A level and each of FBS, serum creatinine and serum albumin.[28]

Cardiovascular risk factors like obesity, hypertension, hypercholesterolaemia, diabetes and smoking are associated with atherosclerosis at different sites and with an increased risk of coronary heart disease. [3] Several population-based studies have investigated the association between cardiovascular risk factors and coronary calcification. In asymptomatic adults, these cardiovascular risk factors were strongly associated with the amount of coronary calcification[29]. Also *Oei et al. (2002)* found that age and male sex are the most important risk factors for coronary calcification. Cardiovascular risk factors assessed 7 years before EBT (electron-beam tomography) scanning were strongly associated with coronary calcification. Associations of blood pressure and cholesterol with the calcium score attenuated when risk factors were measured concurrently to EBT scanning. Although cardiovascular risk factors are strongly associated with the amount of coronary calcification in asymptomatic subjects, almost 30% of the men and 15% of the women without cardiovascular risk factors have extensive coronary calcification.[30]

In this study, calcium and phosphorus levels showed insignificant differences between both subgroups; patients with and without calcifications. Also there was insignificant difference between both subgroups as regards bone ALP level and history of calcium intake. These are concordant with *Stenvinkel et al. (2005)* who found insignificant differences between patients with CVD (n=90) and those without (n=168) as regards serum calcium (2.53±0.02 vs. 2.56±0.03) and phosphate levels (1.96±0.05 vs. 1.79±0.07).[7]

On the other hand in our study, Fetuin A level was significantly lower among patients with calcifications (p<0.05). This agree with *Katsuhito et al. (2012)* investigated the association between fetuin-A and calcified coronary artery disease in 92 participants without diabetes nor renal dysfunction and found that fetuin-A levels were significantly lower in patients

with coronary artery calcification compared with those without coronary artery calcification (257.1±49.7, 288.0±63.1 μg/ml, respectively; $P = 0.010$).[31]

Also these results agrees with *Mohty et al. (2010)* who concluded from their study that reduced level of Fetuin A was associated with enhanced valvular calcification and faster stenosis progression rate among elderly patients. Also *Baun et al. (2012)* concluded that a high concentration of Fetuin-A correlated with a lack of progress in coronary calcification. In that study Fetuin-A was identified as an antiinflammatory biomarker decelerating coronary artery calcification. [32]

Conclusion

low serum Fetuin-A level may be considered as a risk factor for coronary artery disease, even before evident coronary calcification and in the absence of abnormal serum creatinine. Vascular calcification with low serum Fetuin-A starts in early stages of chronic kidney disease before the rise of serum creatinine which may indicate a causal relationship rather than uremia associated consumption of fetuin A.

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