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Serum Fetuin–A Level Association with Coronary Artery Disease

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ABSTRACT

Objective: *End stage renal disease is commonly associated with vascular calcium deposition, and its relation to cardiovascular risk factors and fetuin –A levels thoroughly studied. The aim of this present study is to assess the direct association between serum fetuin-A level and coronary artery disease (CAD) in patients with no renal impairment.*

Patients and Methods: *A total of 54 patients constituted the study sample (29 males and 25 females). Full history and clinical examination, transthoracic echocardiography, coronary angiography, and biochemical investigations were performed to all patients. Serum fetuin-A level was measured using EDI (Epitope Diagnostics, Inc.) human fetuin-A ELISA (Enzyme-linked immunosorbent assay) kit. A Low level of serum fetuin-A is defined as <0.59 g/L. All patients with renal impairment or valvular heart disease were excluded.*

Results: *Out of 54 patients included in the study 35 (64.8%) were diagnosed with CAD. Low fetuin-A level was significantly associated with CAD (Mann-Whitney U= 151.5, p=0.001) with an adjusted odds ratio of 12.4 (95% CI: 2.3-68.6) for having low level of fetuin-A versus normal level. Also, a significant correlation was noted between fetuin-A level and serum triglycerides (r= 0.3, p= 0.034), high density lipoprotein (HDL) (r=0.3, p= 0.038) and low-density lipoprotein (LDL) (r= -0.416, p= 0.002).*

Conclusion: *Patients with low serum levels of fetuin-A were associated with higher risk of developing CAD. Whether lowered serum fetuin-A is a risk factor or an outcome for CAD, it remains to be investigated.*

Keywords

Fetuin-A, Coronary artery disease, Cardiovascular risk factors.

Summary

Our research article objective was to assess the direct association between serum fetuin-A level and coronary artery disease (CAD) in patients with no renal impairment, considering that this assessment has not been thoroughly studied before for such group of patients. Fetuin-A has been identified as a potent circulating inhibitor of calcification. Transthoracic echocardiography, coronary angiography, and biochemical investigations were performed to all patients. Our series shows that patients with low serum levels of fetuin-A were associated with higher risk of developing CAD. Moreover, a novel finding in this study is that increasingly higher prevalence rates of low serum fetuin-A were observed as the number of affected coronary vessels increased documented by coronary angiography.

Introduction

Atherosclerosis is the corner stone in developing acute coronary events. The understanding of its pathogenesis has been rapidly evolving, and includes the study of factors that either promote or inhibit calcium deposition in the systemic vasculature [1]. Accelerated atherogenesis has been linked to both vascular calcification and inflammation [2,3]. This calcification process is an active cellular process, and the burden of calcified atherosclerotic plaque is an important predictor of subsequent cardiovascular events [4] and coronary artery disease [1].

Fetuin – A (alpha-2-Heremans Schmid glycoprotein {AHSG}) is a protein produced by the liver. It is secreted into serum in high concentrations (0.5 – 100 g/L) during fetal life [5]. Fetuin-A has been identified as a potent circulating inhibitor of calcification, and accounts for roughly 50% inhibition of ectopic calcification that is a frequent complication of many degenerative diseases [6].

Fetuin-A is highly effective in the formation and stabilization of protein-mineral colloid, referred to as calciprotein particles (CPP). Albumin and acidic protein in general greatly enhance the fetuin-A triggered formation of secondary CPPs and this substituted substantial amounts of fetuin-A without loss of inhibition of calcium phosphate precipitation [7]. Moreover, fetuin – A reacts as a negative acute phase protein, thus, down-regulation may occur in acute and chronic inflammatory status [8].

Several studies involving patients with end stage, renal disease have shown an association between fetuin-A concentrations and vascular calcification [9-11].

Another study was done by Kaden et al. [12] on aortic stenosis and found that serum fetuin-A levels were lower in patients with calcific aortic stenosis; further more specific staining of fetuin-A was found in stenotic valves but not in healthy control valves.

Merx et al. [13] concluded that independent of arterial stiffness, calcification is associated with myocardial stiffness characterized by cardiac fibrosis, diastolic dysfunction, impaired tolerance to ischemia, and catecholamine resistance, thus, it may constitute an underestimated cardiovascular risk factor that contributes to cardiac failure in calcification prone state.

However, to the best of our knowledge, what is known about the physiological significance of fetuin – A regarding atherosclerosis in other conditions, rather than chronic kidney disease, is still limited to some degree. Furthermore, the association between low levels of fetuin-A and coronary calcification with or without plaque disruption remains a speculation [14].

This study aims to investigate the association between serum fetuin–A level and coronary artery disease in patients with no renal impairment.

Subjects & Methods

The research followed a consecutive study sample. Patients presented to our hospital during the period from January to July 2017 were assessed for eligibility to be included into the study.

A total of 82 patients (after their approval to be involved in our study) were recruited. They underwent full history taking, thorough clinical examination, electrocardiogram (ECG), resting trans-thoracic echocardiogram, coronary angiography, and biochemical investigations.

Exclusion criteria comprised age >60 years and patients with renal impairment, cardio myopathy, or valvular heart disease. Consequently, 28 patients were excluded, and a total of 54 patients constituted the study sample (35 with CAD and 19 without). Their ages ranged from 47 to 59 years.

Serum fetuin-A level was measured using EDI human fetuin-A ELISA kit. According to Ix et al. [15], a cut off of 0.59 g/L was considered. below which low levels of serum fetuin-A was diagnosed.

All study participants underwent trans-thoracic Echocardiographic (TTE), TTE was performed for every patient using GE Vivid E9 set (part no GA 091568, Norway 2010 – Chicago, Illinois, United States by GE Healthcare) using 5 MHz transducer. Images were taken while the patient was supine or in left lateral position, utilizing two-dimensional (2D), M-mode and Doppler echocardiographic techniques. The entire echocardiographs were performed according to the recommendation of American Society of Echocardiography [16].

Coronary Angiography

Invasive coronary angiography ICA was performed by interventional cardiologist in the cardiac cath-lab using the Judkins approach via the femoral artery acquiring standardized projections [17]. Coronary stenosis was analyzed using a well validated commercially available software package (Xcelera, Phillips Xper FD, and Phillips Medical Systems Nederland B.V the Netherlands). ICA images where analyzed using a dedicated software (Xcelera Cardiology information management system). Visual estimation was applied and the qualitative analysis was based on the angiographic projection showing the most severe narrowing. A coronary stenosis was defined as significant based on visual inspection when the degree of stenosis was more than 50% [18]. Analysis of coronary artery lesions was performed by interventional cardiologist. Each coronary artery was visualized by multiple projections and assessed for diameter reduction.

Biochemical investigations

Fasting 7 ml venous blood sample was withdrawn from patients without venous stasis into plain tube and serum separated by centrifugation (850-1500Xg for 10 min.). Serum samples were stored frozen at -200C to measure fetuin-A, cardiophase hsCRP using BN system (Dade Behring, USA), total cholesterol, high density lipoproteins, cholesterol, and triglycerides levels using Daimension RXL (Dade Behring USA). Low-density lipoprotein cholesterol (LDL-C) concentrations were estimated by the Friedewald equation [19].

Serum fetuin-A was measured by using EDI human fetuin-A ELISA kit (Epitope Diagnostic, Inc., USA). The assay utilizes the two-site sandwich technique with two selected goat antihuman fetuin-A polyclonal antibodies that bind to different epitopes of human fetuin-A. The intra- and inter-assay coefficients of variation are 4.5% and 5.7%, respectively. The assay range 0.05- 3.5 g/L. (20)

Statistical analysis

Collected data were verified and coded prior to computerized data entry. The statistical package for social sciences (SPSS version, 25) was used for data analysis. Descriptive statistics and significance testing using the appropriate test statistics were applied. A statistically significant level was considered at $p<0.05$.

Results

Table 1 shows that coronary artery disease was diagnosed in 35

patients out of 54 (64.8%). The sample had 25 (46.3%) males and 29 (53.7%) females. Single vessel coronary artery disease was present among 8 (22.9%) of patients diagnosed with CAD; two vessel diseases was present among 13 (37.1%), while triple vessel coronary artery disease was present among 14 (40%). 32 patients (59.3%) were diabetic, while 24 (44.4%) were hypertensive.

Variable		No CAD (n=19)		CAD (n=35)		p-value
		No.	%	No.	%	
Age (median, IQR)		50	48-55	52	49-54	p= 0.344
Fetuin A (median, IQR)		0.88	0.59-1.02	0.56	0.51-0.59	p=0.001
Fetuin A	Normal	14	63.3	8	36.4	X^2= 13.177
	Low	5	15.6	27	84.4	p<0.001
Gender	Female	7	24.1	22	75.9	X^2= 3.352
	Male	12	48.0	13	52.0	p= 0.067
Diabetes mellitus	No	15	68.2	7	31.8	X^2= 17.724
	Yes	4	12.5	28	87.5	p<0.001
Hypertension	No	17	56.7	13	43.3	X^2= 13.658
	Yes	2	8.3	22	91.7	p<0.001
Affected coronary vessels	One	NA		8	22.9	NA
	Two			13	37.1	
	Three			14	40	

Table 1: Characteristics of patients included in the study.
IQR: Interquartile range.

The univariate analysis showed statistically significant difference between patients diagnosed with CAD and those without in fetuin-A level (Mann-Whitney U= 151.5, p=0.001), with CAD patients showing lower fetuin-A levels. Diabetes mellitus (X2=17.724, p<0.001) and hypertension (X2=13.658, p<0.001) also showed statistically significant differences. No significant difference was found regarding serum cholesterol level (p=0.183), triglycerides (p=0.11), HDL (p=0.077) and LDL (p=0.611) as shown in table (2).

Variable	No CAD (n=19)		CAD (n=35)		p-value*
	Median	IQR	Median	IQR	
Cholesterol (mg/dl)	208	170-217	199	188-210	p= 0.183
TG (mg/dl)	185	168-195	172	168-181	p=0.110
LDL (mg/dl)	117	110-130	120	105-135	p=0.611
HDL (mg/dl)	48	45-50	45	39-50	p=0.077

Table 2: lipid profile of patients included in the study.
IQR: Interquartile range, TG: Triglycerides, LDL: Low density lipoprotein, HDL: High density lipoprotein. *Mann-Whitney U test.

However, a significant correlation was noted between fetuin-A level and serum triglycerides (r= 0.3, p= 0.034), HDL (r=0.3, p= 0.038) and LDL (r= -0.416, p= 0.002).

Subsequent multivariate logistic regression analysis selected only fetuin-A level (B=2.521, p=0.004) and diabetes (B=2.953, p=0.001) to be significant independent predictors of CAD after adjusting for other variables with adjusted odds ratios of 12.4 (95% CI: 2.3-68.6) and 19.2 (95% CI: 3.5-106.3) respectively.

Discussion

Prior studies have addressed that calcifying atherosclerosis is an active process which is controlled by calcification inhibitors and inducers [21,22], it has already been established that the magnitude of vascular calcification is associated with increased vascular mortality [23]. Fetuin-A, a glycoprotein synthesized by hepatocytes with a molecular weight of about 60 Kda, is the most powerful circulating calcification inhibitor of hydroxyapatite formation till date. Fetuin-A reacts as a negative acute phase protein; thus, down regulation may occur in acute and chronic inflammatory states [16].

In End stage renal disease patient's serum fetuin-A concentration was associated with increased coronary arterial and vascular calcification scores [24]. Several studies have been published regarding the relationship between fetuin-A concentration and cardiovascular mortality in dialysis patients [25,26] and renal transplant recipients. All studies established that a lower serum fetuin-A was associated with increased mortality. However, it's still not clear whether fetuin-A deficiency should be regarded as inflammation dependent or independent risk predictor, as results vary in this context.

In our study we focused on the association of serum fetuin-A with coronary artery disease (as confirmed by coronary angiography) in patients with no real impairment.

This study revealed that serum fetuin-A levels were not significantly different according to personal characteristics of patients (i.e., age or gender), which is not in accordance with that reported by Joachim et al. [29], who demonstrated that patients with higher fetuin-A concentrations were younger and more frequently women. Furthermore, Zheng et al. [27] found no association between age and fetuin- A level, while Wigger et al. [28] did his study on healthy children and concluded that fetuin-A concentration is independent of age and gender.

In our study, we found out that there were significantly low levels of serum fetuin-A among diabetics, hypertensive, and ischemic heart disease patients. These findings are in agreement with Aroner et al and Keskin et al. [30,31]. Lim et al. [32], concluded that low concentrations of the anti-inflammatory mediator "fetuin–A" are strongly associated with death within 6 months in patients with ST segment elevation myocardial infarction, which further supports our results regarding the association between low fetuin-A and significant affecting the coronary vessels and leading to subsequent coronary syndrome. Moreover, a novel finding in this study is that increasingly higher prevalence rates of low serum fetuin-A were observed as the number of affected coronary vessels increased documented by coronary angiography. Our results are compatible to some extent with Mori et al. [33], who conducted a study to establish the relation between fetuin –A and calcified CAD in 92 patients without diabetes or renal dysfunction. He concluded that fetuin –A levels are inversely correlated with advanced calcified

coronary artery disease and it decreased significantly in patients with severe three vessel coronary artery disease, compared to those without stenosis (24.5 ± 50.9, 289.9 ± 71.8 µg /ml) respectively ($p < 0.05$).

The present study finding is in accordance with Ix et al. [10], who concluded that fetuin-A may be an important inhibitor of dystrophic calcification in persons with coronary heart disease and low fetuin-A level might be associated with significant coronary artery disease. Stenvinkel et al. and Chen X et al. [2,34] found that a low fetuin-A level is associated with malnutrition, inflammation, and atherosclerosis (carotid plaques), as well as with increased cardiovascular and all-cause mortality. Furthermore, Bilgir et al, Basar N et al, and Ix JH1 [35-37] demonstrated that serum fetuin-A levels decreased instable angina and myocardial infarction patients. Additionally, it might play a role in the pathophysiology of Coronary Artery Disease.

However, in contrast to our findings, Weikert et al. [38] suggested a link between high plasma fetuin-A levels and an increase risk of both myocardial infarction and ischemic stroke. Our explanation for discrepancies across studies may be the difference in study sample selection or variation in the assays used for the measurement of fetuin –A.

Our results regarding correlation between fetuin-A level and serum triglycerides HDL, and LDL showed a significant correlation ($r= 0.3$, $p= 0.034$), ($r=0.3$, $p= 0.038$), ($r= -0.416$, $p= 0.002$) respectively. This is compatible with findings of Mehrotra et al. [39]. They demonstrated a significant direct relationship between serum fetuin-A and serum triglycerides ($r = 0.27$, $P = 0.01$), also with Joachim et al. [5] concluding that higher fetuin-A levels were strongly and independently associated with higher low-density lipoprotein, non-high-density lipoprotein (HDL), and triglyceride concentration.

The present study owns some notable strengths. Firstly, during selection of our study sample we excluded patients with any degree of renal impairment owing to established association with low fetuin –A level. Secondly, in our results increasingly higher prevalence rates of low serum fetuin-A were observed as the number of affected coronary vessels increased such flinging to best of our knowledge not elaborated by others before.

Conclusion

In conclusion, Patients with low serum levels of fetuin-A are at a higher risk of developing CAD. The severity and number of coronary vessels affected are inversely proportional with serum fetuin-A levels. However, further studies with large sample size are needed to better understand whether low serum fetuin-A is a risk factor or an outcome for CAD. Nevertheless, we admit that our study sample appears relatively limited.

Abbreviations

CAD: Coronary Artery Disease; EDI: Epitope Diagnostics, Inc.; ELISA: Enzyme-linked Immunosorbent Assay; HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein; AHSG: Fetuin – A Alpha-2-Heremans Schmid glycoprotein; CPP: Calciprotein Particles; ECG: Electrocardiogram; TTE: Echocardiographic; 2D: Two-dimensional; SPSS version, 25: Statistical package for social sciences; IQR: Interquartile Range.

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