ORIGINAL RESEARCH

Cigdem U. Afsar, MD¹
Hafize Uzun Professor, PhD²
Selen Yurdakul, MD²
Cuneyt Muderrisoğlu MD¹
Mecdi Ergüney, MD¹
Bulent Demir, MD³
Aram Aslan, MD⁴
Hale Aral, MD⁴
Sibel Özyazgan, PhD⁵

¹Coronary Intensive Care Unit, Ministry of Health İstanbul Education and Research Hospital, İstanbul, Turkey

²Department of Biochemistry, Cerrahpaşa Faculty of Medicine, İstanbul University, İstanbul, Turkey

³Department of Cardiology, Dr Sadi Konuk Education and Research Hospital, İstanbul, Turkey.

⁴Department of Biochemistry, Ministry of Health İstanbul Education and Research Hospital, İstanbul, Turkey

⁵Department of Pharmacology and Clinical Pharmacology, Cerrahpaşa Faculty of Medicine, İstanbul University, İstanbul, Turkey

Association of serum fetuin-A levels with heart valve calcification and other biomarkers of inflammation among persons with acute coronary syndrome

Abstract

Purpose: Fetuin-A is a multifunctional hepatic secretory protein that inhibits dystrophic vascular and valvular calcification. Our aim was to evaluate the relationship among fetuin-A levels, heart valve calcification and other biomarkers of inflammation in patients with acute coronary syndrome (ACS).

Methods: The associations among serum fetuin-A concentrations, mitral annular (MAC) and aortic valve calcification and other biomarkers of inflammation (hs-CRP, ferritin, fibrinogen, white blood cell count (WBC), erythrocyte sedimentation rate (ESR), albumin levels) were evaluated in ACS patients and healthy controls. The study included 95 patients (mean age 61.8 ± 12.10 years) and 81 healthy controls (mean age 48.33 ± 9.19 years).

Results: Fetuin-A levels were significantly lower in patients with ACS than in healthy controls (0.76 ± 0.23 and 1.10 ± 0.45 g/L, respectively; $p < 0.001$). Fetuin-A was lower in patients with mitral annular calcification ($p = 0.007$) and aortic ($p = 0.001$) valve calcification. In patients with ACS, there was a negative correlation among serum urea ($r = -0.377$; $p < 0.001$) and creatinine ($r = -0.232$; $p = 0.024$) levels and fetuin-A, and a negative correlation among WBC ($r = -0.156$; $p = 0.132$), ESR ($r = -0.214$; $p = 0.037$), hs-CRP ($r = -0.220$; $p = 0.032$) levels and fetuin-A. A positive correlation was seen between albumin and fetuin-A ($r = 0.362$; $p < 0.001$). Multivariate logistic regression analysis revealed that fetuin-A was the variable that had a significant effect on ACS ($p = 0.020$ OR = .015; (95% CI) (0.000–0.520).

Conclusion: Fetuin-A levels decrease in patients with acute coronary syndromes, independent of heart valve calcification. Fetuin-A may therefore act as a negative acute phase protein after myocardial infarction.

Manuscript submitted 20th March, 2012

Manuscript accepted 18th June, 2012

Clin Invest Med 2012; 35 (4): E206-E215.

Correspondence to:

Sibel Özyazgan
Department of Pharmacology and Clinical Pharmacology
Cerrahpaşa Faculty of Medicine
İstanbul University,
34303 İstanbul, Turkey
e-mail: ozyazgans@yahoo.com

Atherosclerotic coronary artery disease is the major cause of mortality and morbidity in developed countries. Some risk factors, such as hypertension, smoking, obesity, diabetes and high LDL cholesterol levels, have been identified for atherosclerosis, but in more than 50% of patients who had atherosclerotic disease, none of these risk factors has been detected. Thus, factors other than these traditional risk factors may play a role in the atherosclerotic process.

A correlation between heart valve calcification and acute coronary syndrome has been known for a long time. Mitral annular calcification (MAC), a fibrotic, degenerative calcification of the mitral annulus, is caused by age, hypertension and chronic renal failure [1, 2]. Like MAC, aortic valve calcification, defined as aortic stenosis (AS), is also affected by the same factors. Since the risk factors such as hypertension, hyperlipidemia, diabetes and obesity are common risk factors for MAC and coronary artery disease [3, 4], MAC can be considered as an indicator of the subclinical atherosclerosis. Furthermore, it has been shown that vascular calcification is a risk factor for coronary artery disease [5, 6].

Fetuin-A ($\alpha 2$ Heremans Schmid Glycoprotein, Ahsg) is a 59 kD glycoprotein that is secreted mainly from the liver in adults, and which has a serum concentration of 0.5-1 g/L [7]. Fetuin-A has recently been described as an inhibitor of vascular and valvular calcification and it is a major inhibitor of calcium and phosphate precipitation. At the same time fetuin-A regulates the bone mineralization by preventing the ectopic calcification [7].

Recent studies have shown fetuin-A clustered inside the calcified vascular lesions of patients with renal failure [8]. Low serum fetuin-A levels and lower activity were detected in dialysis patients [8]. Wang et al. [9] reported an evident correlation among low levels of fetuin-A, increased vascular calcification and inflammation and an inverse correlation between the serum levels of this glycoprotein and the ratio of cardiovascular deaths in dialysis patients. The correlation between inflammation and atherosclerosis were studied in patients with acute coronary syndrome [8].

The main markers of inflammation are the acute phase reactants. In acute coronary syndrome patients, positive acute phase reactants include the erythrocyte sedimentation rate (ESR), white blood cell (WBC) count, ferritin, fibrinogen and high sensitive-C-reactive protein (hs-CRP). In addition to these reactants, the most used parameter of the negative acute phase is albumin. It has been argued that in acute inflammation, the serum level of fetuin-A decreases, as well as the level of albumin, and it has been suggested that fetuin-A should be considered as a negative acute phase reactant. It has been

proved that the concentration of fetuin-A decreases during inflammation and trauma [10]. In addition, there is an inverse correlation between fetuin-A levels and the levels of the pro-inflammatory cytokines such as IL-1 β , IL-6 and TNF- α [11] and an inverse correlation between low levels of fetuin-A and CRP, which is a marker of inflammation in patients with chronic renal failure who were on stable haemodialysis [12].

An inverse association was detected between serum fetuin-A concentrations and MAC among a cohort with coronary heart disease and without severe kidney disease [13]. A similar association was observed between fetuin-A and AS, but was limited to participants without diabetes mellitus [13]. These data are consistent with the hypothesis that fetuin-A functions 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 [13].

It has been hypothesized that fetuin-A is protective against ischemia in cardiomyocytes. Low levels of fetuin-A concentration have a direct deteriorating effect on myocardial functions. Catecholamine resistance and tolerance to ischemia and diastolic functions were found to be reduced in the hearts of fetuin knock-out mice [14].

The aim of this study was to evaluate the relationship among serum fetuin-A levels, heart valve calcification and other biomarkers of inflammation in patients with coronary heart disease.

Materials and Methods

Demographic features of patients

A total of 95 patients, 68 men and 27 women, with acute coronary syndrome (ACS) between the ages of 37-84 years were included in the study. Our control group was 32 healthy men and 49 healthy women between the ages of 32-76 years. Acute coronary syndrome was diagnosed according to universal definition of myocardial infarction [15]. The patients were asked for their gender, medical history (type 2 DM, HT, smoking and medications) and measured for systolic blood pressure (SBP), diastolic blood pressure (DBP) and glomerular filtration rate (GFR).

Exclusion criteria included trauma history, major surgical history, chronic inflammatory disease, acute infection, chronic kidney disease with a creatinine clearance under 15 mL/min, alcoholic hepatitis, acute toxic hepatitis, chronic autoimmune hepatitis, fatty liver, alcoholic and primer biliary cirrhosis, lung disease, known liver disease, known malignancy, known rheumatic heart valve disease, congenital heart disease including

bicuspid aorta, dilated cardiomyopathy and known osteoporosis.

Serum preparation

Blood was drawn in the morning after 12 -14 hours of fasting. Serum was obtained after at least 30 minutes of clotting by centrifugation at 2500xg for 15 minutes. Serum was used directly or stored at -80°C until assayed. All icteric or haemolytic blood samples were discarded. All parameters were analyzed in all samples together in a single batch at the termination of the experimental protocol (control and patient samples were analysed in the same batch).

Measurement of serum fetuin-A levels

Serum fetuin-A levels in the patients with acute coronary syndrome were measured at the 48th hour and in the control group in the serum obtained from the venous blood. A commercial kit (Assaypro, USA, cat no: EG 63501-1), based on a quantitative sandwich ELISA, was used and results was determined with ELX 800 UV version ELISA reader and calculated in grams per liter. Mean intra-assay and inter-assay coefficients of variation were less than 4.9 % (n:10) and 6.7 % (n:10).

Measurement of other biochemical parameters

Levels of serum glucose, urea, creatinine, total cholesterol, triglyceride, HDL, LDL, parathyroid hormone (PTH), calcium, phosphorus, white blood cell (WBC) count, ferritin, albumin, fibrinogen, hs-CRP, creatine kinase-MB(CK-MB), troponin T and erythrocyte sedimentation rate (ESR) were measured in the patient and control groups using the same biochemistry laboratory in our hospital.

Transthoracic echocardiography

Echocardiographic examinations of all patients were done during rest using a Vivid-3 (General Electrics) echocardiography machine with a 3.5 MHz probe. Two dimensional echocardiographs and Doppler examinations were done in all standard positions. Echocardiographical examinations of all patients were performed by one experienced cardiologist who was not informed about the clinical status of the patients. In the images of paracentral long axis, apical four ventricles and paracentral short axis, mitral annular calcification (MAC) was defined as a hyperechoic formation inside the atrioventricular junction and mitral posterior leaflet [16, 17]. Aortic calcification was also defined as increased thickness and bright echoes of the valve leaflets. The pressure gradient measured on the aortic valve was

calculated as a maximum and average gradient according to the Bernoulli equation and the area of the aortic valve was calculated according to the continuity equation principle [18, 19]. Aortic stenosis was defined as the current velocity measured on the aortic valve above 2.0 m/s, the maximum gradient above 15 mmHg and aortic valve area under 2 cm^2 [20].

Statistical analysis

Statistical analyses (Mann-Whitney U-test, Student t test) were performed with SPSS 17 (Statistical Package for Social Sciences). The difference in gender, smoking, HT, DM, calcium channel blocker use, MAC and aortic stenosis groups were analyzed by the Chi-square test. Pearson correlation test was used for correlating fetuin-A and the biochemical parameters, age, BMI, systolic and diastolic pressure and GFR. Multivariate logistic regression model was performed to determine the effect of independent risk factors for acute coronary syndrome. P-values < 0.05 were considered significant.

Results

Clinical data of patients are summarized in Table 1. In the patient group, the percentage of men ($p<0.001$), smoking ($p<0.001$), hypertension ($p<0.001$), diabetes mellitus ($p<0.001$) and calcium channel blocker usage ($p<0.01$) was significantly higher than in the control group.

There was no statistically difference ($p > 0.05$) in heart valve calcification among the patients and healthy controls [Table 2].

In Table 3, the averages of age ($p<0.001$) and the diastolic and systolic arterial pressures ($p<0.001$) are statistically higher in the patient group than in the healthy controls. There was no statistically difference in BMI ($p>0.05$), GFR ($p>0.05$), cholesterol and LDL level averages ($p>0.05$) among the patient group and the healthy controls. Even though the triglyceride levels were significantly higher in the patient group ($p<0.01$), the HDL levels were significantly higher in healthy controls ($p<0.001$). In the patient group, the averages of fasting blood glucose levels ($p<0.001$), urea and creatinine levels ($p<0.001$) were statistically higher than the healthy control group, yet the urea and creatinine levels were within the normal range. The levels of CKMB ($p<0.001$), WBC ($p<0.001$), fibrinogen ($p<0.001$), ESR ($p<0.001$) and ferritin ($p<0.01$) were significantly higher in the patient group than the control group, whereas the average calcium and of albumin levels were higher ($p<0.001$) in the healthy controls than the patient group. There was no significant difference between PTH and phosphorus levels among two groups. The average of hs-CRP levels

TABLE 1. Comparison of demographics and medical history in patient and control groups

	Control group		Patient group		Q-square	p
	n	%	n	%		
Gender						
Male	32	39.5	68	71.6		
Female	49	60.5	27	28.4	18.33	0.000***
Smoking						
Yes	34	42.0	64	67.4		
No	47	58.0	31	32.6	11.42	0.001***
HT						
Yes			49	51.6		
No	81	100.0	46	48.4	57.89	0.000***
DM						
Yes			35	36.8		
No	81	100.0	60	63.2	37.25	0.000***
Calcium channel blocker use						
Yes			10	10.5		
No	81	100.0	85	89.5		0.002**

were statistically higher ($p < 0.001$) in the patient group but the averages of fetuin-A levels were significantly lower (0.76 ± 0.23 and 1.10 ± 0.45 g/L, respectively; $p < 0.001$) in the patient group than the control group.

In Table 4, there was a negative correlation between serum urea ($r = -0.377$; $p = 0.000$) and creatinine ($r = -0.232$; $p = 0.024$) levels and fetuin-A in the patients. There was a negative correlation between WBC ($r = -0.156$; $p = 0.132$), ESR ($r = -0.214$; $p = 0.037$), hs-CRP ($r = -0.220$; $p = 0.032$) levels and fetuin-A in patients; however, there was a positive correlation between albumin and fetuin-A ($r = 0.362$; $p = 0.000$). Serum fetuin-A ($r = -0.29$, $p < 0.01$) showed a negative moderate correlation with age in the patients and in both groups combined [Table 4].

Fetuin-A was lower in patients with mitral annular ($p = 0.007$) and aortic ($p = 0.001$) valve calcification [Table 5].

In the patient group and in total, there is a negative weak correlation between fetuin-A levels and age ($r = -0.291$, $p < 0.01$; $r = -0.327$, $p < 0.001$). There is a negative correlation between urea and creatinine levels and fetuin-A but there is a positive correlation between GFR and fetuin-A in patient group. In

patient group there is a positive correlation between triglyceride levels and fetuin-A. There is a positive correlation between fetuin-A and albumin and calcium levels while there is a negative correlation between ESR, WBC and fetuin-A.

Fetuin A is negatively correlated with hs-CRP and troponin T in patient group. Among patients with aortic stenosis, fetuin-A levels are found to be lower but with only a weak significance. Between MAC and fetuin-A, there was a lower correlation with a weak significance.

The results of multivariate logistic regression analysis, including OR for each of the demographic factors, biochemical parameters and fetuin-A are presented in [Table 6]. Significant risk factors were related to SBP [$p = .028$, OR(95%CI = 1.098(1.010-1.194))], CKMB [$p = .003$, OR(95%CI = 1.148(1.047-1.259))], fetuin-A [$p = .020$, OR(95% CI = .015(0.000-0.520))], and ferritin [$p = .004$, OR(95%CI = 1.015(1.005-1.025))] levels.

TABLE 2. Comparison of MAC and aortic stenosis in patient and control groups

	Control group		Patient group		Q-square	p
	N	%	n	%		
MAC						
Yes	72	88.9	83	87.4		
No	9	11.1	12	12.6	0.09	0.756
AORTIC STENOSIS						
Yes	76	93.8	87	91.6		
No	5	6.2	8	8.4	0.32	0.570

TABLE 3. Comparison of the groups according to age, BMI, systolic pressure, diastolic pressure, GFR, biochemical parameters, acute phase reactants and fetuin A levels

	Control group		Patient group		p
	Median	SD	Median	SD	
Age	48.33	9.19	61.80	12.10	.000***
Systolic pressure	114.38	11.82	137.84	24.19	.000***
Diastolic pressure	69.88	7.90	81.16	14.88	.000***
GFR	117.75	7.11	114.84	12.33	.062
Glucose	93.93	11.07	151.67	90.22	.000***
Urea	28.65	7.40	46.97	27.18	.000***
Creatinine	.80	.13	1.15	.59	.000***
Cholesterol	202.25	39.52	192.39	48.07	.143
Triglyceride	132.98	52.40	168.35	108.79	.008**
HDL	46.26	11.68	35.22	9.37	.000***
LDL	129.25	34.54	123.77	39.87	.336
CKMB	13.58	7.97	103.74	139.74	.000***
WBC	7459.26	2221.19	11721.05	3924.50	.000***
Fibrinogen	328.58	72.82	374.26	132.63	.006**
ESR	15.37	9.77	20.99	20.63	.026*
Albumin	4.12	.28	3.47	.48	.000***
Calcium	9.60	.37	9.26	.45	.000***
Phosphorus	3.32	.53	3.42	.97	.397
PTH	53.50	17.72	65.98	60.42	.075
Ferritin	59.7963	67.2252	233.1873	489.8118	.002**
Hs-CRP	.66	1.33	4.66	6.12	.000***
Fetuin-A	1.10	.45	.76	.23	.000***

TABLE 4. Correlations among fetuin-A and biochemical and physiological parameters in patient and control groups

	Control group		Patient group		Total	
	r	p	r	p	r	p
Age	-.021	.855	-.291	.004**	-.327	.000***
Systolic pressure	.269	.015*	.211	.040*	-.082	.281
Diastolic pressure	.360	.001***	.212	.039*	.002	.975
GFR	.187	.094	.214	.037*	.211	.005**
Glucose	-.055	.628	.035	.738	-.166	.028*
Urea	-.133	.238	-.377	.000***	-.346	.000***
Creatinine	-.162	.149	-.232	.024*	-.275	.000***
Cholesterol	-.208	.062	.144	.165	-.001	.991
Triglyceride	.020	.860	.333	.001***	.047	.533
HDL	.065	.567	-.035	.736	.230	.002**
LDL	-.268	.015*	.078	.453	-.073	.333
CKMB	-.037	.742	.045	.664	-.161	.033*
WBC	-.259	.020*	-.156	.132	-.370	.000***
Albumin	-.024	.831	.362	.000***	.378	.000**
Calcium	.002	.989	.391	.000***	.294	.000***
Phosphorus	.062	.583	-.141	.174	-.060	.428
PTH	.082	.468	-.221	.032*	-.133	.077
Fibrinogen	-.040	.724	-.085	.411	-.136	.072
Sedimentation	-.120	.285	-.214	.037*	-.195	.010*
Ferritin	-.122	.277	-.076	.466	-.146	.054
hsCRP	-.144	.201	-.220	.032*	-.282	.000***
Troponin T			-.219	.033*		

TABLE 5. Comparisons of fetuin-A and troponin T levels in the patients with aortic calcification and MAC

Aortic calcification	No		Yes		p
	Median	SD	Median	SD	
Fetuin-A	.95	.39	.59	.10	.001***
Troponin T	2.25	3.64	2.08	2.24	.898
MAC	No		Yes		p
	Median	SD	Median	SD	
Fetuin-A	.95	.40	.71	.20	.007**
Troponin T	2.35	3.71	1.47	1.81	.423

TABLE 6. Logistic regression analysis of the association between fetuin-A and demographic, physiological and biochemical parameters

Variables	OR	P
Gender	.977	.968
Smoking	1.511	.493
BMI	.862	.233
Systolic pressure	1.098	.028
Diastolic pressure	1.097	.084
HDL	.950	.363
LDL	1.000	.999
CKMB	1.148	.003
Fibrinogen	1.001	.932
ESR	1.029	.497
Calcium	.072	.051
Phosphorus	1.286	.669
PTH	1.011	.590
Ferritin	1.015	.004
Fetuin-A	.015	.020

Discussion

In our study, it was found that, serum fetuin-A levels decreased, regardless of the degree of calcification of the heart valve, in patients with acute coronary syndrome. After myocardial infarction, the serum fetuin-A levels were negatively correlated with hs-CRP levels, which is an acute phase reactant. In other words, fetuin-A may be a negative acute phase reactant showing a diagnostic and prognostic importance in patients with acute coronary syndrome.

Inflammation is a common feature of all different stages of atherosclerosis. Fetuin-A is an anti-inflammatory mediator that is produced by the liver and participates in macrophage deactivation. Epidemiological studies, demonstrating the correlation between fetuin-A and dystrophic calcification, which have been performed on the patients with end-stage renal failure, showed a correlation between low level of serum fetuin-A, valvular and vascular calcification and mortality [9, 21, 22]. Ix et al. [13] have observed an inverse association between fetuin-A and mitral annular calcification among individuals with coronary heart disease. An inverse association was also observed between fetuin-A and calcific aortic stenosis among participants without diabetes mellitus. In another study performed by the same researchers, serum fetuin-A levels of the coronary

heart disease patients with normal and moderate renal dysfunction were found to be similar [23]. In this study, patients with coronary heart disease, with normal renal function and healthy patients as the control group, were examined and fetuin-A levels of the control group were determined to be significantly higher than of the patient group. No statistically significant difference was found between the patient and the control groups in terms of MAC or AS. Serum fetuin-A levels of the patients without MAC and AS, and of the control groups, were found to be statistically higher.

Although no significant difference between the control and patient groups was determined in terms of MAC and AS, higher level of serum fetuin-A in the control group compared with the patients with acute coronary syndrome raises a new question: why does serum fetuin-A level decrease in the patients with acute coronary syndrome? In a study performed by Merx et al. [14] on the hearts of fetuin-A knockout mice, an increase in cardiac fibrosis and calcification, impaired diastolic function, tolerance to the ischemia and catecholamine resistance were detected. Inflammation is the main predictor of the plaque rupture mechanism in patients with ST segment elevation myocardial infarction (STEMI). Following necrosis, inflammation is maintained in the residual myocardium. Results of this may be both useful (such as improvement and recovery in functions) and harmful (such as acute cardiac rupture, chronic dilatation, heart failure) [24].

Impairment of the balance between the anti-inflammatory and proinflammatory mediators can be harmful. Anti-inflammatory mediators decrease the production of the proinflammatory cytokine production through several different ways [25]. On the other hand, fetuin-A is considered as a preformed cofactor that opsonizes the cationic macrophage deactivating molecules [26]. During inflammation, the proinflammatory cytokines, whose synthesis is generally inhibited by fetuin-A, block the synthesis of fetuin-A in the liver [11]. Decreased fetuin-A level in the circulation affects, and thereby prevents, the activities of several anti-inflammatory molecules [27]; however, in the presence of acute coronary syndrome, low serum fetuin-A concentration facilitates ongoing inflammatory processes and causes over-production of the cardiotoxic cytokines such as TNF [11, 28]. This causes patients to be exposed to remodeling of left ventricle and to a high risk of relapse of acute coronary syndrome. Postinfarct inflammation reaches to its highest level on the 3rd day. In this study, serum fetuin-A levels were measured in patients on the 2nd day post-infarction. Fetuin-A concentration reflects the stage of inflammatory imbalance, since fetuin is activated as a result of the interaction between proinflammatory and anti-

inflammatory cytokines. This crucial finding causes the fetuin-A level to be considered as a clinical risk score.

Fetuin-A acts as a negative acute phase reactant and its level decreases in chronic inflammation [10]. Inflammation plays a major role in the pathophysiology of coronary artery disease and atherosclerosis is defined as a chronic inflammatory disease [29]. Many pro-inflammatory markers have been shown to contribute to the development of atherosclerosis [30]. In addition, anti-inflammatory cytokines, produced during inflammation, also play an important role in controlling the inflammatory response. The results from different studies regarding the relationship between the levels of fetuin-A, which is an anti-inflammatory cytokine, and the presence of cardiovascular diseases are controversial [31, 32]. High levels of serum fetuin-A have been found to be associated with a high risk of myocardial infarction and ischemic stroke [32]. In our study, the fetuin-A levels in patients with acute coronary syndrome are lower than in the control group. Acute myocardial infarction is a pro-inflammatory condition, and the CRP levels have been shown to increase in patients with ACS [33]. Although it is an anti-inflammatory marker, fetuin-A can stimulate the inflammatory response by increasing the production of cardiotoxic cytokines such as tumor necrosis factor [34]. It has also been shown that fetuin-A induces the expression of cytokine in human monocytes and reduces the expression of adiponectin, which has atheroprotective properties in animals [32]. Basar et al. [35] have found low fetuin-A levels to be associated with an impaired coronary flow in patients with STEMI who underwent primary percutaneous coronary intervention. In the same study, the one year major adverse cardiac events (MACE) were higher in patients with low fetuin-A levels than those with high fetuin-A levels. Similarly, in a recent study, hs-CRP, which is a positive acute phase reactant, was associated with an increase in mortality in patients with ACS [36]. In another study of ACS, Lim et al. [37] have demonstrated that low serum fetuin-A levels in patients with STEMI were associated with a poor prognosis. As determined in our study, the fetuin-A levels in patients with ACS, as an indicator of the severity of the inflammatory response, are low and may indicate a poor prognosis.

There is also an inverse correlation between fetuin-A and CRP levels and between fetuin-A and inflammation [9]. In our study, a statistically significant negative correlation between ESR, WBC and hs-CRP levels and fetuin-A was determined in patients with ACS and overall. These results support the theory that fetuin-A is a negative acute phase reactant. The negative correlation between fetuin-A and the positive acute phase reactants is also consistent with this theory.

In our study, MAC was established in 12 patients with ACS, while AS was detected in eight patients. In the control group, MAC was also detected in nine patients and AS in five patients. No statistically significant difference between these groups was detected in terms of coronary valve calcification; however, in patients with mitral annular calcification and aortic valve calcification, the serum fetuin-A levels were lower than in patients without detected calcification of the heart valves. This finding is consistent with the results of Ix et al. [13], which showed that MAC and AS and low fetuin-A level are related to each other in patients with coronary artery disease. In other words, fetuin-A is an important inhibitor of dystrophic calcification, and with decreasing fetuin-A serum levels, the probability of the calcification of the heart valves increases. In patients with end-stage renal failure, correlations were determined between low level fetuin-A concentration and increased vascular and valvular calcification, increased cardiovascular events and mortality. It is unknown whether there is an association between the dystrophic calcifications and serum fetuin-A levels in other populations [9, 21, 22, 24].

Our study has several limitations. One of the major limitations of the study is the relative scarcity of the patients with ACS, compared with the general patient population. In addition, the relationship between the success of the reperfusion therapy and the serum fetuin-A levels was not assessed. Similarly, the relationship between serum fetuin-A levels and the parameters such as the Syntax and Gensini scores, which show the extent of atherosclerosis and the complexity of the lesion, was not assessed. Another important limitation is the lack of the assessment of the relationship between the serum fetuin-A levels and in-hospital MACE and mortality. In addition, the lack of the assessment of the relationship between the serum fetuin-A levels and the short-and the long-term prognosis in patients with ACS is another limitation.

Conclusion

Serum fetuin-A level may prove to be useful as a marker of inflammation and calcification for cardiovascular events. Since the serum levels may vary in metabolic syndrome, DM, hepatic steatosis, inflammatory diseases and in general healthy population, it is obvious that further study is required. Further studies are also needed for patients with ACS. Future detailed studies will show us the importance of fetuin-A and its functions in human body in different metabolic, inflammatory pathways.

Financial Support

This work was supported by The Research Fund of Istanbul Education and Research Hospital.

References

1. D'Cruz IA, Panetta F, Cohen H, Glick G. Submitral calcification or sclerosis in elderly patients: M-mode and two dimensional echocardiography in "mitral anulus calcification". *Am J Cardiol* 1979; 44: 31-8.
2. Hirschfeld DS, Emilson BB. Echocardiogram in calcified mitral anulus. *Am J Cardiol* 1975; 36: 354-6.
3. Boon A, Cheriex E, Lodder J, Kessels F. Cardiac valve calcification: characteristics of patients with calcification of the mitral annulus or aortic valve. *Heart* 1997; 78: 472-4.
4. Savage DD, Garrison RJ, Castelli WP, et al. Prevalence of submitral (anular) calcium and its correlates in a general population-based sample (the Framingham Study). *Am J Cardiol* 1983; 51: 1375-8.
5. Iribarren C, Sidney S, Sternfeld B, Browner WS. Calcification of the aortic arch: risk factors and association with coronary heart disease, stroke, and peripheral vascular disease. *JAMA* 2000; 283: 2810-5.
6. Keelan PC, Bielak LF, Ashai K, et al. Long-term prognostic value of coronary calcification detected by electron-beam computed tomography in patients undergoing coronary angiography. *Circulation* 2001;104: 412-7.
7. Heiss A, DuChesne A, Denecke B, et al. Structural basis of calcification inhibition by alpha2-HS glycoprotein/fetuin-A. Formation of colloidal calciprotein particles. *J Biol Chem* 2003; 278: 13333-41.
8. Hermans MM, Brandenburg V, Ketteler M, et al. Association of Serum Fetuin-A Levels with Mortality in Dialysis Patients. *Kidney Int* 2005;67:2295-304.
9. Wang AY, Woo J, Lam CW, et al. Associations of serum fetuin-A with malnutrition, inflammation, atherosclerosis and valvular calcification syndrome and outcome in peritoneal dialysis patients. *Nephrol Dial Transplant* 2005;20: 1676-85.
10. Lebreton JP, Joisel F, Raoult JP, et al. Serum concentration of human alpha 2 HS glycoprotein during the inflammatory process: evidence that alpha 2 HS glycoprotein is a negative acute-phase reactant. *J Clin Invest* 1979; 64: 1118-29.
11. Kelly RA, Smith TW. Cytokines and cardiac contractile function. *Circulation* 1997; 95: 778-81.
12. Ketteler M. Fetuin-A and extraosseous calcification in uremia. *Curr Opin Nephrol Hypertens* 2005;14:337-42.
13. Ix JH, Chertow GM, Shlipak MG, Brandenburg VM, Ketteler M, Whooley MA. Association of Fetuin-A With Mitral Annular Calcification and Aortic Stenosis Among Persons With Coronary Heart Disease. *Circulation* 2007;119:2533-9.
14. Merx MW, Schafer C, Westenfeld R, et al. Myocardial stiffness, cardiac remodeling, and diastolic dysfunction in calcification-prone fetuin-A-deficient mice. *J Am Soc Nephrol* 2005;16:3357-64.
15. Thygesen K, Alpert JS, White HD. Universal definition of myocardial infarction. *Circulation*. 2007;116:2634-53.
16. Meltzer RS, Martin RP, Robbins BS, Popp RL. Mitral annular calcification: clinical and echocardiographic features. *Acta Cardiol* 1980;35:189-202.
17. Barasch E, Gottdiener JS, Larsen EK, Chaves PH, Newman AB, Manolio TA. Clinical significance of calcification of the fibrous skeleton of the heart and aortosclerosis in community dwelling elderly: the Cardiovascular Health Study (CHS). *Am Heart J* 2006;151:39-47.
18. Skjaerpe T, Hegrenaes L, Hatle L. Noninvasive estimation of valve area in patients with aortic stenosis by Doppler ultrasound and two-dimensional echocardiography. *Circulation* 1985;72:810-8.
19. Zoghbi WA, Farmer KL, Soto JG, Nelson JG, Quinones MA. Accurate noninvasive quantification of stenotic aortic valve area by Doppler echocardiography. *Circulation* 1986;73:452-9.
20. Cowell SJ, Newby DE, Prescott RJ, Bloomfield P, Reid J, Northridge DB, Boon NA. A randomized trial of intensive lipid-lowering therapy in calcific aortic stenosis. *N Engl J Med* 2005;352:2389-97.
21. American Heart Association: Heart and Stroke Facts: 1995 Statistical Suppl. Dallas, American Heart Association 1994.
22. Moe SM, Reslerova M, Ketteler M, et al. Role of calcification inhibitors in the pathogenesis of vascular calcification in chronic kidney disease (CKD). *Kidney Int* 2005;67:2295-304.
23. Ketteler M, Bongartz P, Westenfeld R, et al. Association of low fetuin-A (AHSG) concentrations in serum with cardiovascular mortality in patients on dialysis: a cross-sectional study. *Lancet* 2003;361:827-33.
24. Stenvinkel P, Wang K, Qureshi AR, et al. Low fetuin-A levels are associated with cardiovascular death: impact of variations in the gene encoding fetuin. *Kidney Int* 2005;67:2383-92.
25. Abbate A, Bonanno E, Mauriello A, et al. Widespread myocardial inflammation and infarct related artery patency. *Circulation* 2004;110:46-50.
26. Kilic T, Ural D, Ural E, et al. Relation between proinflammatory to anti-inflammatory cytokine ratios and long-term prognosis in patients with non-ST elevation acute coronary syndrome. *Heart* 2006;92:1041-6.
27. Wang H, Zhang M, Bianchi M, Sherry B, Sama A, Tracey KJ. Fetuin (alpha2-HS-glycoprotein) opsonizes cationic macrophage deactivating molecules. *Proc Natl Acad Sci USA* 1998;95:14429-34.
28. Ombrellino M, Wang H, Yang H, et al. Fetuin, a negative acute phase protein, attenuates TNF synthesis and the innate inflammatory response to carrageenan. *Shock* 2001;15:181-5.
29. Ross R. Atherosclerosis-an inflammatory disease. *N Engl J Med* 1999;340:115-6.

30. Theroux P, Armstrong PW, Mahaffey KW, Hochman JS, Malloy KJ, Rollins S, et al. Prognostic significance of blood markers of inflammation in patients with ST-segment elevation myocardial infarction undergoing primary angioplasty and effects of pexelizumab, a C5 inhibitor: a substudy of the COMMA trial. *Eur Heart J* 2005;26:1964-70.
31. Rittig K, Thamer C, Haupt A, et al. High plasma fetuin-A is associated with increased carotid intima-media thickness in a middleaged population. *Atherosclerosis* 2009;207: 341-342.
32. Weikert C, Stefan N, Schulze MB, et al. Plasma fetuin-a levels and the risk of myocardial infarction and ischemic stroke. *Circulation*. 2008;118:2555-62.
33. Elabbassi W, Al-Nooryani A. Acute coronary syndrome. An acute inflammatory syndrome. *Saudi Med J*. 2006;27:1799-803.
34. Bilgir O, Kebapcilar L, Bilgir F, et al. Decreased serum fetuin-A levels are associated with coronary artery diseases. *Intern Med*. 2010;49:1281-5.
35. Basar N, Sen N, Kanat S, et al. Lower fetuin-A predicts angiographic impaired reperfusion and mortality in ST-elevation myocardial infarction. *J Investig Med*. 2011;59:816-22.
36. Ahmed K, Jeong MH, Chakraborty R, et al. Prognostic impact of baseline high-sensitivity C-reactive protein in patients with acute myocardial infarction undergoing percutaneous coronary intervention based on body mass index. *Korean Circ J*. 2012;42:164-72.
37. Lim P, Collet JP, Moutereau S, et al. Fetuin-A is an independent predictor of death after ST elevation myocardial infarction. *Clin Chem* 2007; 53:1835-40.