Association of mitral annular calcification with endothelial dysfunction, carotid intima-media thickness and serum fetuin-A: an observational study

Mitral anüler kalsifikasyonun endotel fonksiyon bozukluğu, karotis intima mediya kalınlığı ve serum fetuin-A düzeyi ile ilişkisi: Gözlemsel bir çalışma

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ABSTRACT

Objective: Mitral annular calcification (MAC) is characterized by degenerative calcification of the mitral valve annulus. Atherosclerosis plays role in progression of MAC. Fetuin A is the inhibitor of pathological calcification. In the present study, we investigated the relationship between MAC and fetuin A with carotid intima media thickness (CIMT) and endothelial dysfunction.

Methods: In this observational cross-sectional study, 40 patients with documented MAC on transthoracic echocardiography (TTE) and 40 without MAC were included. All patients had coronary artery disease (CAD). Endothelial functions were assessed by brachial artery Doppler ultrasound (USG) and carotid artery Doppler USG. Serum fetuin-A level was also measured. Linear regression analysis and receiver operator curve (ROC) analysis were performed.

Results: Endothelial derived vasodilatory response (EDVR) was significantly decreased and CIMT value was increased in MAC group. There was a strong positive correlation between EDVR and serum fetuin-A value. There was a strong negative correlation between CIMT and EDVR, moderately negative correlation between CIMT and serum fetuin-A level. Simple linear regression analysis revealed that CIMT (β=0.367, p=0.001) and serum fetuin-A level (β=-0.291, p=0.009) were independent factors associated with MAC. The area under the curve (AUC) for serum fetuin-A level was 0.731 (95% 0.620-0.824) and AUC for CIMT was 0.724 (95% CI 0.613-0.818).

Conclusion: We observed that MAC is closely related with CIMT and serum fetuin-A level. Serum fetuin-A and CIMT can be used as independent markers in the diagnosis of MAC. We suggest that MAC can be used as an early determinant of CAD. *(Anadolu Kardiyol Derg 2013; 13: 752-8)*

Key words: Endothelial function, fetuin-A, mitral annular calcification, regression analysis, diagnostic accuracy, specificity, sensitivity

ÖZET

Amaç: Mitral anüler kalsifikasyon (MAK), mitral kapak anulusunun dejeneratif kalsifikasyonu ile karakterize bir hastalıktır. MAK gelişiminde aterosklerotik mekanizmaların rol oynadığı düşünülmektedir. Fetuin-A, patolojik kalsifikasyonun inhibitörlerindendir. Çalışmamızda; MAK ile, endotel fonksiyon bozukluğu, karotis intima media kalınlığı (KİMK) ve serum fetuin-A düzeyleri arasındaki ilişkiyi inceledik.

Yöntemler: Bu gözlemsel, kesitsel çalışmaya, transtorasik ekokardiyografide MAK saptanan 40 hasta ile saptanmayan 40 hasta dahil edildi. Tüm hastalar koroner arter hastası idi. Endotel fonksiyonları brakiyal arter ultrasonografisi (USG), karotis arter Doppler USG ile değerlendirildi. Hastaların Fetuin A düzeyi ölçüldü. Lineer regresyon analizi ve "ROC" analizi ile istatistiki değerlendirmeler yapıldı.

Bulgular: MAK grubundaki ortalama endotele bağımlı vazodilatasyon yanıtı (EBVY) kontrol grubuna göre anlamlı olarak azalmış, ortalama karotis intima media kalınlığı ise artmıştı. EBVY ile serum fetuin-A düzeyi arasında güçlü derecede pozitif korelasyon saptandı. KİMK ile EBVY arasında güçlü derecede negatif korelasyon, KİMK ile fetuin-a arasında ise orta derecede negative korelasyon saptandı. Regresyon analizleri sonucunda KİMK (beta katsayısı 0,367, p=0,001) serum fetuin-A düzeyinin (beta katsayısı-0,291, p=0,001) MAK için bağımsız risk faktörleri olduğu saptandı. Eğri altındaki alan (AUC) fetuin-A düzeyi için 0,731 (güvenlik aralığı %95), KİMK için AUC değeri 0,724 (güvenlik aralığı %95) saptandı.

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Sonuç: Çalışmamız sonucunda MAK'ın, KİMK ve fetuin-A düzeyi ile yakın ilişkili olduğunu göziedik. Serum fetuin-A düzeyi ve KİMK'ın, MAK tanısı için bağımsız belirteçler olabileceğini ve ekokardiyografik inceleme ile kolayca tanısı konulabilen MAK'ın, koroner arter hastalığının erken belirteci olarak kullanılabileceğini düşünmekteyiz. *(Anadolu Kardiyol Derg 2013; 13: 752-8)*
Anahtar kelimeler: Endotel fonksiyonu, fetuin-A, mitral anüler kalsifikasyon, regresyon analizi, tanısal değer, özgüllük, duyarlılık

Introduction

Mitral annular calcification (MAC) is a chronic, degenerative process, which is characterized by fibrosis and calcification of the mitral valve annulus (1). The prevalence of MAC has been reported to be approximately 15% in population-based studies and up to 35% in patients with severe coronary artery disease (CAD) (2, 3). It has been demonstrated that MAC is associated with atherosclerotic risk factors (4). MAC is also correlated with age, infective endocarditis, cardiovascular disease, stroke, and congestive heart failure (5-11).

Table 1. Baseline characteristics

Variables	MAC (n=40)	Control (n=40)	*p
Age, years	65.6±7.2	64.3±9.6	0.49
Male gender, n (%)	27 (67)	28 (70)	0.81
Systolic blood pressure, mm Hg	118.5±9.7	122.5±12.1	0.10
Diastolic blood pressure, mm Hg	73.0±7.2	74.7±7.8	0.30
BMI, kg/m^2	28.1±3.0	28.6±4.2	0.51
Pulse pressure, mm Hg	45.5±9.5	47.7±11.4	0.34
Hypertension, n (%)	29 (73)	28 (70)	0.17
Diabetes mellitus, n (%)	18 (45)	21 (52)	0.37
Smoking, n (%)	16 (40)	13 (32)	0.15
Hypercholesterolemia, n (%)	32 (80)	36 (90)	0.46
Heredity, n (%)	10 (25)	11 (27)	0.80
Nitrate use, n (%)	12 (30)	12 (30)	1.0
Beta blocker use, n (%)	26 (65)	29 (72)	0.47
ACEI/ARB use, n (%)	22 (55)	24 (60)	0.65
Statin use, n (%)	18 (45)	18 (45)	1.0
Glucose, mg/dL	122.6±42.7	134.6±57.0	0.29
BUN, mg/dL	18.2±5.7	19.7±9.1	0.38
Creatinine, mg/dL	0.95±0.27	0.88±0.23	0.24
Total cholesterol, mg/dL	192.0±54.0	181.4±42.8	0.33
HDL cholesterol, mg/dL	42.5±9.0	40.9±10.3	0.47
LDL cholesterol, mg/dL	125.4±49.0	111.5±39.0	0.16
Triglyceride, mg/dL	145.2±70.1	156.4±109.6	0.58
Calcium, mg/dL	9.1±0.9	9.1±0.98	1.0
Inorganic phosphorus, mg/dL	3.72±0.5	3.49±0.57	0.09
Albumin, g/dL	4.0±0.47	4.1±0.41	0.30
GFR, mL/dk	78.7±17.9	85.4±20.6	0.12
Hs-CRP, mg/L	40.1±25.2	28.2±19.9	0.21

Data are presented as mean±SD and number (percentage)
*Student- t test, Chi-square and Fisher's exact tests
ACEI - angiotensin converting enzyme inhibitor, ARB - angiotensin receptor blocker, BUN - blood urine nitrogen, GFR - glomerular filtration rate, HDL - high density lipoprotein, hs-CRP - high sensitive C-reactive protein, LDL - low density lipoprotein

The endothelium is a monolayer organ having autocrine, paracrine, and endocrine functions. Endothelial cells produce several vasoactive substances, which maintain vascular homeostasis and normal vasomotor tone (12). Endothelial dysfunction is known to be the key factor in the pathogenesis of arteriosclerosis and disruption of endothelial homeostasis, predisposing to vasoconstriction, inflammation, leukocyte adhesion, thrombosis, and proliferation of vascular smooth muscle cells (13, 14).

Atherosclerotic changes of carotid arteries are closely related to increased cardiovascular morbidity and mortality (15). Carotid intima media thickness (CIMT), is a marker of atherosclerosis and assessment of CIMT is a validated approach for atherosclerosis progression, and has been shown to be highly predictive of future cardiovascular events and death (16, 17).

Fetuin-A is a multifunctional hepatic secretory glycoprotein that inhibits dystrophic vascular and valvular calcification. It was previously demonstrated that serum fetuin-A level is decreased in high vascular calcification conditions such as chronic renal failure (18). In addition, close relationship between decreased serum fetuin-A level and atherosclerosis has been revealed in patients without severe renal impairment (19).

Previous studies have reported a strong correlation between the presence of MAC and the risk of atherosclerosis.

In the present study we aimed to mention the association between the presence of MAC and endothelial dysfunction and a novel atherogenic marker, fetuin A, in order to demonstrate its role in the atherogenic process.

Methods

Study design and population

In this observational cross-sectional study, subjects were selected from the patients who were hospitalized in our cardiology department between January 2007 and May 2009 and having angiographically diagnosed CAD. Patients with rheumatic heart disease, prosthetic heart valves, aortic calcification, chronic renal failure (CRF), hyperparathyroidism, hypercalcemia, hyperphosphoremia, congenital heart disease, hepatic failure and malignity were excluded. MAC group was consisted of 40 patients having MAC with CAD and the control group included sex and age matched 40 subjects having CAD without MAC.

The study protocol was approved by local ethics committee and written informed consent was obtained from all subjects.

Study protocol

Clinical and demographic features of the patients and the control group are presented in Table 1. Venous blood samples for

biochemical analyses were drawn from all subjects after an overnight fast between 8:00 p.m. and 8:00 a.m. Triglyceride (TG), total cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL), blood urea nitrogen (BUN), creatinine, sodium, potassium, calcium, phosphorus, albumin and plasma glucose level and high sensitive C-reactive protein (hs-CRP) levels were analyzed. Serum extracts of the venous blood samples were stored at -80^{0}C for further analysis of fetuin-A. Glomerular filtration rates (GFR) of all subjects were calculated. Standard transthoracic echocardiography (TTE) was performed. Endothelial function was assessed by endothelial dependent vasodilatory response (EDVR), and nitrate dependent vasodilatory response (NDVR) via brachial artery (BA) using brachial artery Doppler ultrasonography (USG). All subjects' CIMT were measured by carotid artery Doppler USG.

Ultrasonographic examinations

Echocardiographic examination

All of patients and control group were underwent TTE by using "Siemens Sequoia"(C256; Mountainview, CA, USA) and "General Electric Vivid 3" (USA) ultrasound systems using 2.5 and 3.5-MHz transducers. Complete 2-dimensional echocardiograms, including Doppler examination were obtained in all standard views (parasternal long -axis, parasternal short- axis, apical four-chamber, apical two- chamber). TTE was performed by experienced cardiologists who were blinded to subjects' clinical status. MAC was defined as an echo-dense structure observed in the margin of the atrioventricular groove and the posterior leaflet on the apical four-chamber view and the parasternal long-axis view on TTE or an echo-dense structure observed on the posterior leaflet in parasternal short-axis view. Subjects having MAC were divided in tertiles according to mitral annular thickness (MAT) measured on the apical four chamber view. Mitral annular thickness of 1-2 mm was reported as mild, 2-5 mm as moderate and >5 mm as severe MAC.

Endothelial function

Subjects were evaluated after an overnight fast, without smoking, drinking alcohol/coffee, taking vasoactive drugs or taking antioxidant vitamins 12 hours prior to testing. Subjects were examined in the supine position after 15 minutes of rest in a dark and quiet room with temperature of 20-25^{0}C. Endothelium-dependent dilation of the BA was measured noninvasively by "General Electric Vivid 3 and Vivid-I (General Electric, USA), 8 MHz linear array transducer" high resolution ultrasound system. The left arm was immobilized in the extended position to allow consistent access to the brachial artery for imaging. Baseline arterial diameter (BAD) was recorded twice at intervals of 1 minute. Following baseline establishment, a blood pressure cuff was placed over the ipsilateral upper arm just above the transducer and inflated for 5 minutes at 50 mmHg greater than systolic blood pressure. The cuff was then deflated suddenly and blood flow velocity was measured immediately after deflation of the cuff (hyperemic blood flow) as well as 60, 75, 90, and 120 seconds later. Maximal BA diameter observed during this time period was used to calculate flow mediated dilatation (FMD). EDVR was calculated as follows:

EDVR = [(FMD-BAD)/BAD] x 100 (20).

After 10 minutes of rest endothelium-independent dilation of BA was measured by using nitroglycerine. Four minutes after sublingual administration of 400 µg glycerol trinitrate, nitroglycerine mediated dilatation (NMD) was measured. NDVR was calculated as follows:

NDVR= [(NMD-BAD)/BAD] x 100 (21).

Measurement of carotid intima-media thickness

Longitudinal images of the common carotid artery (CCA), 1 cm proximal to the bifurcation in which the far wall intima-media interface was clearly defined, were magnified and recorded for further analysis. The distance between the leading edge of the intima and the media-adventitia interface was measured with ultrasonic calipers. Three measurements were obtained from both the right and left CCAs, and a mean CIMT was calculated.

Laboratory measurements

The following parameters were measured at Florence Nightingale Hospital Biochemistry Laboratory: TG, total cholesterol, LDL, HDL, BUN, creatinine, sodium, potassium, calcium, phosphorus, albumin and plasma glucose levels from fasting blood samples were measured with "Roche Hitachi Cobas 6000; Japan" system by using standard enzymatic methods. High sensitive CRP (hs-CRP) was measured at Florence Nightingale Hospital Microbiology Laboratory with "Boeki Medical System Prestige 24i; Japan" by using immunoturbidimetry method.

Subjects underwent body mass index (BMI) (kg/m^2) calculation. Renal functions were determined using Mayo clinic Cockcroft-Gault formula: estimated glomerular filtration rate =[(140-age) x weight (kg)]/72 x serum creatinine (mg/dL) (x0.85 if female).

Serum Fetuin-A measurement

Blood samples were stored under standardized conditions at -80°C until analysis. Serum fetuin-A levels were measured by "Biovendor Research and Diagnostic Products; Czech Republic" ELISA kit method according to the manufacturer's protocol. The intra- and interassay variations were 5.3% and 7.1%, respectively.

Statistical analysis

Statistical analyses were conducted with a commercially available software package (SPSS version 15.0, SPSS, Chichago, IL). In this study, data are expressed as mean±SD for continuous variables and as counts and percentage for categorical variables. Student`s t- test was used for comparison of continuous variables, Chi-square and Fisher's exact tests were used for comparison of categorical variables. Correlations of continuous variables were evaluated using Pearson correlation analysis. Linear regression analysis was performed to explore independent factors associated with MAC development. Receiver

operator curve (ROC) analysis were used to determine the diagnostic accuracy of fetuin-A level and CIMT for prediction of MAC development.

Results

Clinical and demographic characteristics of the MAC and control groups are presented in Table 1. There were no statistically significant differences in baseline characteristics between the two groups (Table 1).

In the MAC group; (70%) 28 subjects had mild, (25%) 10 subjects had moderate and (5%) 2 subjects had severe MAC.

Relation between mitral annular calcification and endothelial function

Endothelial dependent vasodilatory response was significantly decreased in MAC group compared to the control group (5.8±2.9% vs. 7.6±3.7%, p=0.02). However NDVR was similar between two groups (6.8±3.6% vs. 8.3±6.9% in p=0.22) (Fig. 1).

Relation between mitral annular calcification and carotid intima media thickness

Mean CIMT was markedly increased in MAC group compared to the control group (1.13±0.2 mm vs 0.94±0.3 mm, p<0.0001) (Fig. 2.)

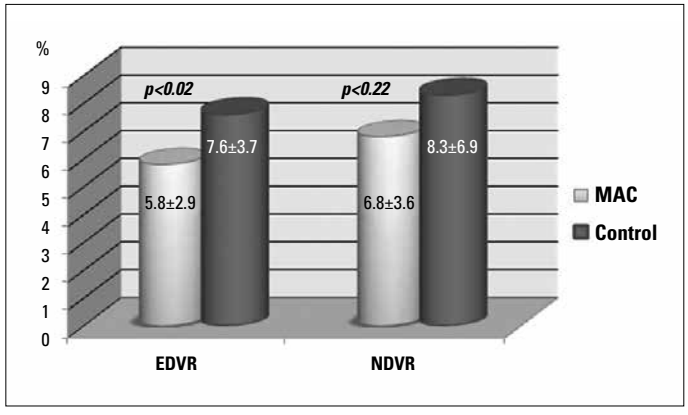

Figure 1. Mean EDVR and NDVR values of MAC and control groups (Student t test)
EDVR - endothelium- dependent vasodilatory response, MAC - mitral annular calcification, NDVR - nitrate-dependent vasodilatory response

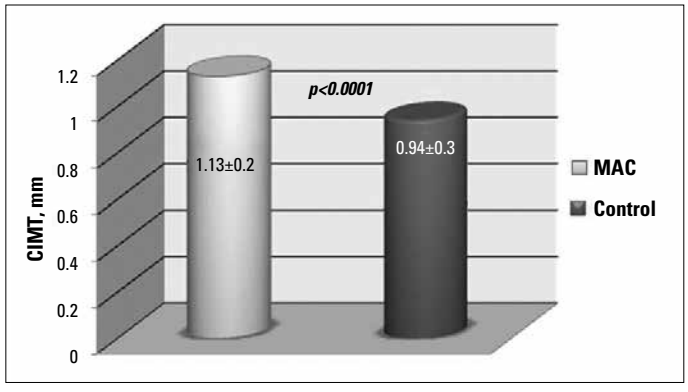

Figure 2. Mean CIMT of MAC and control groups (Student t-test)
CIMT - carotid intima media thickness, MAC - mitral annular calcification

Relation between mitral annular calcification and fetuin-A level

Mean serum fetuin-A level was significantly lower in the in MAC group compared to the control group (2.9±0.1 ng/mL vs. 3.0±0.2 ng/mL, p<0.0001) (Fig. 3)

Mitral annular calcification degree was inversely correlated with serum fetuin-A level. Mean serum fetuin-A level was 2.91±0.10 ng/mL in mild MAC group, 2.85±0.13 ng/mL in moderate MAC group and 2.83±0.13 ng/mL in severe MAC group. However, differences between groups were not statistically significant (Fig. 4).

Correlation and regression analysis

Correlation analysis marked a strong positive correlation between EDVR and serum fetuin-A level (r=0.682, p<0.0001). There was a strong negative correlation between EDVR with CIMT (r=-0.514, p=0.001) and hsCRP (r=-0.545, p<0.0001). There was a moderate negative correlation between CIMT and serum fetuin-A level (r=-0.482, p=0.002) (Fig. 5). Simple linear regression analysis revealed that, only CIMT and serum fetuin-A level were independent risk factors for MAC (Table 2).

ROC analysis

The ROC curves for accuracy of serum fetuin-A level and CIMT for predicting MAC are shown in Figure 6A and 6B. The

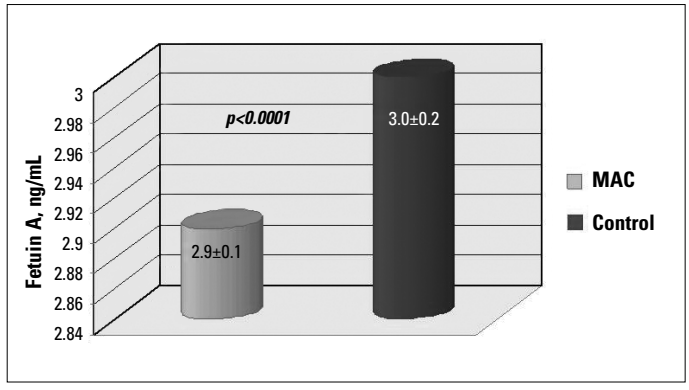

Figure 3. Mean serum fetuin-A levels of MAC and control groups (Student t-test)
MAC - mitral annular calcification

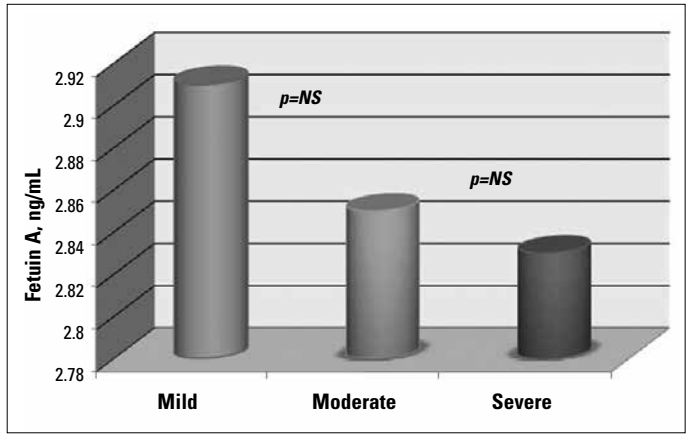

Figure 4. Relation between MAC severity and serum fetuin-A level ("$\chi 2$" and "Fisher's exact" tests)
MAC - mitral annular calcification

area under curve (AUC) for serum fetuin-A level was 0.731 [95% CI 0.620-0.824]. A cut off value of 2.93 ng/mL for serum fetuin-A level was associated with 72.5% sensitivity and 62.5% specificity in prediction of MAC. The AUC for CIMT was 0.724 (95% CI 0.613-0.818). A cut off value of 1 mm for CIMT was associated with 67.5% and 65% specificity for prediction of MAC.

Discussion

In the present study, we investigated the relationship between MAC and cardiovascular risk factors. We revealed that CIMT and serum fetuin-A level were independent factors associated with MAC, which can be used as an early determinant of CAD.

Table 2. Linear regression analysis results

Variables	*β	*p
Serum fetuin-A	-0.291	0.009
CIMT	0.367	0.001
EDVR	-0.145	0.18
NDVR	-0.087	0.45
Age	0.063	0.57
Gender	-0.035	0.75
Systolic blood pressure	-0.168	0.14
Diastolic blood pressure	-0.018	0.87
BMI	0.054	0.64
Smoking	0.047	0.66
Diabetes	-0.097	0.36
Hypertension	-0.066	0.55
Hyperlipidemia	0.063	0.54
Total cholesterol	-0.104	0.69
LDL cholesterol	0.159	0.55
Hs-CRP	0.194	0.07

*Linear regression analysis
BMI - body mass index, CIMT - carotid intima media thickness, EDVR - endothelial dependent vasodilatory response, hs-CRP - high sensitive CRP, LDL - low density lipoprotein, NDVR - nitrate dependent vasodilatory response

MAC is a degenerative pathology which primarily effects posterior region of the mitral annulus. MAC has been associated with age, cardiovascular diseases, stroke, and congestive heart failure (5, 7-11). Although the mechanism of MAC is not clearly understood, its predictive value in atherosclerosis is supported by several studies (22-24).

It is widely known that endothelium dysfunction is the initial lesion in developing atherosclerosis. Despite the fact the association between aortic calcification and endothelium dysfunction has been shown before by Poggianti et al. (25) there is hardly any literature available investigating MAC and endothelium dysfunction. Hence, in this study we intended to investigate the relationship between MAC and endothelial dysfunction. The patients in the MAC group had statistically lower EDVR than patients with only CAD, while NDVR was slightly impaired, not reaching significance. We demonstrated a strong significant association between the presence of MAC and endothelial dysfunction.

Endothelial dysfunction, which is evaluated by CIMT measurement, is regarded as the manifestation of atherosclerosis (26, 27). There are few studies investigating the association between MAC and CIMT. Sgorboni et al. (28) showed that patients with MAC had higher CIMT measurements. In this pres-

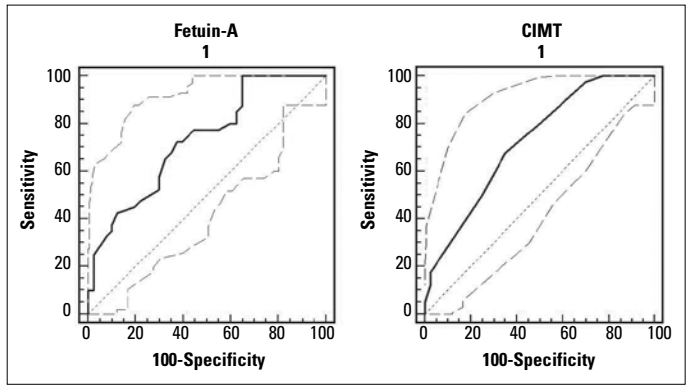

Figure 6. A) ROC curve analysis of the performance of serum fetuin-A level for diagnosing MAC. B) ROC curve analysis of the performance of CIMT for diagnosing MAC
CIMT - carotid intima-media thickness, MAC - mitral annular calcification

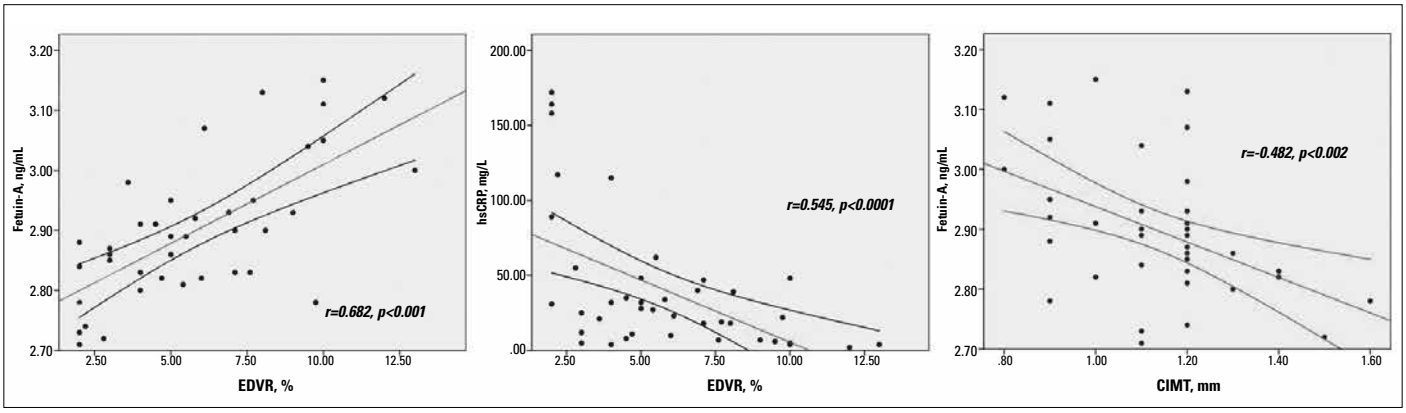

Figure 5. A) Correlation between serum fetuin-A level and EDVR (Pearson correlation analysis). B) Correlation between EDVR and hsCRP (Pearson correlation analysis). C) Correlation between CIMT and serum fetuin-A level (Pearson correlation analysis)
CIMT - carotid intima-media thickness, EDVR - endothelium-dependent vasodilatory response, hsCRP - high-sensitive C-reactive protein

ent study MAC group had statistically higher levels of CIMT measurements than the control group. Also, there was a strong negative correlation between the EDVR and CIMT.

Fetuin-A, a hepatic secretory glycoprotein is accepted as a vascular and valvular calcification inhibitor. Fetuin-A is a negative acute phase reactant having a prognostic importance in patients with acute coronary syndrome. It is suggested to be an anti-inflammatory mediator, which plays an important role in macrophage deactivation and decreased fetuin A level prevents the activities of several anti-inflammatory molecules. Many studies investigated the relationship between fetuin-A and cardiovascular calcification. Stenvinkel et al. (29) showed that decreased fetuin-A level was associated with inflammation, atherosclerosis, cardiovascular mortality and all causes mortality in end stage renal failure patients. Moe et al. (19) demonstrated the negative correlation between serum fetuin-A level and coronary calcification and suggested that fetuin-A plays a prominent role in atherosclerotic process. There are few studies which investigated the relationship between fetuin-A and MAC. Sgorbini et al. (28) observed a negative correlation between MAC and fetuin-A levels in a group of CAD patients with normal or mildly impaired renal functions. Supporting these results Ix et al. (30) revealed a significant, negative association between MAC and serum fetuin-A level. In the present study, fetuin-A level in the MAC group was significantly decreased, compared to the control group. Additionally correlation analysis showed that there is a strong positive correlation between EDVR and serum fetuin-A levels and a strong negative correlation between CIMT and fetuin-A levels. Our results suggest a close relationship between MAC and atherosclerotic process.

Study limitations

In order to make MAC and the control groups homogeneous, subjects with CAD were involved. However, the scarcity of our patient population is the major limitation of our study. Another limitation is the lack of classification of the patients according to their angiographic findings. Further studies with larger cohorts of patients are warranted to reveal the relationship of MAC with atherosclerotic risk factors.

Conclusion

In the present study, we demonstrated that MAC is associated with endothelial dysfunction expressed as impaired flow mediated dilation of the brachial artery as well as CIMT and decrease in plasma fetuin-A level. Our findings may reflect the close association between MAC and cardiovascular risk factors in patients with CAD, based on fetuin A and CIMT evaluation. Consequently, we suggest MAC may prove to be a useful marker of inflammation and calcification regarding patients with CAD.

Conflict of interest: None declared.

Peer-review: Externally peer-reviewed.

Authorship contributions: Concept - M.Z.; Design - Y.T.; Supervision - S.A.; Resource - Ö.Y.; Materials - M.Z.; Data collection&/or Processing - M.Z.; Analysis &/or interpretation -Ş.T.Ş.; Literature search - Ş.T.Ş.; Writing - Ş.T.Ş.; Critical review - S.Y.; Other - S.A.

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