The relationship between coronary calcification and the metabolic markers of osteopontin, fetuin-A, and visfatin

Osteopontin, fetuin-A, visfatin metabolik belirteçleri ile koroner kireçlenme arasındaki ilişki

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Objectives: We investigated whether coronary calcification detected by multislice computed tomography (MSCT) was correlated with plasma osteopontin, serum fetuin-A, and visfatin levels.

Study design: The study included 64 consecutive patients (51 males, 13 females; mean age 49.5±10.9 years; range 33 to 78 years) who underwent MSCT for suspected coronary artery disease. Coronary artery calcification (CAC) scores of the patients were calculated using the Agatston scoring method. Plasma osteopontin, serum fetuin-A, and visfatin levels were measured from fasting blood samples and correlations were sought with calcium scores.

Results: Coronary calcification was detected in 32 patients (50%). The mean CAC score was 146.5±333.7 Agatston units (AU), indicating an intermediate risk for coronary artery disease. In 10 patients (15.6%), the CAC score exceeded 400 AU. The mean fetuin-A, visfatin, and osteopontin levels were 25.6±6.4 ng/ml, 19.7±47.2 ng/ml, and 20.4±16.1 ng/ml, respectively. Serum visfatin (r=0.15, p=0.37) and fetuin-A (r=0.17, p=0.22) were not correlated with the CAC score, whereas plasma osteopontin level showed a moderate correlation with the CAC score (r=0.35; p=0.008). In ROC analysis, the area under the curve for identification of CAC was greatest for osteopontin (0.741; p=0.004), followed by fetuin-A (0.574; p=0.31), and visfatin (0.580; p=0.27). The cut-off value was 18.45 ng/ml for osteopontin, with a sensitivity of 72% and specificity of 73%.

Conclusion: Our results suggest that there might be an association between CAC and plasma osteopontin levels. Research should continue to find out a metabolic parameter that will strongly indicate coronary calcification.

Key words: Atherosclerosis/metabolism; calcification; coronary artery disease/radiography; osteopontin; tomography, X-ray computed.

Amaç: Bu çalışmada, çokkesitli bilgisayarlı tomografi (ÇKBT) ile belirlenen koroner arter kireçlenmesinin, plazma osteopontin, serum fetuin-A ve visfatin düzeyleri ile ilişkili olup olmadığı araştırıldı.

Çalışma planı: Çalışmaya, koroner arter hastalığı şüphesiyle ÇKBT ile incelenen 64 ardışık hasta (51 erkek, 13 kadın; ort. yaş 49.5±10.9; dağılım 33-78) alındı. Hastaların koroner kalsiyum skorları (KKS) Agatston ölçüm yöntemi kullanılarak hesaplandı. Açlık kan örneklerinde plazma osteopontin, serum fetuin-A ve visfatin düzeyleri ölçüldü ve bunların kalsiyum skorlarıyla ilişkisi araştırıldı.

Bulgular: Otuz iki hastada (%50) koroner kireçlenme saptandı. Ortalama KKS 146.5±333.7 Agatston ünitesi (AÜ) bulundu; bu değer koroner arter hastalığı için orta derecede riski göstermekteydi. On hastada (%15.6) skor 400 AÜ'nün üzerindeydi. Ortalama fetuin-A, visfatin ve osteopontin düzeyleri sırasıyla 25.6±6.4 ng/ml, 19.7±47.2 ng/ml ve 20.4±16.1 ng/ml bulundu. Serum visfatin ve fetuin-A düzeyleri kalsiyum skoruyla anlamlı ilişki göstermezken (r=0.15, p=0.37 ve r=0.17, p=0.22), plazma osteopontin düzeyi kalsiyum skoruyla orta derecede ilişki gösterdi (r=0.35; p=0.008). Koroner kireçlenmeyi öngörmek için yapılan ROC analizinde, eğri altındaki en büyük alan osteopontine aitti (0.741; p=0.004); bunu fetuin-A (0.574; p=0.31) ve visfatin (0.580; p=0.27) izlemekteydi. Osteopontin için kesim değeri 18.45 ng/ml (duyarlılık %72, özgüllük %73) bulundu.

Sonuç: Bulgularımız KKS ile plazma osteopontin düzeyi arasında ilişki olabileceğini göstermektedir. Koroner kireçlenmeyi daha güçlü şekilde öngörmeyi sağlayacak metabolik parametreler için araştırmalar sürdürülmelidir.

Anahtar sözcükler: Ateroskleroz/metabolizma; kalsifikasyon; koroner arter hastalığı/radyografi; osteopontin; bilgisayarlı tomografi.

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Coronary artery disease is the dominant chronic disease in many parts of the world. New screening tools have been consistently investigated for detection of atherosclerosis. Because of the noninvasive nature of computed tomography (CT), there is great interest in developing CT-based techniques for detection of coronary artery calcium, a known marker underlying atherosclerosis. Recent studies have demonstrated that there is a strong relationship between vascular calcification and atherosclerosis.[1]

Vascular calcification was previously supposed to be a passive and degenerative process of aging. However, accumulating evidence shows that vascular calcification is an active process in which many biochemical markers may take place.[2] Human fetuin-A (α2-Heremans-Schmid glycoprotein) is a 62-kD glycoprotein secreted from hepatocytes.[3] Fetuin-A inhibits vascular calcification by preventing *de novo* formation of hydroxyapatite crystals.[4] A reverse correlation was shown between cardiovascular mortality and fetuin-A levels in patients undergoing hemodialysis for impaired renal function.[5] Additionally, accumulation of fetuin-A was demonstrated in calcified vessel lesions of patients with renal failure.[6,7] However, studies concerning fetuin-A are generally limited to patients with impaired renal function.

Adipokines have also been recognized to be expressed within atherosclerotic lesions, suggesting local and endocrine effects on atherosclerotic plaques.[8,9] Although visfatin is noticeably expressed in symptomatic atherosclerotic carotid plaques,[10] there is no published study investigating its relationship with coronary atherosclerosis.

Osteopontin is known as cytokine Eta-1 (early T-lymphocyte activation 1) and was first described in 1979.[11] It consists of 314 amino acids and is localized on chromosome 3q14. It is expressed by macrophages in atherosclerotic lesions.[12] Furthermore, it has been shown that osteopontin levels are increased in patients with vascular calcification[13] and in patients with coronary artery disease.[14]

This study was designed to determine whether coronary calcification detected by multislice CT (MSCT) was correlated with plasma osteopontin, serum fetuin-A, and serum visfatin levels.

PATIENTS AND METHODS

Patients. A total of 72 patients from whom MSCT was requested for preliminary diagnosis of coronary artery disease were consecutively enrolled in the study. Eight patients were excluded due to the presence of C–reactive protein level exceeding 10 mg/dl. Sixty-four patients (51 males, 13 females; mean age 49.5±10.9 years; range 33 to 78 years) were finally evaluated.

Before coronary MSCT angiography, a detailed history was obtained and all patients underwent standardized physical examination including measurements of height and body weight. Diabetes mellitus was defined by history of the disease or the presence of fasting glucose ≥126 mg/dl, hypertension was defined by history or the presence of blood pressure ≥140/80 mmHg. The study was approved by the local ethics committee and written informed consent was obtained from each patient.

Multislice computed tomography. All patients were given 0.4 mg of sublingual nitroglycerin shortly before the procedure. Patients with a heart rate >65 beats/min received metoprolol 5-10 mg intravenously before MSCT examination (Somatom Sensation 64, Siemens, Forchheim, Germany). The scan parameters were as follows: gantry rotation time 330 msec, tube voltage 120 kV, tube current 250 mAs, detector collimation 0.6 mm. The contrast agent (80-100 ml; 350 mg iodine/ml) was given intravenously (5.0 ml/sec). Image reconstruction of the raw data was started at 70% of the R-to-R interval using a medium-smooth convolution kernel of B30f. In patients with clinically doubtful diagnosis, segments were individually modified for reconstructions.

Calcium scoring was carried out on the reconstructed image set with commercially available software (Syngo CaScore, Siemens, Forchheim, Germany) using the Agatston scoring method.[14] A standard scoring threshold of 130 Hounsfield units was utilized during the procedure. The overall calcium score was calculated from the scores of the individual calcifications.

Biochemical analysis. Fasting blood samples were collected by venipuncture. The samples were centrifuged at 3000 rpm for 5 min within 20 minutes of collection. Blood samples were stored at -80 °C until analysis. Serum fetuin-A levels were determined with an enzyme-linked immunosorbent assay (ELISA) kit. The assay procedure was performed as described by the manufacturer (Human Fetuin-A ELISA, BioVendor, Heidelberg, Germany). Serum visfatin was measured using a commercially available ELISA kit (Phoenix Pharmaceuticals, Burlingame,

Table 1. Clinical and laboratory characteristics of the patients

	Overall (n=64)			Calcium=0 (n=32)			Calcium>0 (n=32)			
	n	%	Mean±SD	n	%	Mean±SD	n	%	Mean±SD	*p*
Age (years)			49.5±10.9			41.4±9.7			57.6±14.4	**<0.05**
Body mass index (kg/m²)			28.8±5.1			28.5±4.1			29.9±7.9	N S
Systolic blood pressure (mmHg)			131.2±16.4			131.2±16.4			126.5±21.3	N S
Pulse pressure (mmHg)			50.9±12.2			50.9±12.2			53.5±12.1	N S
Smoker	29	45.3		16	50.0		13	40.6		N S
Arterial hypertension	27	42.2		12	37.5		15	46.9		N S
Diabetes	12	18.8		5	15.6		7	21.9		N S
Family history of CAD	25	39.1		11	34.4		14	43.8		N S
Biochemical parameters										
Serum creatinine (mg/dl)			1.1±0.2			1.0±0.3			1.1±0.2	N S
High-sensitive CRP (mg/dl)			2.8±2.5			2.8±2.8			3.0±2.2	N S
Total cholesterol (mg/dl)			212.1±130.4			197.5±51.1			226.8±172.5	N S
LDL-cholesterol (mg/dl)			117.7±43.4			109.6±39.1			125.9±46.4	N S
HDL-cholesterol (mg/dl)			45.1±10.6			42.8±9.8			47.3±11.1	N S
Triglyceride (mg/dl)			176.1±135.6			225.4±228.2			126.8±74.6	N S
Serum fetuin-A (ng/ml)			25.6±6.4			24.8±6.3			26.4±6.6	N S
Plasma osteopontin (ng/ml)			20.4±16.1			14.3±15.4			24.5±15.8	**<0.05**
Serum visfatin (ng/ml)			19.7±47.2			21.8±55.4			17.6±38.2	N S

NS: Not significant; CAD: Coronary artery disease; CRP: C-reactive protein.

CA, USA). Plasma osteopontin was measured with a sandwich ELISA method using a commercially available kit (Human Osteopontin, Assay Designs, Ann Arbor, MI, USA).

Total cholesterol, triglyceride, HDL-cholesterol, high-sensitive C-reactive protein, and other biochemical parameters were measured on a Beckman Coulter LX 20 analyzer (Beckman Coulter, Brea, CA, USA). LDL-cholesterol concentration was calculated using the Friedewald formula.[15]

Statistical analysis. Statistical analyses were performed using SPSS 13.0 statistical package. Continuous variables were expressed as mean±standard deviation, and categorical variables as percentages. Differences between groups were tested using the Student t-test, Mann-Whitney U-test, or chi-square test, as appropriate. The variables were tested for normal distribution with the Kolmogorov-Smirnov test. For calculation of correlations, we used Spearman correlation coefficients. A *p* value of less than 0.05 was considered statistically significant. Receiver operating characteristic (ROC) curves and respective areas under the ROC curve were calculated for all parameters. The cut-off values were determined using the Youden's index, J=sensitivity+specificity-1. The highest J value was accepted as the cut-off value for that parameter. Sensitivity and specificity of osteopontin were determined using the cut-off value. The factors affecting the constitution of coronary calcification were investigated by the analysis of covariance (ANCOVA) test. The effect of age on the coronary artery calcification (CAC) score was eliminated using the ANCOVA test.

RESULTS

The clinical features and biochemical parameters of the patients are summarized in Table 1. The mean CAC score of the patients was 146.5±333.7 Agatston units (AU), indicating an intermediate risk for coronary artery disease. The mean fetuin-A, visfatin, and osteopontin levels were measured as 25.6±6.4 ng/ml, 19.7±47.2 ng/ml, and 20.4±16.1 ng/ml, respectively.

Coronary calcification was detected in 32 patients (50%). In 10 patients (15.6%), the CAC score was over 400 AU. Patients with coronary calcification had a higher mean age and a higher osteopontin level compared to patients without CAC ($p<0.05$).

Neither serum visfatin ($r=0.15$, $p=0.37$) nor fetuin-A ($r=0.17$, $p=0.22$) levels were correlated with the CAC score. However, there was a weak to moderate correlation between the CAC score and plasma osteopontin level ($r=0.35$; $p=0.008$).

In the ANCOVA test and after eliminating the effect of age on the CAC score, no significant effect of gender, diabetes mellitus, hypertension, dyslipidemia, or smoking was found on the constitution of coronary calcification.

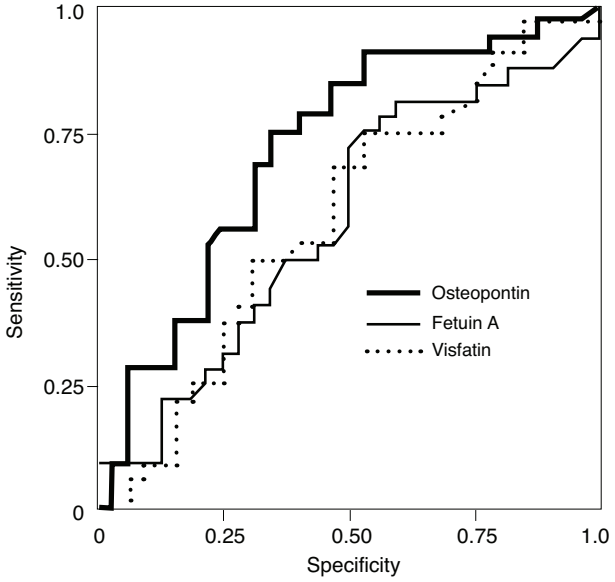

Figure 1. Receiver operating characteristic curves for osteopontin, fetuin-A, and visfatin in the identification of coronary calcification. The area under the curve was 0.741 for osteopontin, 0.574 for fetuin-A, and 0.580 for visfatin.

In ROC analysis, the area under the curve for identification of coronary calcification was greatest for osteopontin (0.741; p=0.004) followed by fetuin-A (0.574; p=0.31), and visfatin (0.580; p=0.27; Fig. 1). The cut-off value was determined as 18.45 ng/ml for osteopontin, with a sensitivity of 72% and specificity of 73%.

DISCUSSION

The major findings of this study are that coronary calcification determined by MSCT-based CAC scoring is associated with the blood level of osteopontin but not with the levels of fetuin-A and visfatin.

The central physiological function of osteopontin is the control of biomineralization by inhibiting calcification in bone.[16] In addition to osteoblasts and chondrocytes, it is biosynthesized by a variety of tissue types including dendritic cells, macrophages, smooth muscle, endothelial cells, brain, kidney, decidua, and placenta. Osteopontin is involved in diverse biological and pathophysiological processes in multiple organs and tissues.[17] Being a cell-secreted protein with pleiotropic functions, osteopontin has been implicated in tissue repair, remodeling, and inflammation.[18] Studies from noncardiac cells demonstrate that osteopontin plays a critical role in tissue remodeling by modulating angiogenesis and extracellular matrix organization. Blood vessels express low levels of osteopontin under normal conditions. The atherosclerotic lesion is highly inflammatory and, like other chronic inflammatory diseases, is characterized by the persistence of macrophages and other immune cells. Advanced lesions become complex; they are filled with smooth muscle cells and are characterized by the presence of an extensive extracellular matrix and a large necrotic core filled with cholesterol clefts. In very advanced lesions, the matrix is often mineralized. Nevertheless, macrophages and foam cells are persistent even in very advanced lesions. It has been shown that osteopontin is highly expressed in human as well as experimental animal atherosclerotic lesions, especially associated with macrophages and foam cells.[12] Our findings demonstrate that osteopontin has a critical role in calcification of the vessel wall during the atherosclerotic process.

It is believed that vascular calcification is a consequence of actively regulated processes including several factors.[2] Identification of these factors are important with regard to the etiopathological mechanisms underlying coronary calcification. To date, many markers have been investigated as histopathological mechanisms of vascular calcification. In human atherosclerotic lesions, osteopontin is expressed in smooth muscle cells in the lesion, in angiogenic endothelial cells, and in macrophages.[19] Under conditions of injury and disease, osteopontin appears to be an important regulator of vascular calcification and is associated with mineralized deposits.[19]

In human carotid arteries from endarterectomy samples, osteopontin was found to be associated with calcium deposits in fibroatheroma lesions.[20] It has also been shown as an indicator of plaque stabilization in carotid artery plaques.[21] In patients with chronic stable angina, plasma osteopontin levels were indicated as an independent predictor for cardiac events.[22]

Ohmori et al.[13] demonstrated that plasma osteopontin levels were associated with the presence and extent of coronary artery disease, suggesting a critical role of osteopontin in the development of atherosclerotic plaques. Similar to our results, they also showed a relationship between coronary calcification and plasma osteopontin levels. However, they calculated the burden of coronary calcification using volumetric measurements of calcified vessel wall on conventional coronary angiograms. We demonstrated, for the first time in the literature, the relationship between osteopontin and coronary calcification determined by the MSCT-based CAC scoring system. Besides its useful-

ness, the advantage of the CT-based CAC scoring system is that, during calculation of calcium burden, it takes into account not only the volume, but also the intensity of the calcification.

Serum concentrations of fetuin-A are depressed in patients with end-stage renal disease, and it has been shown that lower serum concentrations are independently associated with the risk for cardiovascular and all-cause mortality in this population.[5] It has also been demonstrated that fetuin-A serves as an important inhibitor of dystrophic calcification in patients with coronary heart disease.[7] Our findings are parallel to the data of Roos et al.[3] who evaluated the association of the CAC score with plasma fibroblast growth factor 23 and fetuin-A levels. They also did not find any relationship between vascular calcification and fetuin-A levels.

Visfatin is produced by adipose tissue, bone marrow, skeletal muscle, and liver. This adipokine serves actively in many biochemical processes. Zhong et. al.[23] showed increased serum visfatin levels in patients with metabolic syndrome. In our study, we did not find any correlation between serum visfatin levels and the CAC score. However, studies should continue to elucidate the role of visfatin in diverse patient groups.

In conclusion, a powerful metabolic parameter has yet to be found, which will predict CAC with the use of MSCT in patients with suspected coronary artery disease. It seems that there might be an association between CAC and plasma osteopontin levels. Research should continue to find out a metabolic parameter that will strongly indicate coronary calcification.

Study limitations. One limitation of our study is that it was conducted in a relatively small number of patients with suspected coronary artery disease. Therefore, further studies should involve large patient series and diverse patient groups.

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