Increased neopterin levels and its association with angiographic variables in patients with slow coronary flow: an observational study

Yavaş koroner akımlı hastalarda artmış neopterin düzeyleri ve anjiyografik değişkenlerle birlikteliği: Gözlemsel bir çalışma

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ABSTRACT

Objective: Although various inflammatory markers have been studied in patients with slow coronary flow (SCF), serum neopterin levels have not been studied previously. We investigated the serum neopterin and high sensitivity C-reactive protein (hs-CRP) levels and the relationship between neopterin and hs-CRP levels and TIMI flow in patients with SCF.

Methods: The study group consisted of 51 patients with SCF. An age and gender matched control group was composed of 40 subjects. Coronary flow rates of all patients and control subjects were documented by Thrombolysis in Myocardial Infarction (TIMI) frame count.

We measured serum neopterin and hs-CRP levels at the same time in patients with SCF and control subjects in this cross-sectional observational study. Chi-square, Mann-Whitney U and unpaired t tests, Pearson correlation and linear regression analyses were used for statistical analysis.

Results: The TIMI frame counts for all coronary arteries and the mean TIMI frame count were significantly higher in the SCF group than controls. Serum neopterin levels were significantly higher among patients with SCF when compared with control group (2.13±1.03 vs. 1.60±0.50 ng/ml; p=0.004). Serum hs-CRP levels were significantly higher among patients with SCF when compared with control group (2.06±1.32 vs. 0.74±0.40 mg/L respectively; p<0.001). There was a significant association of serum neopterin levels (β=0.60, 95% CI: 4.93-9.06, p<0.001) and serum hs-CRP levels (β=0.29, 95%CI: 0.84-4.33, p=0.004) with mean TIMI frame count independent of potential confounders such as age, gender, body mass index, smoking, glucose and cholesterol levels.

Conclusion: We have shown that serum neopterin and hs-CRP levels were significantly elevated in patients with SCF when compared with control subjects. Serum neopterin and hs-CRP levels were correlated with mean TIMI frame count in patients with SCF.

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Key words: Slow coronary flow, neopterin, high-sensitive C-reactive protein, regression analysis

ÖZET

Amaç: Her ne kadar yavaş koroner akım (YKA)'lı hastalarda çeşitli enflamasyon göstergeleri çalışılmış olsa da, serum neopterin seviyeleri çalışılmamıştır. Yavaş koroner akım (YKA)'lı hastalarda serum neopterin ve yüksek duyarlı C-reaktif protein (hs-CRP) düzeylerini ve serum neopterin ve hs-CRP düzeyleriyle TIMI kare sayısı arasındaki ilişkiyi araştırdık.

Yöntemler: Çalışma grubu 52 YKA hastasından, yaş ve cinsiyet açısından eşitlenmiş kontrol grubu 40 kişiden oluşmaktaydı. Hastaların ve kontrol grubunun koroner akım hızları (Thrombolysis In Myocardial Infarction) TIMI kare sayısı yöntemi ile ölçüldü. Bu enine-kesitli gözlemsel çalışmada, YKA ve kontrol grubunda serum neopterin ve hs-CRP düzeylerini ölçtük. İstatistiksel analizde Ki-kare, Mann-Whitney U ve esleştirilmemiş t testleri, Pearson korelasyon ve doğrusal regresyon analizleri kullanıldı.

Bulgular: Bütün koroner arterlerin TIMI kare sayıları ve ortalama TIMI kare sayısı YKA hastalarında normal koroner arter grubundan daha yüksekti. Serum neopterin düzeyi YKA hastalarında kontrol grubundan daha yüksekti (2.13±1.03'e karşın 1.60±0.50 ng/ml sırasıyla; p=0.004). Serum hs-CRP düzeyi YKA hastalarında kontrol grubundan daha yüksekti (2.06±1.32'e karşın 0.74±0.40 mg/L sırasıyla; p<0.001). Serum neopterin (β=0.60, %95GA: 4.93-9.06, p<0.001) ve serum hs-CRP düzeyleri (β=0.29, %95GA: 0.84-4.33, p=0.004) yaş, cinsiyet, vücut kitle indeksi, sigara içiciliği, ve kolesterol düzeylerinden bağımsız olarak ortalama TIMI kare sayısı ile ilişkili idi.

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Sonuç: Bu çalışmada YKA hastalarında serum neopterin ve hs-CRP düzeylerinin kontrol grubundan anlamlı olarak daha yüksek olduğunu gösterdik. Serum neopterin ve hs-CRP düzeyleri ortalama TIMI kare sayısı ile korelasyon gösteriyordu.
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Anahtar kelimeler: Yavaş koroner akım, neopterin, yüksek duyarlı C-reaktif protein, regresyon analizi

Introduction

Slow coronary flow (SCF) is an angiographic finding characterized by slow antegrade progression of contrast agent to the distal branch of a coronary artery in the absence of obstructive coronary artery disease (1). Several mechanisms have been proposed for etiology of SCF, including microvascular and endothelial dysfunction, small-vessel disease and diffuse atherosclerosis (1-4). It has also been proposed that inflammation is another important etiologic factor (5-10). Its etiopathogenesis is not still clear. The importance of SCF phenomenon results from its association with angina pectoris, acute myocardial infarction, hypertension and sudden cardiac death (11).

Neopterin, a pteridine derivative and a byproduct of the guanosine triphosphate-biopterin pathway, is produced by activated macrophages and is thought to represent a marker of immune activation and macrophage activity (12). Increased serum neopterin levels were found in patients with pronounced peripheral atherosclerosis (13) and in patients with carotid atherosclerosis (14). It has been shown to be elevated in the serum of patients with unstable angina and acute myocardial infarction as compared with control subjects and patients with stable angina pectoris (15, 16). Neopterin levels were associated with CAD extent in stable patients, thereby emphasizing the inherent role of inflammation in atherogenesis itself beyond plaque destabilization (17).

However, the relation between SCF and serum neopterin levels has not been investigated previously.

Accordingly, we aimed to measure the plasma neopterin and high sensitivity C-reactive protein (hs-CRP) levels at the same time in patients with SCF to show the possible role of inflammatory processes (monocyte/macrophage activity) in the underlying pathology of SCF and we also aimed to investigate the relationship between neopterin and hs-CRP levels and thrombolysis in myocardial infarction (TIMI) flow.

Methods

Study design and patients

This was a cross-sectional observational study.

The study group consisted of 51 patients with SCF (27 females, 24 males, mean age 55.2±8.9 years). An age and gender matched control group was composed of 40 subjects (21 females, 19 males with a mean age 56.0±5.4 years).

All the patients with SCF underwent coronary angiography in our clinic for the evaluation of coronary artery disease. The control subjects presented with atypical chest pain for which elective coronary angiography was performed and subsequently were found to have normal coronary arteries.

None of the patients or control subjects were taking any medications (such as statins) affecting neopterin levels at the time of blood sampling. Hypertension was considered to be present if the systolic pressure was >140 mmHg and/or diastolic pressure was >90 mmHg or if the individual was taking antihypertensive medications. Diabetes mellitus was defined as a fasting blood glucose level >126 mg/dl or current use of a diet or medication to lower blood glucose. Patients who were smoking before hospitalization was accepted as smokers.

Patients with coronary artery disease, acute coronary syndromes, previous myocardial infarctions, left ventricular dysfunction, valvular heart disease, renal and hepatic failure, peripheral vascular disease, immunologic or inflammatory diseases, sepsis, active local or systemic infections, a history of recent infection (<3 months before the study), and a history of malignancy were excluded from the study.

The study was approved by the institutional ethics committee, and informed consent was obtained from all patients.

Coronary angiography

Coronary angiography was routinely performed without the use of nitroglycerin. Selective coronary angiography was performed by means of the Judkins technique in multiple projections. We used iohexol (Omnipaque) as contrast agent during coronary angiography in all patients and control subjects. Coronary blood flow was measured quantitatively using the

TIMI frame count which was derived from the number of cine-frames recorded from the first entrance of contrast to its arrival at the distal end of either the left anterior descending artery (LAD), circumflex artery (Cx), or right coronary artery (RCA) (18). TIMI frame count is a quantitative, simple, objective and reproducible index of coronary flow velocity (18). Initial frame count is defined as the frame in which concentrated dye occupies the full width of proximal coronary artery lumen, touching both borders of the lumen, and forward motion down the artery. The final frame is designated when the leading edge of the contrast column initially arrives at the distal end. The last frames used for the LAD, Cx and RCA were those in which the dye first entered the mustache segment, distal bifurcation segment, and first branch of the posterolateral artery, respectively. The final count was then subtracted from the initial count and the exact TIMI frame count was calculated for the given artery. The TIMI frame count of the LAD artery was corrected by dividing the final count by 1.7. The mean TIMI frame count for each patient and control subject was calculated by adding the TIMI frame counts for LAD, Cx and RCA and then dividing the obtained value into three. Coronary angiograms and TIMI frame counting were analyzed by two blinded interventional cardiologists with-

out knowledge of the clinical status and laboratory measurements of the subjects.

Due to different durations required for normal visualization of coronary arteries, the corrected cutoff values were 36.2±2.6 frames for LAD, 22.2±4.1 frames for Cx, and 20.4±3.0 frames for RCA, as has been reported earlier in the literature (18). Any values in excess of these thresholds were considered as SCF.

Biochemical analysis

Venous blood was collected at the time of coronary angiography from an antecubital vein with a 19-gauge needle without venous stasis. Sera of patients and controls were stored at -80°C. Neopterin levels in serum were determined in a competitive inhibition enzyme-linked immunosorbent assay using a commercial kit (DRG Diagnostics, Marburg, Germany) according to the manufacturer's instructions. Briefly, 25 µL of undiluted sera and standards were added to the appropriate wells of a 96-well microtiter plate followed by 100 µL enzyme conjugate. The plate was covered with black adhesive foil and incubated at room temperature for 2 hours on rotary horizontal shaker (200 rpm). After incubation, the contents of all wells aspirated and the plate was washed three times with phosphate-buffered saline (pH: 7.2) and 200 µL of color substrate (tetramethyl benzidine) was added into the wells as a substrate. Plate was incubated at room temperature for 30 minutes on a rotary horizontal shaker. The reaction was stopped with 100 µL of 1 mol/L H2SO4. The optical density was read at 450 nm in a micro titer plate reader and calculated the results using the data reduction system. The minimum limit of detection of serum neopterin was 0.2 ng / ml.

Hs-CRP was measured using chemiluminescent immunometric assay on IMMULITE 2000 Analyzer (DPC Cirrus Inc, Los Angeles, CA USA). The analytical sensivity of hs-CRP was 0.1 mg/L.

Statistical analysis

Data were analyzed with the SPSS software version 10.0 for Windows (SPSS Inc., Chicago, IL, USA). Continuous variables from the study groups were reported as mean±standard deviation, categorical variables as percentages. To compare continuous variables, the Student t-test or Mann-Whitney U test were used where appropriate. Categorical variables were compared with the Chi-square test. The correlations between neopterin, hs-CRP levels and mean TIMI frame count were performed with Pearson correlation analysis. The relationship between serum neopterin levels and mean TIMI frame count and serum hs-CRP levels and mean TIMI frame count were tested in a linear regression model adjusted for confounding factors such as age, gender, BMI, smoking, glucose and cholesterol levels. Standardized beta coefficients and 95% confidence intervals (CI) were calculated. Statistical significance was defined as $p<0.05$.

Results

Clinical characteristics

Clinical and laboratory characteristics of the patients with SCF and control group are presented in Table 1. There were no statistically significant differences between the two groups with respect to age, gender, body mass index, smoking, systolic and diastolic blood pressures, heart rate, ejection fraction and levels of glucose, creatinine, total cholesterol, triglyceride, low-density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, white blood cell. The TIMI frame count for all the epicardial coronary arteries and the mean TIMI frame count were significantly higher in the SCF group than control group.

Serum neopterin levels in SCF

Serum neopterin levels were significantly higher among patients with SCF when compared with control group (2.13±1.03 vs. 1.60±0.50 ng/ml respectively; p=0.004). Serum hs-CRP levels were significantly higher among patients with SCF when compared with control group (2.06±1.32 vs. 0.74±0.40 mg/L respectively; p<0.001).

Table 1. Clinical and laboratory characteristics of the patients with SCF and control group

Variables	SCF (n=51)	Control (n=40)	p*
Age, years	55.2±8.9	56.0±5.4	0.62
Sex, M/F	24/27	19/21	0.56
Smoking, n (%)	15 (29)	12 (30)	0.95
BMI, kg/m^2	28.9±3.1	27.7±3.0	0.06
SBP, mmHg	130.3±15.4	130.9±17.5	0.85
DBP, mmHg	82.2±10.9	82.8±10.7	0.80
Heart rate, beats/min	78.1±13.9	76.7±11.0	0.60
EF, %	65.7±3.0	65.0±2.5	0.19
Glucose, mg/dl	97.8±8.9	100.3±12.2	0.26
Creatinine, mg/dl	0.94±0.17	0.94±0.18	0.99
Total cholesterol, mg/dl	193.5±35.6	197.2±41.8	0.65
Triglycerides, mg/dl	160.4±59.9	142.0±55.4	0.13
LDL-cholesterol, mg/dl	112.1±28.6	117.9±37.5	0.40
HDL-cholesterol, mg/dl	48.0±12.5	47.9±10.2	0.98
WBC, ×10^3 mg/dl	7.1±1.6	7.1±1.8	0.91
hs-CRP, mg/L	2.06±1.32	0.74±0.40	<0.001
Neopterin, ng/ml	2.13±1.03	1.60±0.50	0.004
TIMI frame count			
LADc	40.2±6.2	16.1±4.4	<0.001
Cx	32.0±11.0	18.2±6.8	<0.001
RCA	24.9±2.7	15.4±6.1	<0.001
Mean frame count	31.4±9.1	16.4± 4.8	<0.001

Data are presented as mean±SD and proportions/percentages
*Student t-test and Chi-square test
BMI - body mass index, c - corrected TIMI frame count, Cx- circumflex artery, DBP - diastolic blood pressure, EF - ejection fraction, HDL - cholesterol-high density lipoprotein cholesterol, hs-CRP - high sensitive C-reactive protein, LAD - left anterior descending artery, LDL - cholesterol- low density lipoprotein cholesterol, M/F - male to female, RCA - right coronary artery, SBP - systolic blood pressure, SCF - slow coronary flow, TIMI - Thrombolysis in Myocardial Infarction, WBC - white blood cells

Relationship between serum neopterin levels and TIMI frame count

Serum neopterin levels were correlated with mean TIMI frame count (r=0.58, p<0.001) and serum hs-CRP levels were correlated with mean TIMI frame count (r=0.29, p=0.004) in whole study population (n=91) (Fig. 1-2).

In linear regression analysis, when serum neopterin levels were taken as dependent variable with other study variables which are potential confounders such as age, gender, BMI, smoking, glucose and cholesterol levels, serum neopterin levels were only independently correlated with mean TIMI frame count (β=0.60, 95% CI: 4.93 - 9.06, p<0.001). When serum hs-CRP levels were taken as dependent variable with other study variables which are potential confounders such as age, gender, BMI, smoking, glucose and cholesterol levels, serum hs-CRP levels were only independently correlated with mean TIMI frame count (β=0.29, 95%CI: 0.84 - 4.33, p=0.004).

Discussion

In this study, we found that serum neopterin and hs-CRP levels were significantly higher in patients with SCF compared to control subjects. We also found a significant association of neopterin and hs-CRP levels with TIMI frame count.

Several mechanisms have been proposed for the etiology of SCF, including occlusion of small vessels, increased microvascular resistance, and diffuse atherosclerosis (1-4). However, the exact underlying pathophysiological mechanisms as well as the clinical importance of this angiographic phenomenon are not fully understood at present.

Inflammation has been reported to be a major contributing factor to many cardiovascular event, and demonstrated to be associated with different clinical settings of coronary artery disease (19). Recently, inflammation has been suggested to play a role in the pathogenesis of SCF (5-10). Turhan et al. (5) performed a study, evaluating plasma soluble adhesion molecules; intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and E-selectin as possible indicators of endothelial activation or inflammation in patients with SCF, but with angiographically proven normal coronary arteries. They showed that serum ICAM-1, VCAM-1, and E-selectin concentrations of patients with SCF were significantly higher than those of control subjects with normal coronary flow suggesting the presence of a more severe and extensive chronic inflammation in the coronary circulation in these patients. Madak et al. (6) found increased serum levels of hs-CRP and NT-proBNP (accepted as an acute phase reactant) in patients with SCF when compared to normal subjects. Selçuk et al. (7) showed that serum concentrations of adiponectin, a modulator of the inflammatory response in the vascular wall, were decreased in patients with SCF and inversely correlated with mean TIMI frame count. Adiponectin has multiple protective effects on vascular endothelium through anti-inflammatory and anti-atherogenic properties. It has been suggested that loss of anti-inflammatory effects of adiponectin and increased endothelial expression of proinflammatory factors directly contribute to the endothelial dysfunction by allowing vascular proinflammatory reactions to occur more readily. Li et al. (8) showed that plasma levels of inflammatory factors, CRP and IL-6 in patients with SCF were found to be significantly higher than those of control subjects. Barutçu et al. (9) have found that serum hs-CRP concentration was increased in patients with SCF as compared to control group. On the other hand, Yazıcı et al. (20) found that plasma CRP levels were not elevated in patients with SCF when compared with controls. In our study, we also measured serum hs-CRP levels as an inflammatory marker in addition to neopterin.

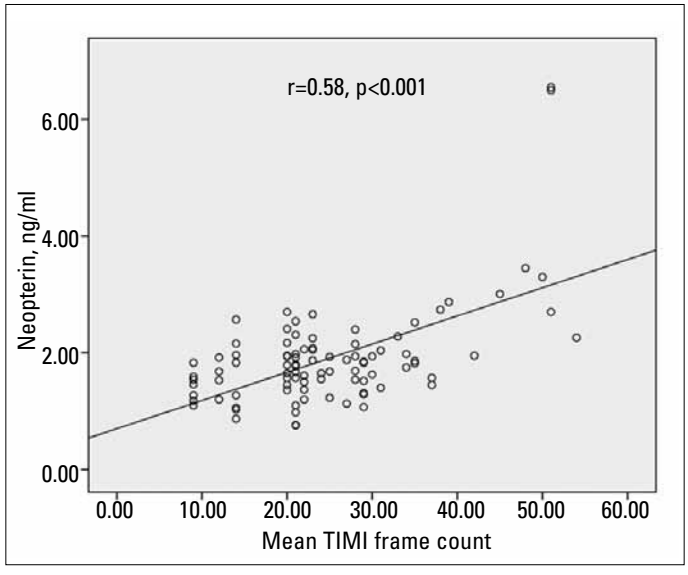

Figure 1. Correlation between serum neopterin levels and mean Thrombolysis in Myocardial Infarction (TIMI) frame count

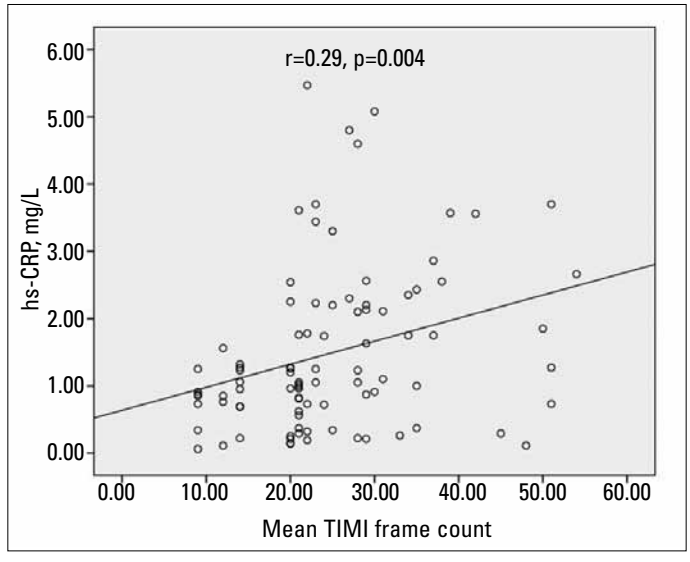

Figure 2. Correlation between serum hs-CRP levels and mean Thrombolysis in Myocardial Infarction (TIMI) frame count
hsCRP - high sensitive C-reactive protein

Neopterin, a pteridine mainly synthesized by activated macrophages, is a marker of inflammation, immune system activation and an active participant in cardiovascular disease.

Elevated, age-dependent, neopterin levels have been shown to be a prognostic indicator of malignancies and chronic inflammatory processes, particularly viral infections and complications in allograft recipients (21). Serum levels of neopterin have been shown to be elevated in patients with coronary artery disease and peripheral artery disease (13, 14). Moreover, neopterin levels have also been shown to be associated with unstable angina and acute myocardial infarction (15, 16). However, the relation between SCF and serum neopterin levels has not been investigated previously. To the best of our knowledge, this is the first study investigating serum neopterin levels in patients with SCF.

Recently we have studied serum neopterin levels in patients with coronary artery ectasia and compared this with serum neopterin levels of patients with coronary artery disease and control subjects. We have found that serum neopterin levels were significantly elevated in patients with isolated coronary artery ectasia and we have also found serum neopterin levels were elevated in patients with coronary artery disease, as in previous studies (22). However, serum neopterin levels did not correlate with the extent of coronary artery ectasia (number of ectatic coronary vessels). Yiğit et al. (23) showed that carotid and coronary artery diameters were increased in patients with SCF (without coronary ectasia) as compared to those with normal coronary arteries. Papadakis et al. (24) have demonstrated that coronary artery ectasia was associated with diminished coronary flow velocity. SCF may be early finding of coronary ectasia. Increased neopterin levels in patients with SCF and also in patients with coronary ectasia suggest that inflammation has an important role in pathogenesis of these two diseases.

Study limitations

There are some limitations of this study that should be noted. The small number of patients was the limitation of the study. Another limitation of this study is that analysis was based on a simple baseline determination that may not reflect the patient status over long periods.

Conclusion

We have shown that serum neopterin and hs-CRP levels were significantly elevated in patients with SCF when compared with control subjects. This study shows that low-grade, chronic inflammation may be involved in pathogenesis of SCF. Further prospective studies with larger sample size are needed to establish the pathophysiological and clinical significance of increased neopterin levels in patients with SCF.

Conflict of interest: None declared.

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