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ORIGINAL ARTICLE / ÖZGÜN ARAŞTIRMA

The evaluation of relationship between neutrophil-to-lymphocyte ratio and slow coronary flow

Nötrofil/Lenfosit oranı ile yavaş koroner akım arasındaki ilişkinin incelenmesi

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ABSTRACT

Objective: The aim of this study was to investigate the correlations between leukocyte counts, the neutrophil-to-lymphocyte ratio (NLR), and slow coronary flow (SCF).

Methods: We evaluated 135 patients undergoing coronary angiography (CAG) within coronary artery disease (CAD) indication. We divided patients into three groups according to the CAG findings. Group 1 consisted of 45 patients with an SCF pattern; group 2 consisted of 45 patients with at least 50% lumen narrowing in at least one epicardial coronary artery; and group 3 (control group) consisted of 45 patients with normal coronary arteries. The quantification of the coronary flow was assessed using the thrombolysis in myocardial infarction (TIMI) frame count method for each of the coronary arteries. Blood samples were collected from the patients after a 12 h overnight fasting. The NLR ratio was calculated from the automated complete blood count.

Results: NLR in CAD was higher than in both the SCF and control groups (p=0.008, p<0.001, respectively). However, there was no statistically significant difference between SCF and control group (p=0.768). Neutrophil counts in CAD were higher than in both SCF and control groups, but only the difference between CAD and SCF groups was statistically significant (p=0.010).

Conclusion: Our study revealed that circulating neutrophil counts and NLR were related to the coronary artery disease, as expected.

Key words: Neutrophil-to-lymphocyte ratio, leukocyte subtype, slow coronary flow, coronary artery disease

ÖZET

Amaç: Çalışmamızda yavaş koroner akım (SCF) ile nötrofil/lenfosit oranı (NLR) ve lökosit alt tipleri arasındaki ilişkiyi araştırmayı amaçladık.

Yöntemler: Çalışmaya; koroner arter hastalığı şüphesiyle koroner anjiyografisi yapılan toplam 135 hasta alındı. Hastalar koroner anjiyografisi sonucuna göre üç gruba ayrıldı: yavaş koroner akım saptanan 45 hasta grup 1, en az bir koroner arterinde 50% ve üzerinde darlık saptanan 45 hasta grup 2 ve normal koroner arterlere sahip 45 kişi ise grup 3'e (kontrol grubu) dahil edildi. Koroner yavaş akım tanısı her bir koroner arterin TIMI kare sayısı hesaplanarak kondu. Tüm hastalardan 12 saatlik açlık sonrası kan örnekleri alındı. NLR oranı tam kan sayımından faydalanılarak hesaplandı.

Bulgular: Nötrofil/lenfosit oranı koroner arter hastalığına sahip olanlarda, yavaş koroner akım ve kontrol grubuna göre anlamlı olarak daha yüksekti (sırasıyla p=0,008 ve p<0,001). Fakat NLR oranı yavaş koroner akım ve kontrol grubunda benzer bulundu (p=0.768). Nötrofil sayıları koroner arter hastalığı grubunda SCF ve kontrol grubuna göre daha yüksekti, ancak sadece koroner arter hastalığı ve yavaş koroner akım grubu arasındaki fark istatistiksel olarak anlamlıydı (p=0,010).

Sonuç: Çalışmamızda nötrofil sayısı ve nötrofil/lenfosit oranı ile koroner arter hastalığı arasında ilişki saptanırken, yavaş koroner akım ile ilişki saptanmadı.

Anahtar kelimeler: Nötrofil/lenfosit oranı, lökosit alt tipleri, yavaş koroner akım, koroner arter hastalığı

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INTRODUCTION

The slow coronary flow (SCF) phenomenon is described as delayed opacification of coronary vessels in the absence of occlusive epicardial coronary artery disease (CAD) in coronary angiography [1]. This phenomenon was first described by Tambe and collegues in 1972 [1]. Since that time, several mechanisms have been proposed for the etiology of SCF including occlusion of small vessels, increased microvascular resistance, and diffuse atherosclerosis [2-4]. However, the exact underlying pathophysiological mechanisms as well as the clinical importance of this unique angiographic phenomenon are not understood at present. Recent data indicated that besides other mechanisms, endothelial activation and inflammation may play a pivotal role in the pathogenesis of SCF [5-8].

The important role of inflammation in cardiovascular disorders has been well established [9]. The presence of systemic atherosclerosis is associated with low-grade systemic inflammatory response and leucocytes play a critical role in this process [10,11]. The neutrophil-to-lymphocyte ratio (NLR) and elevated neutrophil and monocyte counts have been described as potential markers of inflammation in cardiac disorders [12-14]. NLR was found to be a more important parameter than total white blood cell (WBC) count in terms of the presence, severity, and extent of coronary atherosclerosis [15,16]. In our study, we aimed to investigate whether there is a correlation between leukocyte counts, NLR, and SCF.

METHODS

Patient selection

The present study was cross-sectional and observational in nature. Totally 135 patients (mean age: 52±8 years, 76.3% male) with symptoms of chest discomfort who were referred to our outpatient clinic for undergoing a coronary angiography (CAG) for suspected CAD. We divided patients into three groups according to CAG findings. The SCF group (Group 1) consisted of 45 patients (mean age 50±9 years). The diagnosis of SCF was based on the thrombolysis in myocardial infarction (TIMI) frame count (TFC). The CAD group (Group 2) consisted of 45 patients (mean age 52±7 years) with at least 50% luminal narrowing in at least one epicardial coronary artery. The control group (Group 3) consisted of 45 age- and sex-matched individuals (mean age 53±7 years) who had angiographically normal coronary arteries. The indication for CAG was either the presence of typical angina or proven myocardial ischemia according to noninvasive diagnostic tests.

The exclusion criteria of this study were; acute coronary syndromes, serious valvular heart disease, rhythm disturbances, heart failure, left ventricular dysfunction (left ventricular ejection fraction <50%), infectious or inflammatory disease, peripheral artery disease (transient ischemic attack, stroke, intermittent claudication, peripheral revascularization or amputation), a history of early coronary revascularization procedure, history of either coronary artery bypass graft operation or percutaneous coronary intervention, coronary ectasia, acute and chronic renal and/or liver failure, hematological disorders, malignancy, and any abnormality in thyroid function tests. In addition, we excluded patients in whom air embolization occurred during CAG or complications during catheterization. All patients gave written informed consent and this study complied with the Declaration of Helsinki. Moreover, our study was approved by the Institutional Review Board and Ethics Committee.

Blood samples and analysis

Blood samples were collected from the patients after a 12 h overnight fasting. Venous blood samples were centrifuged at 3000 rpm for 10 minutes to collect serum samples. Biochemical tests were conducted on serum samples with Cobas-C 501 (Roche, USA) biochemical analyzer using Roche kits. Neutrophils, lymphocytes, and monocytes counts, hematocrit (Hct), and WBC were measured from venous blood samples collected in K3 EDTA tubes using Mindray BC-500 device by the optical laser method. NLR was calculated as the ratio of neutrophils to lymphocytes, both obtained from the same automated blood sample at admission to the study.

Coronary angiography

Coronary angiography was performed with a femoral approach using Judkins catheters. Coronary arteries were visualized in the left and right oblique planes, and at the cranial and caudal angles. Left ventriculography was performed in the left and right anterior oblique views. Injection of contrast medium (Iopromide, Ultravist-370; Schering AG, Berlin, Germany) was carried out by an automatic injector

at a speed of 3-4 ml/sec for the left coronary artery and 2-3 ml/sec for the right coronary artery. Angiographies were recorded at a speed of 30 frames/sec.

TIMI frame counts (TFC) and definition of slow coronary flow

Coronary blood flow was measured quantitatively using TFC, which was derived from the number of cine-frames recorded from the first entrance of contrast to its arrival at the distal end of the left anterior descending artery (LAD), left circumflex artery (LCx) or right coronary artery (RCA). TFCs for the LAD were divided by 1.7 to calculate the corrected TFC because the normal frame counts for the LAD artery are 1.7 times greater than the mean for the LCx and RCA, as described previously. Patients with a corrected TFC greater than two standard deviations from the normal range mentioned for the particular vessel were considered as having an SCF pattern, while those whose corrected TFC fell within two standard deviations were accepted as having normal coronary flow. The mean corrected TFC was further calculated by averaging the sum of the corrected TFCs for each coronary artery [17]. TFCs were evaluated by two experienced observers blinded to the study design.

Statistical analysis

Statistical analysis was performed using SPSS (Statistical Package for the Social Sciences, SPSS Inc., IL, USA) software version 17 for Windows. The variables were investigated using visual (histograms, probability plots), and analytical methods (Kolmogorov-Smirnov test) to determine whether or not they were normally distributed. Descriptive analyses were presented as mean±standard deviation (SD) and categorical variables were expressed as percentages. Groups were compared using the Kruskal-Wallis test, one-way ANOVA and the Chi-square test. The Mann-Whitney U test was performed to test the significance of pairwise differences using Bonferroni correction to adjust for multiple comparisons. Spearman's correlation was used to evaluate the association between NLR and TFC. The inter-rater agreement between the two observers in determining the TFC was investigated using the kappa test. An overall 5% type I error level was used to infer statistical significance.

RESULTS

Clinical features, biochemical analysis, and TFC counts of all three groups are given in Table 1.

Table 1. Demographic characteristics and laboratory parameters in three groups

		Group1 (n=45)	Group2 (n=45)	Group3 (n=45)	p value
Age (years)		50±9	52±7	53±7	0.363
Sex (male, %)		80	82.2	66.7	0.172
Body mass index (kg/m^2)		28±3	29±4	29±4	0.690
Diastolic blood pressure (mmHg)		74±9	78±10	75±8	0.100
Systolic blood pressure (mmHg)		125±11	128±15	127±15	0.608
Heart rate (beat/min)		77±9	76±10	75±9	0.489
Smoking (%)		44.4	37.8	37.8	0.757
Diabetes mellitus (%)		11.1	24.4	20	0.253
Hypertension (%)		22.2	42.2	37.8	0.110
Hematocrit (%)		43±3	42±5	41±4	0.082
Glucose (mg/dl)		117±39	113±38	104±17	0.406
Creatinine (mg/dl)		0.9±0.2	0.9±0.2	0.8±0.2	0.052
Total cholesterol (mg/dl)		187±38	206±48	202±48	0.141
LDL (mg/dl)		121±30	138±41	131±42	0.143
HDL (mg/dl)		42±11	39±7	44±11	0.128
hsCRP (mg/dl)		3.9±2.7	4.3±2.5	3.0±1.9	0.232
TIMI frame count	LAD	36±13	25±9	23±5	<0.001
	Cx	34±10	23±5	22±5	<0.001
	RCA	37±16	23±7	21±5	<0.001
	Mean	36±9	24±5	22±4	<0.001

LDL: low density lipoprotein; HDL: high density lipoprotein; hsCRP: high sensitive C-reactive protein; LAD: left anterior descending artery; Cx: circumferential artery; RCA: right coronary artery.

Age, sex, body mass index, smoking, hypertension, diabetes, systolic and diastolic blood pressure, heart rate, and biochemical parameters were not statistically different among the groups. On the other hand TFC for the LAD, LCx, RCA, and the mean TFC were significantly higher in the SCF group than the CAD and control groups (p<0.001 for all), although no statistical difference was shown between the CAD and control groups (p=0.344, p=0.234, p=0.216, and p=0.157, respectively). Our TFC results showed excellent agreement between independent observers (kappa=0.89).

Table 2. Leukocyte and leukocyte subtype counts in the three groups

Cell counts	Group1	Group 2	Group 3	p value
Leukocyte count (10^9 cells/L)	7.6±1.8	8.3±1.8	7.9±2.0	0.192
Neutrophil count (10^9 cells/L)	4.3±1.1	5.1±1.4*	4.5±1.3	0.009
Lymphocyte count (10^9 cells/L)	2.6±0.8	2.4±0.6	2.7±0.8	0.217
Monocyte count (10^9 cells/L)	0.5±0.3	0.6±0.2	0.5±0.2	0.084
NLR	1.8±0.6	2.2±0.8**	1.7±0.4	<0.001

NLR: Neutrophil to lymphocyte ratio; *p=0.01 group 2 vs. group 1; **p<0.01 group 2 vs. group 1 and 3.

There were no significant differences among the three groups with regard to leukocyte, lymphocyte or monocyte counts. Neutrophil counts and NLR were significantly different among the groups. In subgroup analysis, NLR in the CAD group was higher than in both the SCF group and the control group (p=0.008, p<0.001, respectively) (Figure 1). However, there was no statistically significant difference between the SCF group and control group (p=0.768). Neutrophil counts in the CAD group were higher than in both the SCF group and control group, but only the difference between the CAD group and the SCF group was statistically significant (p=0.010).

There was no correlation between NLR and TFC for the LAD, LCx, RCA, and the mean TFC (r=-0.118, p=0.441, r=0.066, p=0.666, r=0.042, p=0.785, and r=-0.076, p=0.618, respectively) in SCF group.

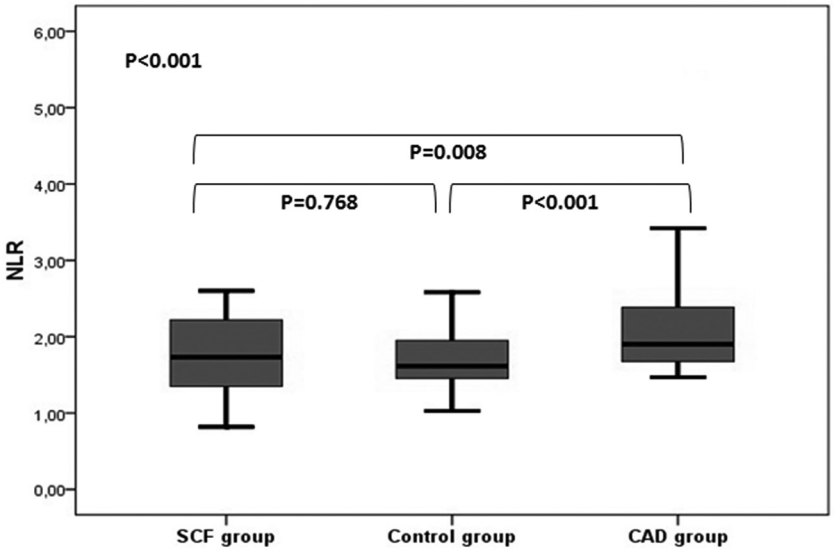

Figure 1. Neutrophil-to-lymphocyte ratio (NLR) was significantly higher in the coronary artery disease group, as compare to both the slow coronary flow and the control groups

DISCUSSION

The purpose of this study was to investigate whether circulating inflammatory cells and inflammation markers were related to SCF. The results of the present study indicate that in the CAD group, neutrophil counts and NLR were statistically higher, as expected, but in the SCF and control group, neutro-

phil counts and NLR were statistically insignificant. On the other hand, there was no correlation between NLR and TFC in the SCF group.

The pathogenesis of SCF is still not well understood. There are some histopathological features associated with SCF. Reduction of luminal diameter and functional obstruction are thought to be the key events in its pathogenesis. Mosseri et al. found medial hypertrophy, myointimal proliferation, and endothelial degeneration with changes of myofibrillar degenerative foci and lipofuscin deposits at the electron microscopic level [2]. Luminal narrowing was attributed to endothelial swelling and degeneration. Mangieri et al. established these findings in SCF patients by showing small-vessel thickening with associated luminal narrowing, dilated interstitial spaces filled with granular fibrillar material, decreased intracellular glycogen, distorted mitochondrial cristae, and patchy myofibrillar disarray at the electron microscopic level [18]. Because of these findings, it was claimed that fibromuscular hyperplasia and medial hypertrophy with a consequent decrease in luminal diameter lead to functional obstruction, ischemia, and SCF.

Impaired coronary flow reserve, which is related to increase resting coronary microvascular tone, is another important characteristic of SCF. With heightened myocardial oxygen demand, the inability to maximize coronary flow can induce persistent and recurrent chest pain in SCF patients. Previous studies have revealed that high small-vascular resistance and increased microvascular tone might cause SCF [2]. It is well known that coronary vascular tone is regulated by the autonomic nervous system. Coronary adrenergic hyperactivity may be the cause of reduction in coronary blood flow and angina. Higher adrenalin and noradrenalin levels and TIMI frame counts have been detected in SCF patients compared to individuals with normal coronary flow. This finding suggests that adrenergic hyperactivity may have a role on the pathogenesis of SCF [19]. In addition, Kurtoglu et al. reported an improvement in microvascular tone and coronary flow with microvascular vasodilators suggesting a functional increase in microvascular resistance [20].

Some studies have implicated that SCF may be manifestation of diffuse atherosclerotic disease [21,22]. In recent years, it has been recognized that atherogenesis is an active, chronic inflammatory process [10,11,23,24]. Inflammation is a central critical feature of atherosclerosis and its clinical manifestations. The role of inflammatory parameters in cardiovascular disease was investigated in several studies [25,26,27]. The total white blood cell count and its subtypes, NLR, can be an indicator of systemic inflammation. The NLR ratio has also been demonstrated to have the greatest predictive value in terms of death, myocardial infarction, and high risk of CAD [25,28].

Cingoz et al. found that the NLR was significantly higher in CAD and SCF group than the controls without any correlation between NLR and mean TFC [29]. Also, Dogan et al. presented that the NLR was higher in SCF group than the healthy controls in contrast to our study [30]. In this study, we did not find any correlation between NLR and TFC in SCF group consistent with Cingoz et al. study [30]. The NLR was no higher in SCF group as compared to healthy controls. These results suggest that the pathophysiological mechanism of SCF may indicate impaired coronary blood flow as seen in small vessel disease or/and microvascular vasomotor dysfunction.

Study limitations

The main limitations of the present study was single-centered and had a relatively small sample size. The lack of information about relation between NLR and coronary artery severity in terms of GENSINI or SYNTHAX score in CAD group and short and long term prognosis were other limitations.

Conclusion

Our results show that, as expected, circulating neutrophil counts and NLR are related to the coronary artery disease. However, neutrophil counts and NLR in the SCF group were similar to control group. This finding may due to different pathological mechanisms, which were postulated the SCF process. Further studies may establish the specific roles of neutrophils, monocyte counts, and NLR in the SCF phenomenon in coronary vasculature.

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