CLINICS

CLINICAL SCIENCE

Increased circulating macrophage migration inhibitory factor levels are associated with coronary artery disease

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BACKGROUND: To evaluate the macrophage migration inhibitory factor and E-selectin levels in patients with acute coronary syndrome.

MATERIALS/METHODS: We examined the plasma migration inhibitory factor and E-selectin levels in 87 patients who presented with chest pain at our hospital. The patients were classified into two groups according to their cardiac status. Sixty-five patients had acute myocardial infarction, and 22 patients had non-cardiac chest pain (non-coronary disease). We designated the latter group of patients as the control group. The patients who presented with acute myocardial infarction were further divided into two subgroups: ST-elevated myocardial infarction (n = 30) and non-ST elevated myocardial infarction (n = 35).

RESULTS: We found higher plasma migration inhibitory factor levels in both acute myocardial infarction subgroups than in the control group. However, the E-selectin levels were similar between the acute myocardial infarction and control patients. In addition, we did not find a significant difference in the plasma migration inhibitory factor levels between the ST elevated myocardial infarction and NST-elevated myocardial infarction subgroups.

DISCUSSION: The circulating concentrations of migration inhibitory factor were significantly increased in acute myocardial infarction patients, whereas the soluble E-selectin levels were similar between acute myocardial infarction patients and control subjects. Our results suggest that migration inhibitory factor may play a role in the atherosclerotic process.

KEYWORDS: Acute Myocardial Infarction (AMI); Macrophage Migration Inhibitory Factor (MIF); E-Selectin; Coronary Artery Disease; Atherosclerosis.

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INTRODUCTION

Atherosclerosis is a chronic inflammatory disease of the arterial wall characterized by an influx of immunocompetent mononuclear cells. Both humoral and cellular immune mechanisms play a major role in the onset and progression of atheromatous lesions (1–3). Various inflammatory mediators, including E-selectin, intracellular adhesion molecule-1 (ICAM-1), integrins and macrophage migration inhibitory factor (MIF), have been found to play a role in the pathogenesis of atherosclerosis. MIF is a pro-inflammatory regulator of many acute and chronic inflammatory diseases (4–7). During events such as acute myocardial infarction (AMI), both hypoxia and oxidative stress induce the release of MIF from cardiomyocytes via an atypical protein kinase C-dependent export mechanism, subsequently resulting in extracellular signal-regulated kinase activation (1,8,9). E-selectin is a cellular adhesion molecule. Cellular adhesion molecules are expressed by the vascular endothelium, resulting in the adhesion and transendothelial migration of circulating leukocytes. Therefore, E-selectin may play a role in the interaction between activated endothelial cells and leukocytes during the pathogenesis of atherosclerosis (10).

The aim of the current study was to determine the severity of arterial damage in coronary artery disease (CAD) and the correlation between inflammatory processes, as reflected by the degree of myocardial ischemia, and the MIF and E-selectin levels.

No potential conflict of interest was reported.

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MATERIALS AND METHODS

Study samples

The present study was designed and performed as a retrospective clinical trial at the İzmir Training and Research Hospital. The study population included 87 subjects consecutively admitted to our internal medicine department from July 2012 to March 2013. Chest pain was the principal complaint among the admitted patients.

The criteria for myocardial infarction were met based on the observation of an increase and/or a decrease in the serum levels of cardiac biomarkers, along with supportive evidence in the form of hallmark symptoms, suggestive electrocardiographic changes, or imaging evidence of the new loss of viable myocardium or a new regional wall motion abnormality.

An elevation in the serum levels of cardiac biomarkers, such as cardiac troponin and the myocardial band fraction of creatine kinase (CK-MB), is an indicator of myocardial injury.

According to these criteria, we examined 87 patients who were admitted with angina pectoris. Twenty-two of the 87 patients had non-cardiac disease (non-cardiac chest pain), and 65 of the patients had AMI. We classified the patients who had AMI into two subgroups according to ECG findings. Thirty of the AMI patients had ST elevation on ECG (STEMI) and thirty five patients had non- ST elevation on ECG (NSTEMI). The 22 subjects who had non-cardiac chest pain were designated as the control group. None of the participants had used statins.

The plasma MIF and soluble E-selectin (sE-selectin) levels were examined in all groups. Patients with a history of any malignancy, osteoporosis, systemic or local infection, or hepatic or renal disease (serum creatinine levels>1.5 mg/dl) and patients receiving systemic glucocorticoids or immunosuppressive therapy were excluded from the study. This study was approved by the local ethics committee of our institution. Written informed consent was obtained from each patient.

Blood collection

Following overnight fasting, venous blood samples were collected in the morning (08:00–09:00 AM) and centrifuged at 2000 x g for 15 minutes prior to laboratory testing. The serum glucose, total cholesterol, low-density lipoprotein (LDL) cholesterol, triglyceride, and high-density lipoprotein (HDL) cholesterol levels were measured using Randox enzymatic kits and a Roche-Hitachi modular system. The LDL cholesterol levels were calculated using the Friedewald equation. The blood samples were divided into aliquots and stored at -70℃ until the analysis was performed.

Measurement of the plasma MIF and E-selectin levels

The plasma MIF levels were measured via a quantitative sandwich human MIF ELISA (R&D Systems, Minneapolis, MN, USA) using a monoclonal antibody against MIF. The plasma concentrations of sE-selectin were determined using enzyme immunoassays from Bender MedSystems Diagnostics (Vienna, Austria). The inter-assay coefficient of variation (CV) was <10%.

Statistical analysis

The data were analyzed using Statistical Package for the Social Sciences (SPSS) for Windows, version 10.0. Categorical variables are expressed as frequencies and percentages. The Kolmogorov-Smirnov (KS) test was used to determine whether continuous variables were normally distributed. According to the KS test, all variables displayed a normal distribution. For comparison of the continuous variables, Student's t-test was used. Differences between the categorical variables were analyzed using Fisher's exact test. $p<0.05$ was considered to be significant.

RESULTS

The demographic characteristics of the AMI and control groups are presented in Table 1. A total of 87 patients were included in this study. Of these patients, the 22 patients lacking coronary occlusion were designated as the control group, whereas the 65 patients with AMI were divided into two subgroups, NSTEMI (n = 35) and STEMI (n = 30). The patients with CAD were compared with the controls (Table 1). No statistically significant difference was found in age, the sex ratio or body mass index (BMI) between the two groups. No significant difference in the plasma sE-selectin levels was observed between the patients with CAD and the control patients. The plasma MIF levels were significantly higher in the AMI group than in the control group ($p<0.001$). In contrast, we did not detect any

Table 1 - Demographic and biochemical characteristics of the patients in the AMI and control groups.

	Control group (n = 22)	AMI group (n = 65)	p[a]
Age, years	62.8±1.1	57.8±11.4	0.716
Female/male, n	10/12	18/47	0.176
Hip circumference, cm	104.6 ± 9.5	108.8±8.4	0.518
Waist circumference, cm	86.1±10.5	91.6±10.0	0.911
Systolic blood pressure, mmHg	131.1±18.9	128.8±11.6	0.823
Diastolic blood pressure, mmHg	74.5±9.1	73.3±7.7	0.587
Glucose, mg/dL	121.0±50.1	116.5±47.4	0.730
Total cholesterol, mg/dL	193.5±32.6	206.4±37.0	0.113
HDL, mg/dL	47.2±7.8	45.77±6.2	0.336
LDL, mg/dL	117.3±23.4	129.8±35.2	0.187
Triglycerides, mg/dL	147.7±30.4	168.5±48.5	0.061
hs-CRP, ng/mL	0.64±0.38	0.90±0.30	0.003*
sE-selectin, ng/mL	55.16±28	50±22.7	0.412
MIF, pg/mL	2.21±23	5.08±23	<0.001*

The results are presented as the means ± SD. [a]An independent-samples t-test was used. A p value of <0.05 was considered to be significant (*). AMI: Acute myocardial infarction; HDL: High-density lipoprotein; hs-CRP: High-sensitivity C-reactive protein; LDL: Low-density lipoprotein; MIF: Macrophage migration inhibitory factor.

significant difference in the MIF or E-selectin levels between the AMI subgroups.

The demographic and biochemical characteristics of the STEMI and NSTEMI patients are presented in Table 2. No significant difference in any demographic characteristic was detected between the two subgroups. We determined that the serum HDL levels were lower in the STEMI patients than in the NSTEMI patients ($p = 0.003$). Common risk factors, such as diabetes mellitus, arterial hypertension and smoking, were similar between the two subgroups.

The use of antihypertensive medications, including angiotensin II receptor antagonists and ACE inhibitors, were similar. The high-sensitivity C-reactive protein (hs-CRP) levels were similar between the STEMI and NSTEMI subgroups. However, the hs-CRP levels in both subgroups were remarkably higher than those in the control group.

DISCUSSION

In the present study, we found that the circulating MIF levels were elevated in subjects with AMI compared to control subjects. Unexpectedly, the plasma sE-selectin levels were not significantly different between the two groups. Furthermore, neither the circulating MIF levels nor the sE-selectin levels were significantly different between the subjects with NSTEMI and STEMI.

It has been shown that the circulating E-selectin levels are valuable for predicting systemic atherosclerosis but that their predictive value is limited for CAD. In addition, studies have demonstrated a relationship between the E-selectin levels and systemic atherosclerosis but not specific coronary atherosclerosis (11,12). In a study by Shyu et al., the E-selectin levels were evaluated in groups of subjects with AMI or unstable angina and in a control group. The circulating E-selectin levels were not significantly different between these three groups (12). Prugger et al. demonstrated that elevated E-selectin levels were independently and significantly associated with ischemic stroke (13). Furthermore, it has been reported that E-selectin gene polymorphisms are associated with an increased risk of CAD (14). In our study, we found that the circulating sE-selectin levels were not significantly different between the subjects in the AMI and control groups or between the subjects with STEMI and NSTEMI. In contrast to our study results, many findings have supported the concept that the E-selectin levels are associated with AMI; our results may be related to the size of our study population (15–17).

Additionally, we found that the MIF levels were significantly higher in the AMI group than in the control group ($p<0.001$). Our data suggest that elevated circulating MIF levels may correlate with myocardial infarction. To evaluate the potential correlation between the MIF levels and the severity of myocardial infarction, we measured the plasma MIF levels in both the STEMI and NSTEMI subgroups. However, no significant difference was observed between the two subgroups. The findings of our study are in accordance with the literature demonstrating the effect of MIF on the atherosclerotic process (6,18,19). Moreover, the circulating MIF levels have been correlated with infarct size based on cardiac magnetic resonance imaging (20).

Increased circulating concentrations of MIF were detected in patients with AMI. MIF may play a role in the pathogenesis of myocardial ischemia. MIF contributes to macrophage accumulation in the infarct area and plays a pro-inflammatory role in macrophage/myocyte-mediated damage during infarction (1,3,8,21). It has been reported that MIF deficiency protects the heart from ischemia-reperfusion injury in mice by suppressing inflammatory responses (22).

The present study contains some limitations: the sample size was relatively small, and we did not evaluate MIF or E-selectin gene polymorphisms.

It has become evident that MIF plays a major role in the development of vascular disease and myocardial damage; therefore, MIF is currently under intense investigation as a therapeutic target (23). These studies may be useful in terms of the prevention of high-mortality diseases related to atherosclerosis. In the future, MIF may be targeted for the prevention of atherosclerotic diseases. It has been demonstrated that MIF plays a role in neointimal lesion formation. The observations that the antibody-mediated inhibition of MIF inhibits neointima formation and is associated with reduced inflammation and cellular proliferation were first identified in mice (24). MIF inhibition has been shown to induce the stabilization and even the regression of atherosclerotic plaques (25,26).

Taken together, our results indicate that the circulating MIF levels were increased in subjects with AMI and that these levels were not significantly different between subjects with NSTEMI and STEMI. The plasma sE-selectin levels were not significantly different between subjects with AMI and control subjects or between subjects with NSTEMI and STEMI. Future studies are needed to generalize these results.

Table 2 - Demographic and biochemical characteristics of the patients with mild or severe coronary artery disease.

	NSTEMI (n = 35)	STEMI (n = 30)	p[a]
Age, years	56.2 ± 12	59.7 ± 10.5	0.221
Hip circumference, cm	108 ± 8.6	109.6 ± 8.2	0.447
Waist circumference, cm	91.7 ± 10.6	91.5 ± 9.5	0.941
Systolic blood pressure, mmHg	123.3 ± 10.4	124.5 ± 13.1	0.686
Diastolic blood pressure, mmHg	73.5 ± 7.1	73.1 ± 8.5	0.852
Glucose, mg/dL	114.3 ± 37.1	119.1 ± 57.7	0.699
Total cholesterol, mg/dL	204.4 ± 30.3	208.7 ± 43.9	0.643
HDL, mg/dL	47.8 ± 5.4	43.3 ± 6.3	0.003*
LDL, mg/dL	129.3 ± 34.1	130.3 ± 35.2	0.910
Triglycerides, mg/dL	147.7 ± 30.4	168.5 ± 36.9	0.114
hs-CRP, ng/mL	0.99 ± 0.48	0.85 ± 0.36	0.887
sE-selectin, ng/mL	53.2 ± 23.6	48.3 ± 21.6	0.392
MIF, pg/mL	5.09 ± 2.4	5.07 ± 2.1	0.970

The results are presented as the means ± SD. [a]An independent-samples t-test was used. A p value of <0.05 was considered to be significant (*). HDL: High-density lipoprotein; hs-CRP: High-sensitivity C-reactive protein; LDL: Low-density lipoprotein; NSTEMI: Non-ST-elevated myocardial infarction; MIF: Macrophage migration inhibitory factor; STEMI: ST-elevated myocardial infarction.

AUTHOR CONTRIBUTIONS

Yüksel A, Bilgir F, Bilgir O, Calam M, and Bozkaya G collected the data and contributed to the discussion. Yüksel A, Bilgir F, and Bilgir O wrote, reviewed and edited the manuscript and contributed to the discussion. Bilgir O and Yüksel A are the guarantors of this work and, as such, have full access to all of the data from this study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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