Decreased Serum Matrix Metalloproteinase-2 Levels in Early Hours of Acute Myocardial Infarcts

Akut Miyokard İnfarktüslerinin Erken Saatlerinde Düşük Serum Matriks Metalloproteinaz-2 Düzeyleri

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ABSTRACT Objective: Matrix metalloproteinases (MMP), which are stimulated by proinflammatory cytokines, play a role in the development of coronary artery diseases. The aim of the present study was to investigate the serum concentrations of matrix metalloproteinase-2 (MMP-2), interleukin-6 and high sensitive C-reactive protein (hs-CRP) before any treatment and to determine any possible relationships between these markers in patients with newly diagnosed acute myocardial infarction (AMI). **Material and Methods:** In order to determine the differences in the early hours of AMI and to prevent the interference of drugs used for treatment, patients (n= 54) were selected from the individuals who admitted to emergency department no more than one hour after they felt a chest pain and diagnosed as AMI by clinical symptoms, electrocardiographic changes and biochemical markers. Venous blood samples of patients were taken before starting any treatment. The control group (n= 30) had no history of cardiovascular, neoplastic, infectious or autoimmune disease that may affect serum MMP-2 levels. Serum levels of MMP-2 and interleukin-6 were measured with ELISA, hs-CRP with chemiluminescent methods. **Results:** There was no difference in age and body mass index between patient and control groups ($p > 0.05$). Serum interleukin-6 and hs-CRP levels of the patient group were significantly higher ($p < 0.05$) while MMP-2 levels were significantly lower compared to the controls ($p < 0.05$). A significant correlation was found between IL-6 and hs-CRP levels in the patient group ($r = 0.580$, $p < 0.05$). **Conclusion:** Decreased levels of MMP-2 in AMI patients before any medication may be mainly associated with patient selection criteria and the time of infarction. Further research on serum MMP-2 levels can provide useful information on tissue remodelling of the post-infarction period.

Key Words: Matrix metalloproteinase 2; interleukin-6; C-reactive protein; myocardial infarction

ÖZET Amaç: Proinflamatuar sitokinler tarafından stimüle edilen matriks metalloproteinazlar (MMP) koroner arter hastalıklarının gelişiminde rol oynar. Bu çalışmada, yeni tanı konmuş akut miyokard infarktüslü (AMI) hastalarda tedavi öncesi serum MMP-2, interlökin-6 (IL-6) ve yüksek duyarlı C-reaktif protein (hs-CRP) düzeylerinin belirlenmesi ve bu belirteçler arasında olası ilişkilerin tespit edilmesi amaçlandı. **Gereç ve Yöntemler:** AMI sonrası erken dönem değişikliklerini belirlemek ve kullanılacak ilaçların interferansını önlemek amacıyla, acil servise göğüs ağrısı başladıktan sonra bir saatten fazla zaman geçirmeden başvuran hastalar (n= 54), klinik semptomlar, elektrokardiyografik değişiklikler ve biyokimyasal belirteçler ile AMI teşhisi alanlar arasından seçildi. Hastaların venöz kan örnekleri herhangi bir tedaviye başlamadan önce alındı. Kontrol grubunun (n= 30) seçiminde serum MMP-2 düzeylerini etkileyebilecek kardiyovasküler, neoplastik, enfeksiyöz ve otoimmün bir hastalık bulunmamasına dikkat edildi. Serum MMP-2 ve IL-6 düzeyleri ELISA, hs-CRP düzeyleri kemiluminisans yöntemle ölçüldü. **Bulgular:** Yaş ve beden kitle indeksi açısından hasta ve kontrol grupları arasında anlamlı bir fark yoktu ($p > 0.05$). Hasta grubunun serum IL-6 ve hs-CRP düzeyleri kontrol grubuna göre anlamlı olarak yüksek iken ($p < 0.05$), MMP-2 düzeyleri anlamlı olarak düşüktü ($p < 0.05$). Hasta grubunda IL-6 ve hs-CRP arasında anlamlı bir ilişki saptandı (sırasıyla; $r = 0.580$, $p < 0.05$). **Sonuç:** AMI hastalarında tedavi öncesi elde edilen düşük MMP-2 düzeyleri başlıca hasta seçim kriterleri ve AMI sonrası geçen kısa süre ile ilişkili olabilir. MMP-2 düzeyleri ile yapılacak ileri çalışmalar infarktüs sonrası dönemde meydana gelen yeniden yapılanma hakkında yararlı bilgiler verebilir.

Anahtar Kelimeler: Matriks metalloproteinaz 2; interlökin-6; C-reaktif protein; miyokard infarktüsü

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Acute myocardial infarction (AMI) is an irreversible myocardial necrosis as a result of prolonged ischemia.[1] Experiments demonstrated that interleukin-6 (IL-6) synthesis is rapidly induced in mononuclear cells and cardiomyocytes of the ischemic myocardium.[2] IL-6 cytokine and it's signalling events have been shown to contribute to both, atherosclerotic plaque development and plaque destabilisation via variety of mechanisms.[3] In response to inflammatory signals, CRP deposits in infarct area and locally activate the complement cascade.[1] Complement activation may play an important role in mediating neutrophil and monocyte recruitment in the injured myocardium which appears to be particularly important during the first hour of reperfusion.[1] CRP was found to have prognostic value even among patients with negative cardiac troponin and patients with elevated plasma concentrations of CRP have higher rate of death.[4]

Matrix metalloproteinases (MMPs) are proteins that participate in extracellular matrix (ECM) degradation which are stimulated by proinflammatory cytokines such as IL-6 and are thought to have important functions in tissue repair and remodelling.[5] MMP activity is inhibited by tissue inhibitors of metalloproteinases (TIMPs). MMP-2 (gelatinase A) which belongs to gelatinase subgroup of MMP family, digest the denatured collagens and gelatins.[6] MMPs play a role in coronary artery disease (CAD) in the aspects of both atherosclerotic plaque and myocardium after myocardial infarction.[5] It was demonstrated that plasma MMP-9 levels were elevated in the infarcted zone after myocardial infarction and correlated to the severity of coronary atherosclerotic plaques, the presence of plaque rupture and the survival rate after successful cardiopulmonary resuscitation.[5] Likewise, it has been reported that MMP-2 levels are significantly elevated in patients with acute coronary syndromes (ACS).[7,8] While no difference in MMP-2 levels was stated in some studies, a significant decrease was observed in other ones.[9,10]

The aim of this study was to determine serum MMP-2, IL-6 and hs-CRP levels and any possible correlations between these markers in newly diagnosed AMI patients before any medication who admitted to emergency department in early hours of myocardial infarction.

MATERIAL AND METHODS

STUDY POPULATION

In order to determine the differences in the early hours of AMI and to prevent the interference of drugs used for treatment, patients were selected from the individuals who admitted to emergency department no more than one hour after they felt a chest pain and diagnosed as AMI by clinical symptoms, electrocardiographic changes and biochemical markers. Patient group consisted of 54 individuals with AMI (42 male, 12 female) and 30 individuals (17 male, 13 female) served as the control group. Patients with concomitant diseases like diabetes mellitus, hypertension and dyslipidemia were recorded and control group was matched for the same ratio of these chronic diseases. The control group had no history of cardiovascular, neoplastic, infectious or autoimmune disease. Controls using steroids, nonsteroid anti-inflammatory drugs within last week or having an inflammatory condition were excluded from the study. The study was approved by the local ethics committee and a written informed consents were obtained from the patients and the controls.

LABORATORY ANALYSES

Before starting any medication, venous blood samples of patients were collected from the antecubital vein in 8 mL evacuated tubes without anticoagulant (Vacuette, Greiner Bio-One, Austria). The use of EDTA was avoided due to concerns about interactions between EDTA and zinc in MMP-2. Each blood sample was centrifuged at 3000 rpm for 10 minutes in room temperature within an hour. After centrifugation, the serum samples were frozen and stored at -80°C until the analysis. Repeated freezing and thawing process weren't performed.

Serum MMP-2 levels were determined by an ELISA kit which is specific for MMP-2, according to manufacturer's instructions (Amersham Biosciences UK Ltd, Buckinghamshire, UK). The analytical sensitivity was 0.37 ng/mL. Within-run CV was

measured below 6.4%. Between-run CV was measured below 12.3%. Serum IL-6 levels were assessed by solid phase Enzyme Amplified Sensitivity Immunoassay (EASIA) (BioSource Europe S.A, Belgium). The analytical sensitivity was 2 pg/mL. Within-run and between-run CV's were measured below 5.7% and 7.6%, respectively. Serum hs-CRP levels were analyzed on Immulite 1000 analyzer (Siemens Healthcare Diagnostics, Deerfield, IL, USA) with immulite kits using solid phase, chemiluminesence immunometric method. Analytical sensitivity was 0.01 mg/dL. Within-run precision CV was below 6.5% and total precision CV was below 10.1%.

STATISTICS

Statistical analysis of data was performed by Statistical Package for Social Sciences (SPSS, version 11.0). Normality was assessed using the Kolmogorov-Smirnov test. Mann Whitney-U test was used to compare hs-CRP and IL-6 between two groups since they were not normally distributed. The median levels and percentiles of hs-CRP and IL-6 were calculated. MMP-2 was normally distributed and independent samples t-test was used to compare the means of patient and control groups. Spearman's correlation coefficient (r) was used to determine the correlation between the parameters. Reported p values were based on two sided tests and $p< 0.05$ was accepted as the level of significance. Data are presented as mean ± SD or median (25%-75% percentiles).

RESULTS

A total number of 84 cases; 54 AMI patients (with a mean age of 62.4 ± 13.2) and 30 control subjects (with a mean age of 64.4 ± 7.7) were included to our study. The mean age and BMI were not significantly different between the two groups ($p> 0.05$) (Table 1). IL-6 and hs-CRP levels in AMI patients were significantly higher ($p< 0.05$) but MMP-2 levels were significantly lower than control group ($p< 0.05$). Demographical and biochemical data of the patient and control groups were shown in Table 1.

There was no correlation between MMP-2 and other parameters (IL-6, and hs-CRP) in patient group. However, a significant, positive correlation was found between levels of IL-6 and hs-CRP ($r= 0.580$, $p= 0.000$) (Figure 1).

TABLE 1: Demographic and biochemical data of the study groups.

	Patient group (n= 54)	Control group (n= 30)	p
Male/female	42/12	17/13	= 0.05*
Age (y)	62.4 ± 13.2	64.4 ± 7.7	> 0.05
BMI (kg/m^2)	25.9 ± 3.6	27.4 ± 6.1	> 0.05
MMP-2 (ng/mL)	968.3 ± 260.0	1099.6 ± 208.5	0.02
IL-6 (pg/mL)	22.9 (9.4-134.0)[&]	5.0 (2.4-9.4)[&]	0.000
hs-CRP (mg/L)	5.1 (2.5-20.3)[&]	2.0 (1.1-6.5)[&]	0.008

[&]Median (25%-75% quartiles),
*According to Fisher's Exact Test,
BMI: Body mass index, MMP-2: Matrix metalloproteinase-2, IL-6: Interleukin-6, hs-CRP: High sensitive C-reactive protein.

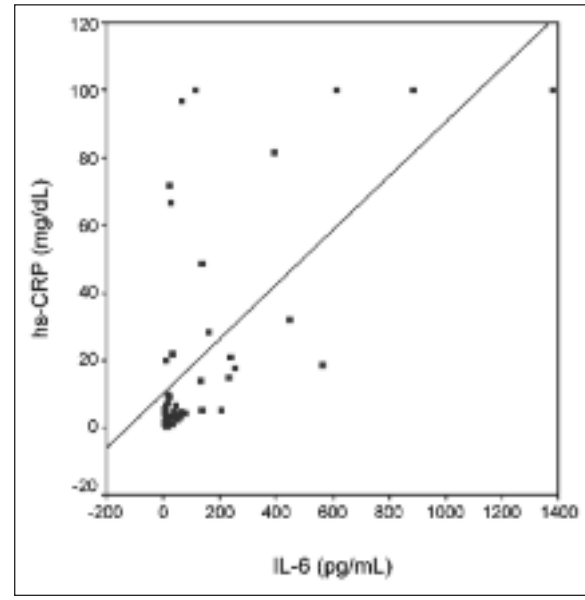

FIGURE 1: The correlation between IL-6 and hs-CRP ($r= 0.580$, $p= 0.000$) (Spearman's correlation).

DISCUSSION

Induction and release of pro-inflammatory cytokines is consistently found in the experimental models of myocardial infarction.[11] Cytokines exhibit a wide range of biological events such as complement activation and reactive oxygen species generation. Extensive experimental evidence demonstrated induction of members of the IL-6 fa-

mily in healing infarcts.[2] Members of the IL-6 family have profound effects on cardiac myocytes by promoting cardiac hypertrophy but also protecting them from apoptosis.[12] IL-6 is capable of modulating the phenotypic characteristics and gene expression of many cell types involved in infarct healing.[13] The primary cytokines upregulated after myocardial infarction are tumor necrosis factor-alpha and members of the IL-1 and IL-6 families.[14] In the present study, IL-6 levels were higher in the patient group compared to controls which reflects the existence of inflammatory activity in the early hours of AMI. The high levels of hs-CRP, which is considered as a sensitive marker of inflammation also confirmed the existence of inflammatory activity, in accordance with the previous studies.[15,16]

In addition to roles in acute inflammatory events, IL-6 was shown to induce MMP synthesis in vitro.[3,14] ECM degradation by MMPs is involved in the pathogenesis of cardiovascular disease, including atherosclerosis, restenosis, post-infarction left ventricular remodeling and dilated cardiomyopathy.[17] There are different MMP-2 results in different studies. A number of studies have demonstrated increased expression and activity of MMP-1, -2, -3 and -9 in human, rat and porcine hearts during the remodeling process after MI, although the data on the exact time course of post-infarction MMP activity is diverse.[18] In clinical studies of AMI patients, there were differences in patient characteristics, interventions, as well as timing and methods of MMP measurement, which could be the reason for discrepancies in findings.[5] In the present study, we found decreased levels of MMP-2 in patients with AMI, contrary to other studies.[7,8,17,19,20] Elevated levels of MMP-2 in the study of Kai may be related to the existence of unstable angina pectoris (USAP) patients, different periods after the beginning of the chest pain, obtaining blood samples after medication and designing of the control group. Since the effects of USAP, angioplasty, and medication on MMP-2 levels are unclear yet, the elevated levels of MMP-2 in their study may be related to the factors mentioned above. The presence of USAP patients and usage of medication in other studies prevents us to understand the sole affect on serum MMP-2 levels.[7,20]

Our findings were in agreement with the study reported by Nilsson et al, who found significantly low levels of plasma MMP-2 in 65 males with CAD.[10] However, that study did not reflect MMP-2 levels after myocardial necrosis. Also, blood samples were drawn 1-3 days after beginning of the symptoms. Likewise, Noji found significantly lower levels of MMP-2 in premature coronary artery disease compared to controls.[9] On the other hand, Ferroni et al., reported that MMP2 levels did not differ between myocardial infarction patients and controls although a significant MMP-9 elevation was obtained in patient group.[21]

Results obtained from various studies are specified above. We claim that serum MMP-2 levels, which are low in the early period, may elevate later due to increased blood flow in the necrosis area after revascularization. This may be the main reason of high MMP-2 levels found in the other studies, since MMP-2 levels were not studied as early as our study in any of the studies mentioned above Besides, absence of a significant correlation between MMP-2 and IL-6 in our study also may be due to short elapsed time after the infarction. Although IL-6 levels were increased at the time of blood collection, it is possible that not enough time have passed to elevate MMP-2 levels.

The effect of drugs on MMP levels has not been widely studied and it is therefore difficult to determine whether changes in serum levels of MMPs are due to the effects of the drugs or not. Various medications after AMI may cause an increase or decrease MMP-2 levels by mechanisms unknown yet. It was shown that cardiovascular drugs inhibit MMP-9 activity or aspirin inhibits MMP-2 and MMP-9 expressions and activities.[22,23] It was also shown that calcium channel antagonists raise the levels of MMP-2 in essential hypertension.[24] In our study, only pretreatment samples were examined to exclude the above mentioned effects of drugs. It would be interesting to study the expression levels of MMPs in different time points before and after

the therapy to see whether any differences would occur.

Another reason for decreased levels of MMP-2 may be the over expression of TIMPs which are specific inhibitors of MMPs. Increased serum levels of TIMPs were detected during the first 12 hours after AMI suggesting early activation in extracellular myocardial matrix metabolism.[25,26] In addition to the TIMP family, there are also several other naturally occuring inhibitors of MMPs which may degrade MMPs and decrease their level such as corticosteroids, retinoic acid, heparin and IL-4.[18] Taken together, various MMP inhibitors might decrease the level of MMP-2. We should perhaps have made serial determinations to observe the fluctuations in serum MMP-2 levels as well as determining TIMP-1 levels to put forward the associations between them during the first hours of AMI in a time course manner.

A last possibility may be related with our control group. Age matched control group consisted of people with a mean age of 64.4 ± 7.7 years. Although apparently healthy, with low levels of IL-6 and hs-CRP, atherosclerotic lesions may have developed in some of the individuals of our control group, elevating the mean levels of MMP-2.

The present study was carried out with patients in the early hours of myocardial infarction. This is the most important property that distinguishes our study from the ones with high levels of MMP-2. It is known that ECM metabolism is regulated by distinct mechanisms in the infarct region. TNF-alpha enhances MMP activity in cardiac fibroblasts.[27] Differentiated macrophages contribute to ECM remodeling by producing MMPs.[2] The mast cells also influence infarct healing and tissue remodeling by expressing MMP-2.[28] All these events take place during the later hours, in the infarct healing period. This fact may also be the main reason of lack of a significant correlation between IL-6 and MMP-2, since MMP-2 expression is not yet stimulated. Probably, a significant correlation would be obtained later. Also, significant correlation between IL-6 and hs-CRP shows that it is the very beginning of the post-infarction inflammatory response and there is still time for induction of MMP-2 syntesis.

Decreased levels of MMP-2 in our study may be associated with patient selection criteria and the short time elapsed after infarction. Further research on serum MMP-2 levels can provide useful information on the complex events; inflammatory, proliferative and maturation phases of the post-infarction period.

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