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Plasma Asymmetric Dimethylarginine and Nitric Oxide Levels on Early Prognosis in Patients with Myocardial Infarction

Miyokard İnfarktüslü Hastalarda Plazma Asimetrik Dimetil Arginin ve Nitrik Oksit Düzeylerinin Erken Prognoza Etkisi

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SUMMARY

Objectives: *It is unclear whether abnormalities of nitric oxide (NO) are related to hemodynamic dysfunction and mortality in patients after acute myocardial infarction (MI). We investigated the relationship of plasma asymmetric dimethylarginine (ADMA) and NO levels with early prognosis in patients with MI.*

Methods: *Fifty-nine patients (9 females, 50 males, aged 32-75) hospitalized in the Coronary Care Unit were included in the study. Blood samples were obtained on the first day of admission for routine hematological and biochemical tests and for plasma ADMA and NO levels. NO was determined as the concentration of nitrate plus nitrite (NO metabolites) spectrophotometrically at 430 nm using the Griess reaction. ADMA concentrations were determined by competitive ELISA assay. One month after MI, echocardiography was performed on 56 patients who survived, and left ventricular ejection fraction (LVEF) was investigated as a prognostic marker of cardiac function.*

Results: *There was no correlation between ADMA and NO levels with age, gender, smoking, localization of the infarction, history of hypertension, coronary heart disease or diabetes mellitus, and the levels of total cholesterol, triglyceride, lactate dehydrogenase, white blood cells, and C-reactive protein. Plasma ADMA concentrations were negatively correlated with NO levels (r=-0.72) and creatine kinase -MB levels (r=-0.37). LVEF had no correlation with ADMA or NO levels (p>0.05).*

Conclusion: *In previous studies, decreased NO and high levels of ADMA were proposed as independent risk factors for coronary artery disease. Although MI patients had higher ADMA levels, they showed no correlation with left ventricular systolic function or early cardiac events.*

Key words: *Asymmetric dimethylarginine; endothelial dysfunction; myocardial infarction; nitric oxide; left ventricular dysfunction.*

ÖZET

Amaç: Miyokard infaktüsünden (Mİ) sonra nitrik oksit (NO) metabolizmasındaki anormalliklerin hemodinamik bozukluk ve mortalite ile ilişkisi açık değildir. Bu çalışmada, Mİ'li hastalarda erken prognoz ile plazma asimertrik dimetil arginin (ADMA) ve NO düzeylerinin ilişkisini inceledik.

Gereç ve Yöntem: Koroner yoğun bakım ünitesinde yatırılan 59 (9 kadın, 50 erkek, yaşları 32-75) hasta çalışmaya alındı. Yatış günü rutin biyokimyasal ve hematolojik incelemeler yapıldı, ADMA ve NO değerlerinin ölçülmesi için kan alındı. NO nitrat + nitrit (NO metabolitleri) konsantrasyonu olarak spektrofotometrik yöntemle 430 nm'de Griess reaksiyonu ile ölçüldü. ADMA konsantrasyonları kompetitif ELISA yöntemi ile tayin edildi. Mİ'den bir ay sonra yaşayan 56 hastada kardiyak fonksiyon göstergesi olarak sol ventrikül ejeksiyon fraksiyonu (LVEF) değerlendirildi.

Bulgular: ADMA ve NO düyeyleri ile yaş, cinsiyet, sigara kullanımı, infarktüs yeri, hipertansiyon, koroner arter hastalığı, diyabetes mellitus varlığı, total kolesterol, trigliserid, LDL-K, lökosit ve C reaktif protein değerleri arasında ilişki bulunmadı. Plazma ADMA düzeyleri NO ile (r=-0.72); ve kreatin kinaz -MB düzeyleri ile (r=-0.37) negatif olarak koreleydi. LVEF ile ADMA ve NO arasında ilişki bulunmadı.

Sonuç: Daha önceki çalışmalarda azalmış NO ve artmış ADMA seviyelerinin koroner arter hastalığı için bağımsız risk faktörleri olduğu bildirilmiştir. Her ne kadar Mİ geçirmiş hastalarda daha yüksek ADMA düzeyleri gözlendiyse de ADMA ile LVEF ve erken kardiyak olaylar arasında ilişki görülmedi.

Anahtar sözcükler: Asimetrik dimetil arginin; endotel disfonksiyonu; miyokard infarktüsü; nitrik oksit; sol ventrikül disfonksiyonu.

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INTRODUCTION

Endothelial dysfunction coexists with many cardiovascular diseases and is known as the first step of atherosclerosis, which is probably the most important disease of the world.[1] The endothelium has emerged as the key regulator of vascular homeostasis. In normal vascular physiology nitric oxide (NO) plays a crucial role to maintain the vascular wall in a quiescent state by inhibition of inflammation, cellular proliferation and thrombosis. Endothelial vasodilator dysfunction which is accompanied with decreased NO levels is a characteristic feature of patients who are at risk for coronary artery disease, therefore coronary vasodilatator dysfunction may predict long-term atherosclerosis progression and cardiovascular event rates.[1,2]

Endothelial dysfunction plays a key role in the development of atherosclerosis, hypercholesterolemia, diabetes and hypertension.[1,2] In vascular diseases, the bioavailability of NO can be impaired either by decreased NO production by eNOS or enhanced breakdown due to increased oxidative stress.[3] The hallmark of endothelial dysfunction is impaired endothelium-dependent vasodilation which is mediated by NO. A defect in NO production or activity acts as a major mechanism of endothelial dysfunction which contributes to atherosclerosis. NO is synthesized from L-arginine by different isoforms of nitric oxide synthase (NOS), including nNOS (neuronal), iNOS (inducible), and eNOS (endothelial). NO synthase inhibitors such as L-NMMA (N-monomethyl-L- arginine) and L-ADMA are naturally occurring compounds which are found in very small amounts. But they can also be present at higher concentrations in specific tissues and individuals. ADMA inhibits NOS and elevated ADMA levels are associated with endothelial dysfunction.[4,5]

ADMA levels had a strong prognostic value for long-term mortality after myocardial infarction, beyond traditional risk factors and biomarkers.[2,6] In critically ill patients, plasma ADMA concentration was found as a strong and independent risk factor for mortality.[7] In another study, ADMA levels were the strongest independent predictor of 30-day mortality. [8] We aimed to see the relationship of ADMA and NO levels, on the first day of admission, with early prognosis of patients including left ventricular ejection fraction (LVEF) one month after myocardial infarction.

MATERIALS AND METHODS

Patients

This study was carried out at Coronary Care Unit in our hospital. Fifty-nine consecutive patients (9 females, 50 males, mean age 58.1±10.2) who were diagnosed and treated for acute myocardial infarction were included in the study. Subjects with non Q myocardial infarction, with known chronic renal, hepatic disease, infections, rheumatic disorders and malignancies were excluded. Personal and family histories were recorded. Two patients died on the first hours of admission because of fatal arrhythmia, and one patient died because of cardiogenic shock. Streptokinase was given to 35 patients. All patients were followed up for angina, reinfarction, and hospitalization for other cardiac events. Our study was conducted in accordance with ethical guidelines and Helsinki Declaration. Informed consent was obtained from all participants.

Routine or emergent coronary angiographic findings, percutaneus transcoronary angioplasty (PTCA), intracoronary stent, coronary artery by-pass graft (CABG) and other medical therapies were recorded. Localization of infarction was as follows: anterior 22, inferior 22, inferior plus right ventricular 14, posterior infarction in 1 patient. 56 patients underwent coronary angiographic evaluation. 1 patient had main coronary artery disease (CAD), 39 patients had multi vessels, 16 patients had 1 vessel involved.

After one month of myocardial infarction, echocardiography was performed on 56 patients, who survived, by the same cardiologist with HP Somos 1000 (Hewlett-Packard Company, Massachusetts, USA). Left ventricular ejection fraction (LVEF) was calculated as an indicator of ventricular function.

Analytical Methods

Blood samples were obtained on the first day of admission for routine hematological and biochemical tests including total cholesterol (TC), triglyceride (TG), lactate dehydrogenase (LDH), creatin kinase-MB (CK-MB), C-reactive protein (CRP) and

complete blood count (CBC). Whole blood (10 ml) was sampled from an indwelling cannula into EDTA tubes following an overnight fasting and the plasma was separated immediately by centrifugation at 2000 x g. The plasma was stored at -70 ºC until assay for NO and ADMA.

Assay of plasma nitrate plus nitrite (NO): NO metabolites were determined as the concentration of nitrate plus nitrite in the plasma. Nitrate was reduced to nitrite by nitrate reductase, the sample was deproteinized with $ZnSO_4$, and the concentration of nitrite was measured spectrophotometrically at 430 nm using the Griess reaction with a commercial kit (Boehringer Mannheim, GmBH, Germany). The intra- and interassay coefficients of variation were 4.6% and 5.0%.

Assay of plasma ADMA: Plasma ADMA concentrations were determined by competitive ELISA assay (*ADMA-ELISA DLD Gessellschaft für diagnostika und medizinische Gerate mbH, Hamburg*). ADMA in samples is acylated and competes with solid phase bound ADMA for a fixed number of rabbit anti-ADMA antiserum binding sites. The intra- and interassay coefficients of variation were 3.2% and 4.3% for ADMA.

Statistical Analysis: Data are expressed as mean ± SEM. The comparisons were performed with Chi-square test, Student's test, Mann-Whitney U test and Pearson correlation test using SPSS for Windows 12.0. A probability value of less than 0.05 was considered statistically significant for comparisons.

RESULTS

1. There were 59 patients (50 males and 9 females) in the study population. 41 of patients were current smokers. Fourteen patients had a history of hypertension, 10 patients had diabetes 10 patients had established coronary heart disease and one patient had been followed up for Burger disease. This was the first cardiovascular event for 32 patients. Presenting characteristics of the patients: Mean age (years): 58.1±10.2, TC (mg/dL): 203.00±45.08, TG (mg/dL): 124.71±59.46, CRP (mg/L): 4.07±7.14, ADMA (μmol/L): 0.78± 0.44, NO (μmol/L): 35.64±0.44.

2. There was not any correlation of ADMA and NO levels with age, gender, smoking, localization of infarction, history of hypertension, coronary heart disease or diabetes mellitus and the levels of total cholesterol, LDH, triglyceride, WBC, and CRP. Plasma ADMA levels were negatively correlated with NO levels ($r=-0.72$, $p<0.001$) and CK-MB levels ($r=-0.35$, $p=0.007$).

3. No difference was found in ADMA and NO levels between the patient subgroup who needed emergent invasive procedures (such as emergent angiography, PTCA, coronary stent or CABG, n=38) and the other subgroup who did not need (n=18) ($p=0.153$, $p=0.213$ respectively).

4. The groups were compared with respect to early complications (reinfarction, repeat hospitalization, ventricular arrhythmia and death). There was not a significant difference of CRP, ADMA and NO levels between subgroups who had developed complications or not ($p>0.05$). The subgroups did not either present significant difference with regard to extend of coronary angiographic lesions ($p>0.05$).

5. At the end of the first month when patients were investigated with echocardiography, LVEF had no correlation with ADMA and NO levels ($p>0.05$). LVEF was negatively correlated with age, TC, TG and LDH ($r=-0.40$, $p=0.002$; $r=-0.29$, $p=0.033$; $r=-0.30$, $p=0.024$; $r=-0.27$, $p=0.046$, respectively). When patients were divided into two subgroups with respect to early complications, significant differences were observed in age and LVEF ($p=0.006$, $p=0.024$) (Table 1).

DISCUSSION

Many of the traditional risk factors that predispose a person to the development of atherosclerosis such as hypercholesterolemia, hypertension, smoking, diabetes and a positive history of premature coronary artery disease are also associated with endothelial dysfunction.[4] Schächinger et al.[9] followed up 147 patients for a median period of 7.7 years. Patients suffering from cardiovascular events (cardiovascular death, unstable angina, myocardial infarction, transluminal coronary angioplasty, coronary by pass grafting or peripheral artery revascularization, and ischemic stroke) had significantly increased vasore-

Table 1. Comparison of complications with age, laboratory parameters and LVEF

	Without complication	With complication	p
Age (years)	55.93±9.75	64.00±9.45	**0.006**
WBC (10^3 /mm^3)	14.05±4.43	13.70±3.96	0.781
CK-MB (U/L)	159.28±104.38	242.50±280.33	0.099
CRP (mg/L)	3.97±7.39	4.36±6.67	0.857
NO (µmol/L)	34.60±10.08	38.44±11.21	0.213
ADMA (µmol/L)	0.83±0.45	0.65±0.40	0.153
EF (%)	50.12±9.26	43.15±10.11	**0.024**

activity. They concluded coronary endothelial vasodilatator dysfunction, which was previously shown to correlate with increased ADMA levels, predicts long-term atherosclerotic disease progression and cardiovascular event rates. In our study, we followed up our patients one month after myocardial infarction and could not find a relation between ADMA levels and early prognosis, in other words, new cardiovascular events. According to Valkonen's studies,[10] among nonsmoker men, those who were in the highest quartile for ADMA had a 3.9 fold increase in risk of acute coronary events compared with the other quartiles. A study in patients with mild nonobstructive coronary arterial disease found that severe endothelial dysfunction significantly increased the risk of cardiac events over an average follow-up of 28 months. In contrast, patients with mild dysfunction or normal function experienced no cardiac events.[4]

Zhang et al.[11] pointed out higher plasma ADMA levels in current smokers. In contrast Zeller et al.[6] showed slightly lower levels of ADMA in smokers than nonsmokers. In our study, similar levels were measured in smoker and nonsmoker group in agreement with Zeller's study.

Exposure to risk factors such as hypertension or hypercholesterolemia decreases the bioavailability of endothelium-derived NO and impairs endothelium-dependent vasodilatation. It was shown that ADMA levels were positively correlated with age, hypertension, sigma glucose (an index of glucose tolerance), and intima-media thickness of the carotid artery in asymptomatic 116 subjects who were taking no medication.[12] Significantly elevated ADMA levels were found in individuals with type-1 diabetes and hypercholesterolemia.[13] Böger reported that ADMA was a novel risk factor independent of other confounding factors including age, smoking, hypercholesterolemia and others.[14]

CRP, as an acute phase reactant, is a recognized biomarker of prognosis after MI.[6] In stable coronary artery disease, an elevated hs-CRP level was found a significant predictor of adverse cardiovascular events.[15] Won Bae et al.[16] from Korea observed a strong association between ADMA and CRP levels. There was no correlation between CRP levels and early cardiac events in our study.

A study from Japan pointed out a significant inverse correlation between plasma NO and LVEF, as estimated by echocardiography, in patients with heart failure of various aetiology.[17] Elevated ADMA plasma concentrations were associated with adverse cardiovascular outcome in patients suffering chronic heart failure.[18] No significant association was found between ADMA and cardiovascular risk factors at the end of Zellers study.[6] In our research there was not a significant correlation between ADMA levels and age, gender, smoking, history of hypertension, diabetes, levels of TC and CRP. We did not find a relationship with respect to ADMA levels and ejection fraction in our group in the short term.

Several studies have shown that a single measurement of endothelial function in both coronary and peripheral circulation can be of prognostic value in a number of cohorts which include patients with established coronary disease. But it should be appreciated that endothelial function has intrinsic biologic variability, and thus a single measurement may also give limited information.[2]

There are many limitations of our study. First the group is small. There was a preponderance of men. We do not have a control group. Although ADMA levels of subjects with/and without early complications were not different, they were higher than reference values of the kit in accordance with other studies.[19,20] We did not evaluate arginine/ADMA ratio which is supposed to be physiologically important. We did not either measure ADMA levels one month after myocardial infaction. It might have showed us whether there was a temporal increase during early period. So we cannot know if the basal levels influence the prognosis. There is a significant difference with regard to age of patient with and without complications. This may also be a confounder. We could not obtain blood from patients who died on the first few ours at the coronary care unit. So we may have lost the subjects who might have the highest ADMA levels which could affect our results. There may also be the potential influence of previously prescribed medicines on ADMA concentrations such as beta blockers, ACE-inhibitors, metformin and clopidogrel.[16] We could not perform echocardiography at admission. So we can not compare the results as the biochemical values.

CONCLUSION

Many studies mentioned above pointed out high ADMA levels as a cardiovascular risk factor in the long-term. But few researches are available for acute coronary syndromes. Although we could not find a relationship between ADMA levels and cardiovascular events after myocardial infarction, it may provide prognostic information beyond CRP, NT-pro-BNP and LVEF. However further studies are needed to evaluate ADMA in specific subgroups. Our data do not allow a specific conclusion to be drawn; so short and long term studies should also be conducted with large patient populations in this field.

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