Asymmetrical dimethylarginine and severity of erectile dysfunction and their impact on cardiovascular events in patients with acute coronary syndrome

Meryem Aktoz[1], Tevfik Aktoz[2], Ersan Tatlı[1], Mustafa Kaplan[2], Fatma Nesrin Turan[3], Ahmet Barutçu[1], İrfan Hüseyin Atakan[2], Muzaffer Demir[4], Armağan Altun[1]

[1]Department of Cardiology, School of Medicine, Trakya University, Edirne, Turkey
[2]Department of Urology, School of Medicine, Trakya University, Edirne, Turkey
[3]Department of Biostatistics, School of Medicine, Trakya University, Edirne, Turkey
[4]Department of Internal Medicine, School of Medicine, Trakya University, Edirne, Turkey

Corresponding author:
Meryem Aktoz, MD
Department of Cardiology
School of Medicine
Trakya University
Edirne 22030, Turkey
Phone: 90 284 235 76 41
Fax: 90 284 235 27 30
E-mail:
meryemaktoz1@yahoo.com

Abstract

Introduction: Coronary artery disease (CAD) and vascular erectile dysfunction (ED) are related to endothelial dysfunction. Elevated asymmetrical dimethylarginine (ADMA) levels and ED are common in patients with increased cardiovascular risk. Our aim was to investigate whether ADMA has a predictive role for major adverse cardiovascular events (MACE) in acute coronary syndrome (ACS). The secondary aim of this study was to investigate whether severity of ED predicts MACE in these patients.
Material and methods: Follow-up data were available for severity of ED in 71 patients with ACS. Plasma ADMA levels were determined by ELISA in 57 patients. Erectile dysfunction was assessed by the International Index of Erectile Function-6 (IIEF-6) score. Major adverse cardiovascular events (reinfarction, all-cause hospitalisation, stroke and all-cause death) was evaluated after a median of 10 months.
Results: Severe ED had no significantly increased hazard ratio for cardiovascular events compared with mild, mild to moderate, and moderate ED (0.259 [95% CI 0.041-1.6], $p = 0.147$; 0.605 [95% CI 0.095-3.8], $p = 0.594$; 0.980 [95% CI 0.233-4.1], $p = 0.978$; and 0.473 [95% CI 0.052-1.3], $p = 0.508$). The patients who had ADMA levels ≥ 0.32 μmol/l had no significantly increased hazard ratio for cardiovascular events compared with patients who had ADMA levels < 0.32 μmol/l (2.018 [95% CI 0.615-6.6], $p = 0.247$).
Conclusions: Severity of ED and ADMA did not increase the risk of cardiovascular events in follow-up patients with ACS in our study. Larger prospective studies are necessary to evaluate whether ADMA predicts cardiovascular events in patients with ACS.

Key words: acute coronary syndrome, asymmetric dimethylarginine, erectile dysfunction, major adverse cardiovascular events.

Introduction

Erectile dysfunction (ED) is associated with increased vascular risk of coronary heart disease and stroke [1]. Increased prevalence rates of ED have been reported in patients with vascular disorders such as myocardial infarction and stroke [2]. Men with ED have a two-fold increased risk for acute myocardial infarction [3]. Because ED is a marker of subclinical

coronary artery disease (CAD), many ED patients carry a risk of acute coronary syndrome (ACS). Although the association between cardiovascular risk factors and ED is well established, the predictive role of ED for future cardiovascular events in CAD patients who present with ACS is not known clearly.

Asymmetrical dimethylarginine (ADMA) has recently aroused interest as an endogenous competitive inhibitor of nitric oxide synthases and an independent marker for cardiovascular disease [4]. Generated ADMA is dependent on the extent of arginine methylation in proteins and in the rates of protein turnover and it is extensively metabolized by dimethylarginine dimethylaminohydrolase [5]. It has the potential to produce considerable biological effects, particularly in the endothelial system. Over the last years, several studies have suggested that plasma concentrations of ADMA provide a marker of risk for endothelial dysfunction [6]. Asymmetrical dimethylarginine levels are significantly elevated in men who have early stage coronary atherosclerosis and ED [7]. Assessment of ADMA might aid cardiovascular risk assessment. We investigated whether ADMA has a predictive role for major adverse cardiovascular events (MACE) in CAD patients who presented with ACS. The secondary aim of this study was to investigate whether severity of ED predicts MACE in these patients.

Material and methods

A total of 121 men who presented with ACS and underwent coronary angiography were prospectively enrolled between January 2007 and July 2007 for this study. Thirty-one patients refused participation or returned the International Index of Erectile Function-6 (IIEF-6) questionnaire incomplete. After evaluation of exclusion criteria (heart failure, end stage renal failure, severe valvular heart disease, concomitant chronic severe disease), 90 patients were enrolled into the ongoing study. In 20 patients laboratory values for ADMA were missing and 13 patients' follow-up data of MACE were not available. Asymmetrical dimethylarginine analysis was performed for the remaining 57 patients. For analysis of ED severity, 19 patients' follow-up data of MACE were not available. The remaining 71 subjects with complete baseline and follow-up data were included in the analysis.

Inclusion criteria for ACS were: chest pain consistent with myocardial ischaemia, elevation of troponin-I or creatine kinase above upper limit of normal, and one of the following findings: electrocardiographic evidence of ischaemia, or wall motion abnormality. At the time of inclusion, demographic data, clinical characteristics and current medications were recorded. All patients were evaluated with physical examination and underwent laboratory tests, including lipid profile and fasting glucose. Risk factors were defined as follows: hypertension as blood pressure > 140/90 mm Hg or taking antihypertensive drugs; hypercholesterolaemia as total cholesterol level > 190 mg/dl and LDL cholesterol level > 130 mg/dl; diabetes as fasting glucose level > 125 mg/dl or taking antidiabetic drugs. The study protocol was approved by the Local Ethics Committee and all patients gave informed consent.

Assessment of erectile dysfunction

Erectile dysfunction was assessed by the IIEF-6 score [8]. All men were asked to complete the IIEF questionnaire. Erectile function is addressed by 6 questions. Each question is scored 0 to 5. Erectile dysfunction is defined as value < 26. The severity of ED was classified into 5 categories: no ED (IIEF score 26 to 30), mild (IIEF score 22 to 25), mild to moderate (IIEF score 17 to 21), moderate (IIEF score 11 to 16), and severe (IIEF score < 10) [8].

Assessment of coronary artery involvement

Coronary angiography was carried out in all patients by the Judkins standard technique. Significant angiographic narrowing was defined as > 50% diameter stenosis. Coronary artery disease was scored with 1-2-3 vessel disease. Our study included cases with ACS, and because of acute total occlusion, previously modified Gensini score was used for coronary artery involvement [9].

Laboratory investigations

After an overnight fast, venous blood was collected and serum separated and stored at –70°C until batch analysis. All blood samples were taken within 1 week of diagnosis of ACS and before coronary angiography. Blood levels of blood glucose, total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides were measured by standard laboratory methods. ADMA was analysed by the ELISA method (ADMA direct ELISA, Immundiagnostik AG, Bensheim, Germany) according to the manufacturer's guidelines.

Study end points and follow-up

The study end point was the occurrence of a first MACE consisting of reinfarction, re-hospitalisation, stroke and all cause death. All patients were then prospectively followed by office visit or by telephone contact at 3-month intervals for the occurrence of first MACE. Outcome was evaluated by 2 observers (M. A., A. B.) who were blinded with respect to the patients' baseline clinical and laboratory data.

Statistical analysis

Continuous data are presented as medians (min-max values) for both ADMA and IIEF-6 score.

Categorical data are given as counts (percentages). Mann-Whitney U test was applied for univariate comparison of continuous data and Spearman rank correlation for assessment of associations between continuous variables. ROC analysis was used for ADMA cut-off values. Event-free survival rates until the first cardiovascular adverse event according to ADMA levels (ROC analysis) and IIEF score were calculated using the Kaplan-Meier method and compared by the log rank test. Multivariate Cox proportional hazards analysis was used to assess the independent effect of ADMA and IIEF on the composite end point and to adjust for potential confounders. Continuous variables were included as confounders if these variables were associated with ADMA and IIEF according to Spearman rank correlation coefficients. Categorical variables were entered into the model if they influenced ADMA and IIEF according to univariate analysis. In addition, adjustment for traditional cardiovascular risk factors was performed. Results of the Cox proportional hazards model are presented as hazard ratio and 95% confidence interval (CI) as well as survival curves according to cut-off values of ADMA and IIEF. P values < 0.05 were considered to indicate statistical significance.

According to a retrospective power analysis for ADMA level in patients, when 57 patients and HR = 2.018 are used, power is calculated as 0.275 (α = 0.05). Data were analysed using NCSS 2008 Demo for Windows (NCSS, Kaysville, UT, USA).

Results

Ninety patients were eligible for the study. In 20 patients laboratory values for ADMA were missing and 13 patients' follow-up data of MACE were not available. Asymmetrical dimethylarginine analysis was performed for the remaining 57 patients. For analysis of ED severity, 19 patients' follow-up data of MACE were not available. The remaining 71 subjects with complete baseline and follow-up data were included in the analysis. Follow-up duration was median 10 months (range: 1-13 months).

Asymmetrical dimethylarginine and survival

Median ADMA plasma concentration of all patients was 0.50 µmol/l (IQR, 0.06 to 1.41). Factors potentially influencing ADMA concentrations such as the presence of risk factors of atherosclerosis, medication, coronary vessels involvement, and revascularisation were not significantly different (Table I). Asymmetrical dimethylarginine was elevated in the patients without diabetes mellitus and the group without antidiabetic therapy and correlated negatively with age and Gensini score (Table II). During follow-up, 5.3% reinfarction, 12.3% hospitalisation, and 8.8% all-cause death occurred

in 57 patients, and the revascularisation rate was 63.2%. When the cut-off value of ADMA 0.32 µmol/l was accepted according to ROC analysis, > 0.32 µmol/l yielded a sensitivity of 70% and a specificity of 28% for detecting CAD which presented ACS. Thus, according to ADMA cut-off value 0.32 µmol/l, 57 subjects were divided into 2 groups. Twenty-nine percent MACE in the patients who had ADMA levels < 0.32 µmol/l and 17.5% MACE in other patients occurred during the follow-up period (p = 0.236, χ^2 = 1.405, log rank test). The patients who had ADMA levels ≥ 0.32 µmol/l had no significantly increased hazard ratio for cardiovascular events compared with patients who had ADMA levels < 0.32 µmol/l after adjustment for possible confounders (2.018 [95% CI 0.615-6.626], p = 0.247 (Table III).

Severity of erectile dysfunction and survival

During follow-up 20 (28.5%) MACE occurred in 71 patients including 7.1% reinfarction, 14.3% hospitalisation, and 7.1% all-cause death, and the revascularisation rate was 64.3%. When patients were divided into 5 groups according to severity of ED, no ED had no significantly increased hazard ratio for cardiovascular events compared with mild, mild to moderate, moderate ED, and severe ED after adjustment for possible confounders (0.259 [95% CI 0.041-1.610], p = 0.147; 0.605 [95% CI 0.095-3.843], p = 0.594; 0.980 [95% CI 0.233-4.121], p = 0.978; 0.473 [95% CI 0.052-1.345], p = 0.508, respectively). (According to multivariate Cox regression analysis) (Table IV).

Discussion

Our findings showed that serum ADMA concentrations and severity of ED did not predict major cardiovascular events after adjustment of confounder factors in patients with CAD who presented with ACS in this study population. No difference, in terms of serum ADMA and severity of ED, was seen between the MACE group and the group without MACE.

Over the last decade, several studies have shown that circulating concentrations of ADMA can be influenced by many factors. Treatment with rosuvastatin [10], rosiglitazone, metformin, ACE inhibitors and AT-2 receptor blockers have decreased ADMA concentrations in humans [11, 12]. In addition, ADMA is a well characterized marker that has been associated with many traditional risk factors and vascular disorders, as demonstrated for hypertension [13], hyperhomocysteinaemia [14], peripheral artery disease [15], and stroke [16]. Barylski et al. [17] showed that renal failure may be related to impaired endothelial function. Vallance et al. [18] demonstrated that accumulation of

Table I. Median plasma concentrations (interquartile range) of ADMA and IIEF score grouped according to categorical baseline variables

	N	ADMA [μmol/l]	P value	N	IIEF (score)	P value
Risk factors						
Hypertension						
Yes	21	0.44 (0.06-1.30)		26	18 (1-30)	
No	36	0.55 (0.09-1.41)	0.155	44	20 (1-30)	0.363
Diabetes mellitus						
Yes	9	0.16 (0.06-0.84)		15	11 (1-26)	
No	48	0.54 (0.09-1.41)	0.001	53	21 (1-30)	0.002
Hyperlipidaemia						
Yes	6	0.34 (0.08-0.71)		7	26 (15-30)	
No	51	0.50 (0.06-1.41)	0.233	63	19 (1-30)	0.116
Smoking current						
Yes	33	0.55 (0.09-1.41)		41	20 (1-30)	
No	24	0.45 (0.06-1.28)	0.070	29	15 (1-30)	0.031
Smoking former						
Yes	46	0.50 (0.08-1.41)		57	19 (1-30)	
No	11	0.47 (0.06-1.28)	0.537	13	20 (1-30)	0.867
Medication						
β-Blocker therapy						
Yes	14	0.52 (0.08-1.13)		16	24 (1-30)	
No	43	0.47 (0.06-1.41)	0.882	54	18.5 (1-30)	0.187
ACE inhibitors therapy						
Yes	15	0.49 (0.06-1.30)		17	21 (1-30)	
No	42	0.52 (0.08-1.41)	0.906	53	19 (1-30)	0.283
AT2 receptor blocker therapy						
Yes	5	0.43 (0.08-0.97)		7	22 (1-30)	
No	52	0.52 (0.06-1.41)	0.401	63	19 (1-30)	0.549
Calcium antagonist therapy						
Yes	4	0.78 (0.38-1.13)		4	15.5 (1-28)	
No	53	0.49 (0.06-1.41)	0.142	66	19.5 (1-30)	0.470
Diuretic therapy						
Yes	1	1.30 (1.30-1.30)		1	30 (30-30)	
No	56	0.49 (0.06-1.41)	0.070	69	19 (1-30)	0.143
Statin therapy						
Yes	5	0.58 (0.28-1.13)		7	26 (9-30)	
No	52	0.49 (0.06-1.41)	0.671	63	19 (1-30)	0.242
Acetylsalicylic acid therapy						
Yes	18	0.46 (0.06-1.41)		20	21.5 (1-30)	
No	39	0.54 (0.12-1.28)	0.589	50	19 (1-30)	0.293
Clopidogrel therapy						
Yes	5	0.50 (0.28-1.13)		6	20.5 (1-30)	
No	52	0.49 (0.06-1.41)	0.838	64	19 (1-30)	0.976

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Table I. Median plasma concentrations (interquartile range) of ADMA and IIEF score grouped according to categorical baseline variables

	N	ADMA [µmol/l]	P value	N	IIEF (score)	P value
Anticoagulant therapy						
Yes	1	0.49 (0.49-0.49)		1	24 (24-24)	
No	56	0.50 (0.06-1.41)	0.982	69	19 (1-30)	0.686
Oral antidiabetic therapy						
Yes	6	0.06 (0.08-0.84)		9	12 (1-26)	
No	51	0.54 (0.06-1.41)	0.017	61	20 (1-30)	0.032
Insulin therapy						
Yes	3	0.15 (0.06-0.84)		3	1 (1-18)	
No	54	0.50 (0.08-1.41)	0.259	67	20 (1-30)	0.058
Coronary vessels involvement						
One-vessel disease						
Yes	17	0.54 (0.16-1.28)		21	24 (1-30)	
No	40	0.47 (0.06-1.41)	0.958	49	18 (1-30)	0.053
Two-vessel disease						
Yes	18	0.61 (0.18-1.26)		20	18.5 (1-30)	
No	39	0.47 (0.06-1.41)	0.066	50	20 (1-30)	0.527
Three-vessel disease						
Yes	22	0.35 (0.06-1.41)		29	18 (1-30)	
No	35	0.55 (0.16-1.28)	0.071	41	20 (1-30)	0.222
Total occlusion						
Yes	13	0.38 (0.06-1.10)		15	18 (1-30)	
No	44	0.54 (0.09-1.41)	0.068	55	20 (1-30)	0.499
Revascularisation						
Yes	36	0.43 (0.06-1.30)		45	21 (1-30)	
No	21	0.54 (0.16-1.41)	0.317	25	15 (1-30)	0.056

Table II. Baseline characteristics and Spearman correlation coefficients (p value) for ADMA and IIEF score and continuous variables of acute coronary syndrome

	Median (min-max)	ADMA (R)	P value	Median (min-max)	IIEF score (R)	P value
Age [year]	58 (35-79)	−0.301	0.02	58 (35-79)	−0.635	0.000
Body mass index [kg/m^2]	26.22 (18.8-42.59)	−0.137	0.31	26.2 (18.8-42.6)	0.139	0.251
Cholesterol [mg/dl]	182 (86-278)	−0.279	0.47	177.5 (86-326)	0.340	0.291
Triglycerides [mg/dl]	128 (50-410)	0.007	0.96	132 (40-410)	0.071	0.573
LDL cholesterol [mg/dl]	113 (29-214)	−0.282	0.04	112 (29-320)	0.056	0.658
HDL cholesterol [mg/dl]	36 (22-50)	−0.342	0.01	35 (22-50)	0.202	0.116
Ejection fraction [%]	50 (57-30.74)	0.102	0.48	57 (30-74)	0.080	0.541
Gensini score	33 (7-117)	−0.291	0.03	39 (7-122)	−0.309	0.009
IIEF score	21 (1-30)	0.247	0.06	19 (1-30)	−	−
ADMA [µmol/l]	0.50 (0.06-1.41)	−	−			

Table III. Cox regression analysis assessing the multivariate hazard ratio for ADMA

	Hazard ratio (95% CI)	*P* value
ADMA < 0.32 μmol/l	1	
ADMA ≥ 0.32 μmol/l	2.018 (0.615-6.626)	0.247

Cut-off value 0.32 Umol/l according to ROC analysis (sensitivity 70%, specificity 27%). Multivariate model: adjustment for age, total cholesterol, LDL cholesterol, fasting blood glucose and Gensini score

Table IV. Cox regression analysis assessing the multivariate hazard ratio for IIEF scoring

	Hazard ratio (95% CI)	*P* value
No ED (IIEF score 26 to 30)	1	
Mild ED (IIEF score 22 to 25)	0.259 (0.041-1.610)	0.147
Mild to moderate ED (IIEF score 17 to 21)	0.605 (0.095-3.843)	0.594
Moderate ED (IIEF score 11 to 16)	0.980 (0.233-4.121)	0.978
Severe ED (IIEF score 1 to 10)	0.473 (0.052-1.345)	0.508

Multivariate model: adjustment for age, diabetes mellitus, current smoking, oral antidiabetic therapy

endogenous ADMA, as an indicator of endothelial dysfunction, is associated with chronic renal failure.

In our study, factors potentially influencing ADMA concentrations and prognosis after ACS were not significantly different, such as the presence of risk factors of atherosclerosis, medication, coronary vessels involvement, revascularisation, and valve disease. In our study, ADMA serum concentrations were low in diabetic patients. This paradoxical finding could in part be explained by administration of oral antidiabetic therapy in half of the diabetic population. Metformin and thiazolidinediones reduce ADMA levels [19, 20]. Furthermore, our results indicate that hypertension, hyperlipaemia, smoking, medications including β-blocker therapy, ACE inhibitors and AT-2 blocker therapy, statins, diuretics, calcium channel blockers and antiaggregant therapy did not influence serum ADMA concentrations. Asymmetrical dimethylarginine concentrations were not different in patients on statin therapy or antihypertensive therapy in the present cohort.

Since the demonstration that concentration of ADMA acts as a marker of endothelial dysfunction, circulating levels of ADMA have been related to the presence of coronary artery disease [21] and the occurrence of acute coronary syndrome [22]. Schnabel *et al.* [23] showed that circulating ADMA adds independent prognostic information in patients with CAD. Valkonen *et al.* [21] demonstrated that high concentrations of ADMA in serum are associated with an increased risk of acute coronary events among non-smoking middle-aged men, especially in men with previous coronary heart disease. A recent study in patients with acute MI reported that the baseline ADMA level has prognostic value for mortality after acute myocardial infarction [24]. Asymmetrical dimethylarginine was not associated with future cardiovascular events in the present study. This could be explained by the fact that our study sample was small and all male. Meinitzer *et al.* [25] demonstrated that ADMA concentrations predict all-cause and cardiovascular mortality in individuals with stable and acute CAD. A difference of their study was inclusion of patients with stable CAD.

Our trial is the first study in the literature to investigate whether severity of ED predicts major

cardiovascular events in patients with coronary artery disease with ACS. Vascular disease and ED have similar pathogenic involvement of the nitric oxide pathway leading to impairment of endothelium dependent vasodilatation and structural vascular abnormalities [26, 27]. Montorsi *et al.* identified ED as a predictor of acute coronary syndromes [28]. They showed that ED occurred before CAD symptoms in 67% of patients with ED. Thus, they reinforced that ED might predict the presence of subclinical vulnerable plaque. Ponholzer *et al.* [29] showed that moderate-to-severe ED was calculated to increase the 10-year relative risk of developing CAD by 65% and stroke by 43%. Another trial showed that ED significantly predicted cardiac events [30]. Montorsi *et al.* [9] found that severe ED was more frequent in patients with multi-vessel as compared to single vessel disease. Thus, severe ED should raise questions about prognosis in patients with CAD. We found that severity of ED did not predict future cardiovascular events in patients with ACS. Recently published data have demonstrated that ED is a powerful predictor of cardiovascular morbidity and mortality in diabetic patients with silent CAD [31]. Large prospective studies are necessary to evaluate whether ED predicts cardiovascular events in patients with ACS.

Our study is limited by the relatively small sample size. The relatively small number of male patients could be the reason why ADMA and severity of ED are not predictive in our study. Bae *et al.* [32] found a significant decrease in ADMA levels after 2 weeks of medical therapy for ACS. Because of this we took blood samples within 1 week after diagnosis of ACS before coronary angiography. Although the majority of published methods for determination of plasma concentrations of ADMA are based on high performance liquid chromatography (HPLC), we determined ADMA by a recently atracted ELISA method that is suitable for the determination of

ADMA in large sample series to be analysed more quickly than HPLC [33]. It may be a limitation of our study. Additionally, only male patients participated in the study, which is a further limitation. A recent study found that a 0.15 µmol/l increase in baseline ADMA levels was associated with approximately 30% increase in incident cardiovascular risk in 880 healthy women [34]. However, whether ADMA concentrations are different in males and females with ACS is not known. After performing power analysis in our study we considered that large prospective studies, also including female patients, are necessary to evaluate whether ADMA predicts cardiovascular events in patients with ACS.

In conclusion, the results of this prospective study suggest that ADMA and severity of ED did not predict future cardiovascular events in this patient group. Larger prospective studies with longer follow-up are necessary to evaluate whether ADMA and ED predict MACE in patients with ACS.

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