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Asymmetric and symmetric dimethylarginine in patients presenting with risk factors for coronary heart disease

Research Article

Marina Deljanin Ilic*[1], Radmila Pavlovic[2], Gordana Lazarevic[3], Tatjana Cvetkovic[4], Gordana Kocic[4], Stevan Ilic[1],Vladisav Stefanovic[5]

1 Institute of Cardiology, Niska Banja, Faculty of Medicine, University of Nis, 18000 Nis, Serbia

2 Institute of Chemistry, Faculty of Medicine, University of Nis, 18000 Nis, Serbia

3 Clinic of Cardiology, Clinical Center, Nis, 18000 Nis, Serbia

4 Institute of Biochemistry, Faculty of Medicine, University of Nis, 18000 Nis, Serbia

5 Institute of Nephrology, Faculty of Medicine, University of Nis, 18000 Nis, Serbia

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Abstract: The aim of the present study was to investigate asymmetric (ADMA) and symmetric dimethylarginine (SDMA) production in patients presenting with one or more risk factor (RF) for coronary heart disease (CHD). Patients and methods: Overall, 113 participants were enrolled in the study, including 45 patients presenting with risk for CHD (27 male and 18 female; aged 55.9 ± 6.4 years), 30 sex and age-matched middle-aged healthy controls (16 male and 14 female; aged 56.3 ± 8.4 years), and 38 young healthy controls (38 male; aged 24.6 ± 3.9 years). Results: No significant differences for ADMA and SDMA were recorded between patients groups presenting with risk for CHD. However, ADMA and SDMA were significantly higher in all examined patient groups (≥3 and 1–2 RF, hypertensive and non-hypertensive, obese and non-obese, diabetics and non-diabetics) compared with both control groups (middle-aged and young controls) ($p<0.001$). ADMA significantly correlated with SDMA in ≥3 RF ($p<0.05$), hypertensive ($p<0.05$), non-obese ($p<0.05$), non-diabetics ($p<0.01$), as well in middle-aged ($p<0.05$) and young controls ($p<0.001$). Conclusion: Significantly higher ADMA and SDMA were found between patients presenting with risk for CHD (≥ 3 and 1–2 RF, hypertensive and non-hypertensive, obese and non-obese, diabetics and non-diabetics) and healthy, middle-aged and young controls. ADMA significantly correlated with SDMA in ≥3 RF, hypertensive, non-obese and non-diabetic patients, as well as in middle-aged and young controls.

Keywords: *Nitric oxide • Asymmetric and symmetric dimethylarginine • Coronary heart disease*

1. Introduction

Endothelial dysfunction plays an important role in the pathogenesis of cardiovascular disease. It is directly related to cardiovascular morbidity and mortality because it predicts long-term progression of atherosclerotic disease and cardiovascular event rates [1,2]. Endothelial dysfunction is believed to be induced by different pathomechanisms, including impaired bioavailability of nitric oxide (NO). NO is a signaling molecule formed from L-arginine by a number of different isoforms of nitric oxide synthase (NOS) [3]. The activity of NO mostly results from its inhibited synthesis or increased consumption by

* *E-mail: marinadi@open.telekom.rs*

reactive oxygen species. Its antithrombotic, antiatherosclerotic, antihypertensive, antioxidant and proapoptotic properties have already been demonstrated.

Asymmetric dimethylarginine (ADMA) an endogenous NOS inhibitor, has been shown to be an independent risk factor and well-established predictor of cardiovascular events and death in patients with coronary heart disease (CHD), advanced renal failure, hypercholesterolemia, insulin resistance, diabetes mellitus and hypertension, and is closely associated with vascular oxidative stress [4-6]. Prospective investigations of ADMA have highlighted its role in increasing vascular resistance, reducing vascular compliance, attenuating cerebral blood flow, increasing sodium retention and decreasing cardiac output, thereby causing adverse cardiovascular events. The predictive power of ADMA was demonstrated to be independent of traditional and widely used risk factors that the risk of all-cause and cardiovascular morbidity and mortality [7].

Not all studies of ADMA and CVD have found significant association with endothelial dysfunction. In an Australian study that included patients with triple-vessel coronary disease, plasma ADMA levels were not significantly different between patients with and without coronary disease [8]. In their study of elderly high-risk men, Eid et al. reported no differences in plasma ADMA levels in men with or without CVD [9]. Finally, in the Ludwigshafen Risk and Cardiovascular Health Study (LURIC), the authors reported no association between 2543 persons with versus 695 persons without angiographically confirmed CAD after multivariable adjustment [7].

However, it remains unknown whether elevated ADMA plasma concentration may be considered simply as a marker for cardiovascular disease or whether increased ADMA levels per se may predispose to the development of vascular disease. Moreover, there is compelling evidence to suggest that ADMA may be suitable not only as a diagnostic marker for the risk assessment, but also to suggest that the biochemical pathways that regulate ADMA may be promising therapeutic approaches for treatment of cardiovascular disease.

Symmetric dimethylarginine (SDMA), an alternative methylation product of N-mono-methylarginine – the immediate precursor and a stereoisomer of ADMA – lacks NOS inhibitory activity, but is independently associated with increased cardiovascular and all-cause mortality. The pattern of risk linked to SDMA is different from that linked to ADMA, suggesting different pathophysiological roles of these 2 methylarginine metabolites [10]. Because SDMA does not inhibit NOS, this structural isomer was thought to be inert [11]. However, recent publications suggest that SDMA has a predictive value for cardiovascular risk similar to that of ADMA [12]. Several explanations have been proposed, including increased formation of reactive oxygen species, competition for l-arginine transport into endothelial cells, or valid reflection of renal function by SDMA [13].

Since these three markers were demonstrated to be significantly associated with an increased risk of incident cardiovascular events and total mortality in subjects at a broad range of global risk, the aim of the present study is to investigate ADMA and SDMA production, not only in patients presenting the risk for CHD but also in healthy middle-aged and young controls.

2. Patients and methods

2.1. Patients

The present study was carried out with of 113 subjects overall, including 45 patients (27 male and 18 female; aged 55.87 ± 6.39 years), without history of previous myocardial infarction but presenting with risk for CHD, recruited from Outpatient Department of the Institute for Treatment and Rehabilitation of Cardiovascular Diseases “Niska Banja”, Nis, Serbia, 30 sex and age-matched middle-aged healthy controls (16 male and 14 female; aged 56.33 ± 8.36 years) and 38 young healthy controls (38 male; aged 24.58 ± 3.89 years). Patients presenting with risk for CHD were assigned into groups according to the frequency of CHD risk factors (RF) (15 patients with 1–2 CHD RF, 30 patients with ≥3 CHD RF) and the history of hypertension (8 non-hypertensive and 37 hypertensive patients), obesity (35 non- obese and 10 obese patients) and diabetes mellitus (35 nondiabetics, 10 diabetics). Major clinical and biochemical characteristics, as well as the presence of RF for CHD, are shown in Table 1. The patients and controls had relatively normal kidney function as estimated by the Cockcroft and Gault formula, which is used to predict an individual’s creatinine clearance. Estimated glomerular filtration rate was 107.3±13.2 ml/min/1.73 m2 in patients, 114.7±12.8 ml/min/1.73 m2m in middle-aged healthy controls, and 124.7±15.2 ml/min/1.73 m2m in young controls.

The study was approved by the local Research Ethics Committee, and an Informed consent was obtained from all subjects enrolled in the study.

3. Methods

3.1. Baseline assessments

A detailed medical evaluation was performed at baseline, with a particular attention to underlying RF, comorbidities

and medical history. According to previously diagnosed comorbidities, including arterial hypertension, diabetes mellitus and/or hyperlipidemia, the concomitant treatment included regular use of cardioprotective (*antiplatelets, β-blockers, angiotensin-converting enzyme inhibitors and/or angiotensin-receptors blockers*), antihyperglycemic (oral antihyperglycemic drugs) and lipid-lowering drugs (statins).

To determine glycemia, lipid profile parameters and high sensitive CRP (Humastar 180 Biochemical analyzer, HUMAN, Germany), blood samples for basic biochemical analyses were taken from an antecubital vein after an overnight fast of 12 hours.

Arterial blood pressure was expressed as the average of 3 consecutive measurements on the left hand with the patient still in the sitting position; heart rate was determined digitally.

3.2. Asymmetric and symmetric dimethylarginine (ADMA and SDMA) determination

ADMA and SDMA were evaluated by high-performance liquid chromatography with fluorescence detection according to the method developed by Paroni et al. [14] .

3.3. Statistical analysis

Data was analyzed using statistical software Jandel SigmaStat® for Windows (version 3.5). The Student`s T-test and Mann-Whitney Rank Sum Test, as well as a Chi-squared Test and Wilcoxon Signed Rank Test were used as appropriate. Data was expressed as means ± SD, absolute values and percents. A p value less than 0.05 was considered statistically significant.

Table 1. Cardiovascular risk factors, clinical and biochemical parameters, ADMA and SDMA.

	Patients (n=45)	Middle-aged controls (n=30)	Young controls (n=38)
CHD risk factors			
Age (years)	55.87 ± 6.39 [D]	56.33 ± 8.36 [D]	24.58 ± 3.89
Male:Female (n,%)	27:18 (60:40) [D]	16:14 (53:47) [D]	38:0 (100:0)
Hypertension (n,%)	37:8 (82:18) [D]	12:18 (40:60) [E]	2:36 (5:95)
Hyperlipidemia (n,%)	33:12 (73:27) [A, D]	10:20 (33:67) [E]	2:36 (5:95)
Diabetes mellitus (n,%)	10:35 (22:78) [D]	5:25 (17:83) [F]	0 (0)
Smoking (n,%)	34:11 (76:34)	21:9 (70:30)	27:11 (71:29)
Obesity (n,%)	10:35 (22:78) [D]	6:24 (20:80) [D]	3:36 (8:92)
Clinical parameters			
Heart rate (bpm)	77.56 ± 11.68	82.00 ± 8.96 [D]	73.68 ± 7.86
Systolic blood pressure (mmHg)	133.69 ± 11.82 [D]	135.83 ± 7.32 [D]	123.03 ± 5.01
Diastolic blood pressure (mmHg)	83.11 ± 5.14 [C, D]	86.00 ± 7.59 [D]	72.89 ± 3.61
Biochemical parameters			
Glycemia (mmol/l)	6.01 ± 1.49 [E]	5.23 ± 0.76	5.18 ± 0.52
Total cholesterol (mmol/l)	6.47 ± 1.27 [A, D]	5.16 ± 0.75	4.97 ± 0.43
HDL-cholesterol (mmol/l)	1.24 ± 0.26 [C, F]	1.39 ± 0.32	1.44 ± 0.39
LDL- cholesterol (mmol/l)	4.07 ± 0.94 [C, D]	3.77 ± 0.16 [D]	2.91 ± 0.45
Triglycerides (mmol/l)	2.14 ± 0.81 [B, D]	1.52 ± 0.68 [F]	1.11 ± 0.42
High sensitive CRP (mg/dl)	12.53 ± 5.25 [A, D]	1.55 ± 0.89	1.49 ± 1.43
ADMA and SDMA			
ADMA (μmol/l)	0.61 ± 0.14 [A, D]	0.26 ± 0.11 [D]	0.30 ± 0.11
SDMA (μmol/l)	0.59 ± 0.24 [A, D]	0.29 ± 0.13	0.28 ± 0.09

CHD, coronary heart disease; CRP, C reactive protein; ADMA, asymmetric dimethylarginine; SDMA, symmetric dimethylarginine.
ADMA values in patients with risk factors for CAD are from an our previous publication15.
A p<0.001 vs. middle-aged controls
B p<0.01 vs. middle-aged controls
C p<0.05 vs. middle-aged controls
D p<0.001 vs. young controls
E p<0.01 vs. young controls
F p<0.05 vs. young controls

4. Results

4.1. Baseline assessments

Baseline clinical and biochemical characteristics, as well as ADMA and SDMA for the entire group of patients presenting with risk for CHD, their age-matched, i.e. middle-aged, as well as young controls, are shown in Table 1. Control groups were free of CHD RF.

4.2. ADMA and SDMA according to the frequency of CHD risk factors

No significant differences for ADMA and SDMA were recorded between the ≥ 3 and 1–2 RF groups of patients presenting with risk for CHD. ADMA and SDMA were significantly higher in both patients groups compared with healthy middle-aged and young controls ($p<0.001$) (Table 2). ADMA and SDMA were significantly correlated in the ≥ 3 RF group ($p<0.05$), middle-aged ($p<0.05$) and young controls ($p<0.001$).

Table 2. ADMA and SDMA according to the number of cardiovascular risk factors, and to the presence or absence of hypertension, obesity and diabetes mellitus.

	ADMA (μmol/l)	SDMA (μmol/l)
CHD risk factors		
≥ 3 risk factors (n=30)	0.63 ± 0.14 A, B	0.61 ± 0.26 A, B
1-2 risk factors (n=15)	0.57 ± 0.12 A, B	0.58 ± 0.21 A, B
middle-aged controls (n=30)	0.26 ± 0.11 B	0.29 ± 0.13
young controls (n=38)	0.40 ± 0.11	0.28 ± 0.09
Hypertension		
hypertensive (n=37)	0.61 ± 0.14 A, B	0.58 ± 0.21 A, B
non-hypertensive (n=8)	0.63 ± 0.13 A, B	0.67 ± 0.36 A, B
middle-aged controls (n=30)	0.26 ± 0.11 B	0.29 ± 0.13
young controls (n=38)	0.30 ± 0.11	0.28 ± 0.09
Obesity		
obese (n=10)	0.63 ± 0.14 A, B	0.68 ± 0.34 A, B
non-obese (n=35)	0.60 ± 0.14 A, B	0.57 ± 0.21 A, B
middle-aged controls (n=30)	0.26 ± 0.11 B	0.29 ± 0.13
young controls (n=38)	0.40 ± 0.11	0.28 ± 0.09
Diabetes mellitus		
diabetics (n=10)	0.63 ± 0.14 A, B	0.61 ± 0.17 A, B
non-diabetics (n=35)	0.60 ± 0.14 A, B	0.59 ± 0.26 A, B
middle-aged controls (n=30)	0.26 ± 0.11 B	0.29 ± 0.13
young controls (n=38)	0.30 ± 0.11	0.28 ± 0.09

CHD, coronary heart disease; ADMA, asymmetric dimethylarginine; SDMA, symmetric dimethylarginine.
ADMA values according to the number of CHD risk factors, and to the presence of diabetes mellitus are from an our previous publication14.
A $p<0.001$ vs. middle-aged controls
B $p<0.001$ vs. young controls

4.3. ADMA and SDMA according to the presence or absence of hypertension

No significant differences for ADMA and SDMA were recorded between hypertensive and non-hypertensive groups of patients presenting with risk for CHD. ADMA and SDMA of patients were significantly higher compared with healthy, non-hypertensive, middle-aged and young controls ($p<0.001$) (Table 2). ADMA and SDMA were significantly correlated in hypertensive patients ($p<0.05$).

4.4. ADMA and SDMA according to the presence or absence of obesity

No significant differences for ADMA and SDMA were observed between obese and non-obese patients presenting with risk for CHD. ADMA and SDMA were, however, significantly higher in patients groups compared with healthy, non-obese middle-aged and young controls ($p<0.001$) (Table 2). ADMA and SDMA were significantly correlated in non-obese CHD patients ($p<0.05$).

4.5. ADMA and SDMA according to the presence or absence of diabetes mellitus

No significant differences for ADMA and SDMA were recorded between diabetics and non-diabetics presenting with risk for CHD. In diabetics and non-diabetics, ADMA and SDMA were significantly higher compared with healthy middle-aged and young controls ($p<0.001$) (Table 2). ADMA and SDMA were significantly correlated in non-diabetic patients ($p<0.01$).

5. Discussion

ADMA was demonstrated to be significantly associated with an enhanced atherogenesis and with an increased risk of incident morbidity and mortality in subjects with low, intermediate and high cardiovascular risk [16]. Some studies of ADMA and CVD have found no significant associations (7,8,9) Recently published results of the different effects of ADMA on various artery types have questioned its role as a confinable and specific cardiovascular risk factor, and clearly suggested the need for further studies to elucidate its prognostic significance for patients with cardiovascular risk [17]. For that reason, one of the aims of the present study is to investigate its production in patients presenting the risk for CHD with regards to the frequency of CHD RF. The

results of the study demonstrated no significant differences for ADMA and SDMA concentrations between ≥ 3 and 1–2 RF groups of patients. However, ADMA and SDMA were significantly higher in th epatient group compared with healthy middle-aged and young controls.

According to the results of the present study, no significant differences for ADMA and SDMA were observed between hypertensive and non-hypertensive patients presenting the risk for CHD. In the patient group, ADMA and SDMA were significantly higher compared with healthy, non-hypertensive, middle-aged and young controls.

Increased circulating methylarginines have been linked to the metabolic syndrome to explain the underlying endothelial dysfunction and overall cardiovascular risk. It has been demonstrated that increased protein turnover in insulin-resistant states contribute to an increase in circulating methylarginines and that obesity, sex and ageing affected methylarginine levels. Data show that ADMA is 29% to 120% higher in obese, and 34% higher in elderly, than in lean subjects, while SDMA is 34% and 20% higher in those subjects, respectively. Elevations of the methylarginines in obese and also ADMA in elderly men are assumed to be related to increased protein turnover and to lesser insulin sensitivity to protein metabolism; it has been suggested that these interrelationships might amplify insulin resistance and endothelial dysfunction [18]. Another study has found, on the contrary, that despite obese women had elevated hsCRP and triglycerides, decreased insulin sensitivity, adiponectin and HDL-cholesterol levels – all of which were closely linked RF for cardiovascular disease – circulating ADMA levels remained unchanged in obese individuals as compared with controls [19]. According to the results of the present study, no significant differences for ADMA and SDMA were recorded between obese and non-obese patients presenting with risk for CHD. ADMA and SDMA were significantly higher in obese and non-obese patients, compared with healthy middle-aged and young controls. The results of the present study have not revealed any significant differences for ADMA and SDMA between diabetics and non-diabetics presenting with risk for CHD.

Abnormal regulation of ADMA plays a role in endothelial dysfunction in different vascular beds and accelerated CHD risk of patients with diabetes mellitus [20]. Reduced NO bioavailability is believed to be crucial for the pathogenesis of endothelial dysfunction in the early stages of diabetes mellitus. For that reason, strategies aimed at increase NO bioavailability are thought to result in restoration of endothelial function [21].

According to the multicenter CARDIAC case control study ADMA can be regarded as a new marker for cardiovascular disease. The plasma concentration of ADMA may represent an additional parameter that, in conjunction with other established cardiovascular risk factors, may help identify patients with an increased risk of cardiovascular disease [22].

In conclusion, the results of the present study have demonstrated no significant differences for ADMA and SDMA between groups of patients presenting with risk for CHD, with respect to the frequency of CHD risk factors and previous history of hypertension, obesity and diabetes mellitus, but did show significantly higher ADMA and SDMA values between patients presenting the risk for CHD and both middle-aged and young controls. ADMA significantly correlated with SDMA in ≥3 RF, hypertensive, non-obese and non-diabetic patients, as well as in middle-aged and young controls.

Our study has limitations; it is a single-center study and confirmation in other cohorts is necessary to validate our findings. Second, the average age of the cohort was 55.9 years and may not apply to younger cohorts. Third, the cohort was relatively small, and the number of events recorded during the study was limited.

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Conflict of interests

It was not declared by the authors.

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