

ASYMMETRIC DIMETHYLARGININE IN HYPER-CHOLESTEROLEMIC PATIENTS

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ABSTRACT:

Background: In the medical literature, data on the levels of ADMA in patients with HH is scarce.

Aim: To study the level of asymmetric dimethylarginine in asymptomatic, marked, never-treated hypercholesterolemia.

Methods: 30 asymptomatic patients with marked, never-treated hypercholesterolemia and 30 controls were evaluated. The plasma level of asymmetric dimethylarginine (ADMA) was tested by ELISA method.

Results: A statistically significant difference was found between patients and controls, regarding all tested parameters, including lipid and non-lipid marker - asymmetric dimethylarginine. ($p < 0.001$)

Conclusion: It is concluded that asymptomatic, marked, never-treated HH is associated with elevated levels of ADMA, and this is related to the proportional increase in total cholesterol.

Key words: LDL-cholesterol, apolipoproteins, endothelium- dependent vasodilation, ultrasound.

INTRODUCTION:

The activity of endogenous nitric oxide synthetase (eNOS) is reduced in HH, resulting in disturbed endothelium-dependent vasodilation and reduced platelet and monocyte adhesion. (3, 4, 5) To a large extent, this is dependent on elevated plasma levels of asymmetric dimethylarginine (ADMA) in hypercholesterolemia, as a competitive inhibitor of eNOS. (1, 6, 7) At this point, it can be accepted that LDL cholesterol increases the expression of ADMA precursor protein and also reduces the activity of the enzyme demethylamino hydrolase, which breaks down ADMA. (2) So far, investigations in patients with mild HH (total cholesterol > 5.5 mmol/l) is scarce. Scientific data is contradictory regarding ADMA metabolic pathway in HH, as evidence from patients with marked HH is limited. (1,6,7)

AIM: Analysis of lipids (total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, Apo-A₁ and B and index ApoB/Apo-A₁) and non lipid (ADMA) markers for atherosclerotic risk in patients compared to control group.

Patients:

Investigated 30 patients with total plasma cholesterol - > 7.5 mmol/l and age above 16 years. Exclusion Criteria: 1. Diabetes mellitus or impaired glucose tolerance – fasting blood glucose > 5.6 mmol/l. 2. Cigarette smoking. 3. History of, clinical and laboratory/instrumental evidence of: 3.1. Coronary artery disease (CAD) in all forms, 3.2 Cerebrovascular diseases 3.3. Arterial Hypertension. 3.4 COPD, Bronchial asthma, 3.5 Chronic arterial insufficiency of the extremities (peripheral arteries) – ABI < 0.9 . 3.6 Chronic renal and hepatic dysfunction 3.7. Systemic disorders of the connective tissue– Collagenosis, Rheumatoid arthritis, SLE 3.8 Neoplasms 3.9 Acute inflammation or chronic inflammatory process requiring active treatment. 4. Prolonged use of NSAID (over the last six months and during the period of investigation), corticosteroids, hormonal medications, psychotropic drugs, lipid regulating medications – fibrates, statins, antioxidants. 4.1. Chronic use of alcohol and drug abuse. The control group consisted of 100 asymptomatic patients without any evidence of dyslipidemia

METHODS:

Laboratory testing was performed at the Central Clinical Laboratory of University Hospital St George. Coulter STKS, USA Hematological Analyzer was used for determination of hemoglobin, erythrocytes, leucocytes, platelets, hematocrit. Urinalysis included relative density, urine glucose, albumin (dry test) and sediment (microscopic direct visualization). Biochemical parameters: blood glucose, total cholesterol, TGL, HDL cholesterol, urea, creatinine, uric acid were investigated

using biochemical analyzer Konelab 60i, Thermo Electron Co, USA. Creatinine clearance was determined using Cockcroft's and Gault's formula - $[(140 - \text{years}) \times \text{weight (Kg)}] / [72 \times \text{serum creatinine}]$ (x 0.85 in women). Fibrinogen was analyzed using Clauss's method. Determination of LDL cholesterol in serum was performed using direct automated analysis and reagents from Thermo Electron Co Konelab™, Finland. Apolipoprotein -A₁ (Apo-A₁) and B (Apo-B) in serum were tested using reagents from Thermo Electron Co Konelab™, Finland and Biochemical analyzer Konelab 60i, Thermo Electron Co, USA. The levels of ADMA were determined by ELISA (Enzyme Linked Immunosorbent Assay) using kits from DLD Diagnostika GMBH, Germany and BenderMed Systems, Germany.

Statistical processing of data was performed, using: analysis of variance (Student's t test and Student's test for independent and paired samples (independent

simple t-test and paired simple t-test). $p < 0.05$. was also applied. All values are expressed as mean±SD, unless otherwise stated. We used linear regression –univariate and multiple regression models. SPSS v.11.0 for Windows was used for statistical analysis.

Prior to the study procedures written informed consent was obtained from patients and controls. The procedures used in this study were approved by the Institutional Ethics Committee at Medical University of Plovdiv.

RESULTS:

Both groups did not differ from each other significantly with respect to age, sex and BMI ($p > 0,05$). In table 1 investigated atherogenic lipids and non lipid biomarker – asymmetric dimethylarginine in patients and the control group with arithmetical mean and standard deviation are seen. (Table 1)

Table 1. Characteristics of the two investigated groups

Biomarkers		N	mean±SD	SEM	t	P
Total Cholesterol (mmol/l)	Patients	30	8.55±0.12	1.50	20.08	<0.001
	Controls	30	4.32±0.04	0.40		
Triglycerides (mmol/l)	Patients	30	1.44±0.41	0.40	2.75	<0.001
	Controls	30	0.56±0.16	0.12		
HDL-cholesterol (mmol/l)	Patients	30	0.91±0.04	0.20	10.76	<0.001
	Control	30	1.24±0.03	0.21		
LDL-cholesterol (mmol/l)	Patients	30	6.65±0.13	1.50	31.34	<0.001
	Controls	30	2.65±0.08	0.30		
Apo- A ₁ (g/l)	Patients	30	1.31±0.12	0.03	20.88	<0.001
	Controls	30	1.74±0.15	0.04		
Apo-B (g/l)	Patients	30	2.11±0.31	0.06	24.14	<0.001
	Controls	30	1.15±0.11	0.03		
Apo B / Apo A ₁	Patients	30	1.71±0.20	0.04	130.64	<0.001
	Control	30	0.50±0.11	0.04		
ADMA (µmol/l)	Patients	30	1.50±0.02	0.30	20.59	<0.001
	Controls	30	0.50±0.02	0.20		

According to the calculated histogram of ADMA, patients were grouped as follows: group 1 - with values $< 1.7 \mu\text{mol/l}$ - mild deviation and group 2 with values $> 1.7 \mu\text{mol/l}$ - severe deviation. This fact gave us to ground to consider the value of $1.7 \mu\text{mol/l}$ as a differential between the two levels of ADMA.

DISCUSSION:

The levels of ADMA in patients with marked HH

in our study are higher than those cited in the literature. (8). Data from a number of studies have documented a double increase in the level of ADMA in HH. This is likely to be due to the significantly higher values of total cholesterol ($> 7.5 \text{ mmol/l}$) in the investigated contingent of patients. In other similar studies, the serum level of total cholesterol is $> 5.7 \text{ mmol/l}$ and $\text{LDL} > 4.1 \text{ mmol/l}$. It is likely that levels of ADMA increase linearly with the increase in total cholesterol.

CONCLUSION:

Asymptomatic, marked, never-treated HH is associated with elevated levels of ADMA, and this is related to the proportional increase in total cholesterol.

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