# APOLIPOPROTEIN-B AS A PREDICTOR OF ASYMMETRIC DIMETHYLARGININE IN HYPER-CHOLESTEROLEMIC PATIENTS

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**ABSTRACT:**

Background: Regarding the correlative dependence of ADMA with biomarkers of atherogenic risk in HH, reviews of the literature reveal contradictory findings.

Aim: Determinate a predictor of high level of ADMA in patients with asymptomatic, marked, never-treated hypercholesterolemia

Methods: Thirthy asymptomatic patients with marked, never-treated hypercholesterolemia and total cholesterol > 7.5mmol/l and age above 16 years. Lipid profil, creatinine, apolipoprotein-$A_1$ and apolipoprotein-B were investigated using biochemical analyzer Konelab 60i, Thermo Electron Co, USA. The plasma level of asymmetric dimethylarginine (ADMA) was tested by ELISA method.

Results: A statistically significant and strong correlation dependence between ADMA and age ($r_{xy}$ = 0.688; p<0,0001) Statistically significant correlation dependence between ADMA and other atherosclerotic biomarkers (cholesterol of lipoproteins with high density (HDL), cholesterol of lipoproteins with low density / HDL-cholesterol, apolipoprotein-B, apolipoprotein-B/$A_1$ ) is found.

Conclusion: It is concluded that ADMA is the basic modulator of %FMD among all tested atherogenic risk biomarkers in asymptomatic, marked, never-treated hypercholesterolemia.

**Key words**: LDL-cholesterol, apolipoproteins, asymmetric dimethylarginine, predictor

**INTRODUCTION:**

To a large extent, this is dependent on elevated plasma levels of asymmetric dimethylarginine (ADMA), as a competitive inhibitor of eNOS. (1, 4, 5, 6) 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. (3) So far, investigations in patients with mild HH (total cholesterol > 5.5 mmol/l ) is scarce. (2) Therefore, seeking relation between these markers is justified. The correlative dependence of ADMA with biomarkers of atherogenic risk in HH, reviews of the literature reveal contradictory findings. In some studies, ADMA is correlated with total cholesterol and LDL cholesterol (2). However, most studies do not document such a relationship. (7, 8). Scientific data is contradictory regarding ADMA metabolic pathway in HH, as evidence from patients with marked HH is limited. (1 - 8)

**AIM:** Determinate a predictor of high levels of ADMA in patients with asymptomatic, marked, never-treated hypercholesterolemia

**PATIENTS:**

Investigated 30 patients with total plasma cholesterol - > 7.5mmol/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.

**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. Urinanalysis 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 Cockroft's and Gault's formula - [(140 - years) x weight (Kg) / [72 x 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$^{TM,}$ Finland. Apolipoprotein -A$_1$ (Apo-A$_1$) and B (Apo-B) in serum were tested using reagents from Thermo Electron Co Konelab$^{TM}$, 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. The procedures used in this study were approved by the Institutional Ethics Committee at Medical University of Plovdiv.

**RESULTS:**

Analysis of correlation between ADMA and age and other biomarkers of atherogenic risk A statistically significant and strong inverse correlation dependence between ADMA and age ($r_{xy}$ = - 0.688; p<0,0001) Statistically significant correlation dependence between ADMA and other atherosclerotic biomarkers (cholesterol of lipoproteins with high density (HDL), cholesterol of lipoproteins with low density LDL/HDL-cholesterol, apolipoprotein-B, apolipoprotein-B/A$_1$ ) is found. (**Table. 1**)There is not statistically significant correlation with total cholesterol, triglycerides, LDL-cholesterol (p>0.05).

**Table 1**. Statistically significant correlation between of ADMA and other biomarkers

|  | $r_{xy}$ | p |
|---|---|---|
| Apolipoprotein-B | 0,972 | < 0,0001 |
| Apolipoprotein B / A$_1$ | 0,510 | < 0,0001 |
| HDL-cholesterol | -0,259 | < 0,01 |
| LDL/HDL -cholesterol | -0,227 | < 0,05 |

A statistically significant strong inverse correlation between ADMA and creatinine clearance ($r_{xy}$ = -0,694, p<0,0001), is observed in the absence of correlation between ADMA and serum creatinine. (p>0.05) Data from linear regression analysis show that Apo-B levels are determinant of ADMA level, whereas backward selection process (include age, Apo-B, Apo-B/Apo-A1, creatinine clearance) selects the most important statistically significant factor related to ADMA - Apo-B. This fact eliminates the renal mechanism for increasing ADMA level in in patients with asymptomatic, marked, never-treated hypercholesterolemia. (**Table 2**)

**Table 2**. Multivariate linear regression analysis of ADMA in relation to independent variables

| Independent variables | B | SE | 95 % CI | p |
|---|---|---|---|---|
| Constant | 0,122 | 0,262 | [1,377; 1,471] | > 0,050 |
| Apo-B | 0,133 | 0,064 | [- 0,008; - 0,006] | < 0,050 |

*In all cases ADMA was dependent variable

**DISCUSSION:**

Regarding the correlative dependence of ADMA with biomarkers of atherogenic risk in HH, reviews of the literature reveal contradictory findings. In some studies, ADMA is correlated with total cholesterol and LDL cholesterol (2). However, most studies do not document such a relationship.(7,8). The mechanism of increased ADMA in HH is not very clear. A number of

hypotheses exist – increased endogenous formation, impaired metabolic degradation or reduced clearance of ADMA. (3). The absence of significant relation between ADMA and the parameters of the routinely tested lipid profile is most probably due to the fact that it does not include the most atherogenic fractions of serum lipids ( OxLDL, small and dense LDL fractions). They are more likely to be related to the increase in ADMA. The strong correlation between ADMA and Apo B in this study shows that ADMA is related to the most atherogenic small and dense LDL fractions in the investigated patients with HH. It is possible that they participate in the mechanisms, determining the increased level of ADMA in HH.

As mentioned above, the level of ADMA correlates with the creatinine clearance but not with serum creatinine level. It is possible that the impaired renal function (decreased creatinine clearance) explains the increased levels of ADMA as it is excreted by the kidneys. The linear regression analysis shows that among all factors linearly correlated to ADMA (age, creatinine clearance, Apo-B, Apo-B/Apo-A1). Moreover, multiple regression analysis determined only Apo B as a predictor of high levels of ADMA. It is evident that ADMA is related to the most atherogenic Apo-B containing parameters of the lipid profile and its elevation is associated with a change in the lipid profile.

## CONCLUSION:

The most important statistically significant factor, as predictor of high levels of ADMA is Apo-B in patients with asymptomatic, marked, never-treated hypercholesterolemia.

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