Biochemical Changes in Asymptomatic Adult Population with Subclinical Atherosclerosis

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In cardiovascular (CV) prevention, the accurate risk evaluation of patients without clinical CV diseases requires multiple investigations, both biochemical and imagistic. This study presents the correlations between various biochemical markers and different parameters of subclinical atherosclerosis assessed in a group of asymptomatic population. Over a 2-year period, 120 subjects were randomized and comprehensively evaluated, with a mean age of 52.01 ± 10.73 years, 66.66% being female. Out of all biochemical markers, the lipid parameters reached the superior limit: total cholesterol (209.77 ± 45.56 mg%), LDL cholesterol (129.96 ± 40.71 mg%) and non-HDL cholesterol (157.27 ± 44.89 mg%). By gender, women presented increased values of fibrinogen, as marker of inflammation ($p=0.031$) while plasma glucose, uric acid and triglycerides were significantly higher in men ($p=0.01$; $p<0.001$; $p=0.043$). Most of the biochemical markers correlated positively with aging. Non-HDL and total cholesterol were associated significantly with intima-media thickness (IMT) ($r=0.22$, $p=0.013$; $r=0.23$, $p=0.009$), pulse wave velocity (PWV) ($r=0.28$, $p=0.003$; $r=0.30$, $p=0.001$) and the presence of aortic atheromatosis ($p=0.001$; $p=0.002$) and carotid plaques ($p=0.001$; $p<0.001$). Increased left ventricular mass index correlated with hypertriglyceridemia ($r=0.18$, $p=0.047$). Uric acid presented significant relation with all markers of subclinical atherosclerosis. A decreased renal function was associated with high IMT ($r=-0.22$, $p=0.015$), PWV ($r=-0.29$, $p=0.002$), carotid plaques ($p<0.001$) and aortic atheromatosis ($p<0.001$). Plasma glucose and hepatic markers presented insignificant correlations. Thus, in asymptomatic patients, the biochemical assessment may provide incremental value for the atherosclerotic burden of each individual.

Keywords: biochemical markers, subclinical atherosclerosis, asymptomatic, cardiovascular

Nowadays, cardiovascular (CV) diseases represent the main cause of mortality worldwide, being responsible for 40% of deaths that occur before the age of 75 years, with increasing trends especially in developing countries [1]. By 2025, CV mortality will surpass all other main diseases, including cancer, infection or trauma [2, 3]. Thus, CV diseases represent a continuous process that causes disability and shorten life years, with poor long term prognosis and expensive therapies.

Along with the publication of the first studies 50 years ago, the conceptual basis for considering certain *cardiovascular risk factors* has emerged. A risk factor is a feature of an individual or a population that is associated with a high risk of developing future disease [4]. Many risk factors have been proven to add an additional CV risk to the majority of individuals, being divided into unchangeable risk factors, acquired behaviors or laboratory values. Age, male sex (until 60 years old), race or early family history of CV events represent unmodified risk factors [5] while smoking, high blood pressure values, obesity, excess caloric intake, chronic alcohol consumption or physical inactivity represent modified parameters that correlate with high levels of coronary risk [6-10]. High values of total cholesterol, LDL cholesterol and triglycerides are the most important biochemical values for CV diseases and represent specific treatment target in the management of CV risk reduction [11, 12]. Increased levels of fasting plasma glucose, inflammatory markers (fibrinogen, C-reactive protein), hyperuricemia or hyperhomocysteinemia prove to be good CV risk predictors and should be treated and included in the biological assessment of each person [13-15].

All the up-mentioned risk factors, along with the predisposition of each individual, contribute to the development of atherosclerosis that represents the main cause of CVD. Nonetheless, many subjects are not aware that they have advanced atherosclerotic development since they present no clinical symptoms and in 30-50% of cases the first manifestation is an acute myocardial infarction often fatal [16]. Current guidelines for CV prevention use multiple risk factor algorithms but they still lack the power to predict accurately the risk in persons apparently healthy. For an efficient CV prevention, international societies have recommended the screening of asymptomatic population for the detection of subclinical atherosclerosis [1, 17]. Very few studies have analyzed the associations between biochemical values and subclinical atherosclerosis in asymptomatic patients. Thus, by measuring directly the atherosclerotic burden, better preventive measured could be taken and the CV risk assessment becomes more accurately.

Experimental part

Material and methods

The aim of the study was to evaluate how biochemical markers correlate with the presence of subclinical atherosclerosis in an adult population free of CV diseases.

The current prospective study included 120 subjects that were investigated in the cardiology department. They were referred to us through the general practitioners after being randomized from the general population. The following inclusion criteria have been used: aged 35-75 years, having an urban residence, women not being pregnant, and, most important, being without any diseases and not having

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followed any chronic treatment in the last 12 months. The University Ethics Committee approved the study and all participants have signed an informed consent before taking part in this research.

A multiple biochemical set has been used in every patient. The inflammatory profile was evaluated by determining serum fibrinogen which is a large and complex, 340 kDa plasma glycoprotein. It acts in the normal blood coagulation cascade and high values are associated with an intense inflammatory response.

Total cholesterol, high- and, respectively, low-density lipoprotein cholesterol (HDL, LDL) and triglycerides were the assessed lipid markers. Cholesterol (or (3β)-cholest-5-en-3-ol) (fig. 1) is an organic molecule with 256 stereoisomers, biosynthesized by all animal cells and a crucial component of cell membranes and precursor of steroid hormones, vitamin D and bile acids. HDL and LDL cholesterol belong to the five major groups of lipoproteins but have different actions. Lipoproteins present a neutral lipid core enclosed by a 20-A° shell made of apolipoproteins, phospholipids and unesterified cholesterol. LDL has a 10-fold larger diameter than normal cholesterol and presents a high cardiovascular risk by invading and oxidizing into the endothelium and promoting the atherosclerotic plaque. HDL is the smallest and densest of lipoprotein particles and by removing the fat molecules from cells it is considered to play an anti-atherogenic role. Moreover, the non-HDL cholesterol is considered to cause atheroma and its measurement has been shown to be a better predictor of CV events than other lipid values [18]. High levels of triglycerides are associated with the atherosclerotic burden and increased risk of CV events. Triglycerides are esters derived from the combination of glycerol and three fatty acids (usually RCO_2H, $R'CO_2H$ and $R''CO_2H$, but not the only ones), based on the following formula:

$$HOCH_2CH(OH)CH_2OH + RCO_2H + R'CO_2H + R''CO_2H \rightarrow$$
$$\rightarrow RCO_2CH_2CH(O_2CR')CH_2CO_2R'' + 3H_2O$$

Glucose is a monosaccharide with the molecular formula $C_6H_{12}O_6$ and exists in nature only as D-isomer form. Its five hydroxyl (OH) groups are arranged along the six-carbon back giving the possibility to arrange both in straight-chain and ring form.

Another assessed biochemical marker was the uric acid (7,9-Dihydro-1H-purine-2,6,8(3H)-trione) that is a diprotic aromatic acid and a product of purine nucleotides metabolism (fig. 2). Hyperuricemia can lead to gout and is associated with increased CV risk.

Serum creatinine and urea were used for the evaluation of renal function. Urea is an organic compound with the chemical formula CH_4N_2O, the molecule having a carbonyl (C=O) functional group linked to two $-NH_2$ groups.

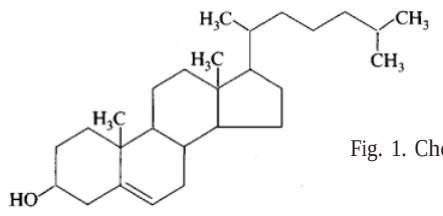

Fig. 1. Cholesterol formula

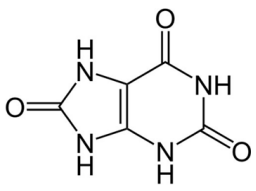

Fig. 3. Uric acid formula

Creatinine (or 2-Amino-1-methyl-5H-imidazol-4-one) is a cyclic derivative of creatine and represent an important indicator of renal function since it is an easily determined muscle metabolism byproduct that is excreted unchanged. Moreover, the renal function was determined by calculating the glomerular filtration rate (GFR) according to the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) formula which has been shown to be more accurate than others [19]:

$$eGFR = 141 \times \min(SCr/k,1)\alpha \times \max(\alpha Cr/k,1)^{-1.209} \times 0.993^{Age} \times [1.018 \text{ if Female}]$$

where SCr is serum creatinine (mg/dL), k is 0.7 for females and 0.9 for males, a is -0.329 for females and -0.411 for males, min indicates the minimum of SCr/k or 1, and max indicates the maximum of SCr/k or 1.

The hepatic function was evaluated by determining: gamma-glutamyl transferase (GGT), alanine transaminase (ALT) and aspartate transaminase (AST). ALT catalyzes the reaction between L-alanine and α-ketoglutarate to form pyruvate and L-glutamate while AST interconverts aspartate and α-ketoglutarate to oxaloacetate and glutamate. GGT is a key component in the gamma-glutamyl cycle by transferring the glutamyl moiety to a variety of amino acids or peptides based on the reaction:

$$\text{(5-L-glutamyl)-peptide + an amino acid} \rightleftharpoons \text{peptide + 5-L-glutamyl amino acid}$$

Atherosclerosis was quantified by multiple modern methods in order to detect accurately the subclinical changes that occur in subjects free of CVD. Carotid intima media-thickness (IMT) and the evaluation of carotid plaques were obtained by carotid ultrasound using an Esaote MyLab50 device. Left ventricle mass index (LVMI) and the presence of aortic atheromatosis have been evaluated by performing a cardiac echography. A novel biomarker for assessing subclinical atherosclerosis was used in our study, the arterial stiffness. An Arteriograph™ device was used and for final analysis we were interested in the aortic pulse wave velocity (PWV) and aortic systolic blood pressure (SBPao). The ankle-brachial index (ABI) was used to evaluate asymptomatic peripheral artery obstruction and decreased ABI values correlated with advanced peripheral artery disease.

Statistical analysis

Data analysis was performed using SPSS 20.0 (Statistical Package for the Social Sciences, Chicago, Illinois). For continuous variables, data were presented as mean ± standard deviation (SD). T-test for independent samples, ANOVA and Pearson's correlation coefficient (r) were applied to assess the relationship between variables. A two-sided p value < 0.05 was considered significant for all data analyses.

Results and discussions

The mean age of the study group was 52.01 years, with one third being male (40 participants). Fibrinogen, as marker of inflammatory process, was in normal ranges. Regarding the lipid profile, total cholesterol was above the normal value while LDL-cholesterol, non-HDL cholesterol and triglycerides were close to the superior limit. These results suggest that the population free of CVD present uncontrolled lipid values. Fasting plasma glucose, uric acid, renal and hepatic function presented normal mean values. Regarding subclinical atherosclerosis, average IMT, PWV,

Variable	Mean	Normal values
Age (years)	52.01 ± 10.73	
Male sex (%)	33.33	
Total cholesterol (mg/dl)	209.77 ± 45.56	< 200
HDL cholesterol (mg/dl)	52.49 ± 14.47	> 50
LDL cholesterol (mg/dl)	129.96 ± 40.71	< 130
nonHDL cholesterol (mg/dl)	157.27 ± 44.89	< 160
Triglycerides (mg/dl)	137.06 ± 81.42	< 150
Plasma glucose (mg/dl)	97.21 ± 12.75	< 106
Fibrinogen (mg/dl)	368.83 ± 77.43	< 400
Uric acid (mg/dl)	4.34 ± 1.59	< 6
AST (mg/dl)	24.14 ± 8.49	< 40
ALT (mg/dl)	26.42 ± 14.48	< 40
GGT (mg/dl)	34.22 ± 24.39	< 45
GFR (ml/min/1.73m²)	89.35 ± 16.54	> 90
IMT (mm)	0.86 ± 0.13	< 0.90
ABI	1.08 ± 0.13	> 0.90
PWV (m/s)	8.28 ± 1.79	< 10
SBPao (mmHg)	128.14 ± 21.05	< 135
LVMI (g/m²)	101.54 ± 23.25	< 105
Aortic atheromatosis (%)	70.83	
Carotid plaques (%)	40	

Table 1
STUDY GROUP CHARACTERISTICS

Parameter	r coefficient	p value
Fibrinogen	**0.19**	**0.043**
Cholesterol total	**0.39**	< **0.001**
HDL cholesterol	0.16	0.073
LDL cholesterol	**0.28**	**0.002**
Non HDL cholesterol	**0.34**	< **0.001**
Triglycerides	**0.24**	**0.008**
Plasma glucose	**0.21**	**0.017**
Uric acid	**0.25**	**0.006**
Creatinine	**0.23**	**0.011**
Urea	**0.40**	< **0.001**
GFR	**-0.54**	< **0.001**
GGT	0.13	0.16
ALT	-0.026	0.77
AST	0.07	0.41

Table 2
CORRELATIONS BETWEEN AGE AND BIOLOGICAL MARKERS

SBPao or LVMI were in normal ranges as well. However, 71% of subjects had aortic atheromatosis while 40% presented carotid plaques (19% unilateral, respectively 21% bilateral carotid plaques). All descriptive data can be found in table 1.

Regarding biochemical modifications by sex, fibrinogen was significantly increased in females (380.36 ± 80.86 vs. 347.95 ± 66.85 mg/dL, p = 0.031). In men, plasma glucose, uric acid and only triglycerides from all lipid markers were higher (101.43 ± 12.94 vs. 91.10 ± 12.20 mg/dL, p = 0.010; 5.48 ± 1.44 vs. 3.77 ± 1.35 mg/dL, p < 0.001; respectively 158.32 ± 99.37 vs. 126.43 ± 69.04 mg/dL, p = 0.043). All hepatic markers (GGT, AST and ALT) were increased in men while no differences by gender were obtained for renal function parameters.

Most of the biochemical markers correlated positively with aging while GFR was negatively associated with advanced age (r = -0.54, p < 0.001) signifying that the renal function was regressing as the population was getting older (table 2).

Out of all lipid markers, total cholesterol presented the best relation with mostly all parameters of subclinical atherosclerosis (IMT, PWV or SBPao) while triglycerides associated additionally with increased LVMI (r = 0.018, p = 0.047). Increased uric acid values correlated significantly with all major markers of asymptomatic atherosclerosis (IMT, PWV, SBPao and LVMI). An impaired renal function (increased creatinine and urea and decreased GFR) correlated with high IMT and PWV values, while inflammatory status was associated only with increased aortic stiffness (r = 0.25, p = 0.013). Out of all biochemical markers, low ABI values correlated only with high lipid values: total cholesterol (p = 0.010), LDL cholesterol (p = 0.022) and non-HDL cholesterol (p = 0.006). All correlation coefficients and p values can be found in table 3.

The presence of aortic atheromatosis correlated with increased values in almost all biochemical markers, especially with lipid profile (total cholesterol, triglycerides, LDL and non-HDL cholesterol), renal function (increased urea and creatinine, decreased GFR), plasma glucose (p < 0.001) and serum uric acid (p < 0.001) (table 4).

Increased total cholesterol, LDL and non-HDL cholesterol were associated with the presence of unilateral and, especially bilateral carotid plaques (p < 0.001; p = 0.004; respectively p = 0.001). Moreover, high glycemic and uric acid values were correlated with carotid atherosclerosis (p = 0.020; respectively p < 0.001) while the GFR was significantly decreasing as the carotid modifications were more severe (96.55 ± 15.91 – absence of carotid plaques, 86.44 ± 14.62 – unilateral plaque, 80.69 ± 14.06 mL/min/1.73m² – bilateral plaques, p < 0.001).

Up to our present knowledge, this is the first study that aimed to correlate multiple and mostly used biochemical

Parameter	IMT	ABI	PWV	SBPao	LVMI
Fibrinogen	r = 0.18 p = 0.06	r = -0.06 p = 0.50	**r = 0.25** **p = 0.013**	r = 0.13 p = 0.19	r = 0.02 p = 0.82
Total cholesterol	**r = 0.23** **p = 0.009**	**r = -0.23** **p = 0.010**	**r = 0.30** **p = 0.001**	**r = 0.30** **p = 0.001**	r = 0.07 p = 0.44
HDL	r = 0.04 p = 0.60	r = 0.03 p = 0.72	r = 0.07 p = 0.43	r = 0.14 p = 0.14	r = -0.03 p = 0.75
LDL	r = 0.16 p = 0.07	**r = -0.21** **p = 0.022**	**r = 0.23** **p = 0.012**	r = 0.18 p = 0.051	r = 0.01 p = 0.87
Non-HDL	**r = 0.22** **p = 0.013**	**r = -0.24** **p = 0.006**	**r = 0.28** **p = 0.003**	**r = 0.26** **p = 0.005**	r = 0.08 p = 0.38
Triglycerides	**r = 0.20** **p = 0.025**	r = -0.17 p = 0.054	**r = 0.19** **p = 0.048**	**r = 0.26** **p = 0.006**	**r = 0.18** **p = 0.047**
Plasma glucose	r = 0.12 p = 0.17	r = -0.07 p = 0.44	r = 0.12 p = 0.18	**r = 0.23** **p = 0.014**	r = 0.14 p = 0.12
Uric acid	**r = 0.37** **p < 0.001**	r = -0.02 p = 0.76	**r = 0.22** **p = 0.021**	**r = 0.24** **p = 0.009**	**r = 0.19** **p = 0.039**
Creatinine	**r = 0.24** **p = 0.009**	r = 0.03 p = 0.74	r = 0.05 p = 0.58	r = 0.10 p = 0.28	**r = 0.19** **p = 0.035**
Urea	**r = 0.22** **p = 0.014**	r = 0.05 p = 0.54	r = 0.14 p = 0.12	r = 0.10 p = 0.29	r = 0.14 p = 0.12
GFR	**r = -0.22** **p = 0.015**	r = 0.11 p = 0.23	**r = -0.29** **p = 0.002**	**r = -0.27** **p = 0.004**	r = -0.10 p = 0.25

Table 3
CORRELATIONS BETWEEN BIOCHEMICAL AND SUBCLINICAL ATHEROSCLEROSIS MARKERS

Parameter	Presence	Absence	p value
Fibrinogen (mg/dl)	377.16 ± 76.10	348.27 ± 78.10	0.085
Total cholesterol (mg/dl)	217.87 ± 44.14	190.12 ± 43.48	**0.002**
HDL (mg/dl)	51.52 ± 14.97	54.85 ± 13.07	0.22
LDL (mg/dl)	135.76 ± 39.18	116.03 ± 41.49	**0.015**
Non HDL (mg/dl)	166.34 ± 42.33	135.26 ± 43.82	< **0.001**
Triglycerides (mg/dl)	153.92 ± 85.59	96.12 ± 51.62	< **0.001**
Plasma glucose (mg/dl)	99.89 ± 12.78	90.69 ± 10.20	< **0.001**
Uric acid (mg/dl)	4.68 ± 1.59	3.53 ± 1.30	< **0.001**
Creatinine (mg/dl)	0.86 ± 0.14	0.74 ± 0.14	< **0.001**
Urea (mg/dl)	31.39 ± 8.36	25.77 ± 5.97	< **0.001**
GFR (ml/min/1.73m²)	85.06 ± 15.79	99.94 ± 13.42	< **0.001**

Table 4
BIOLOGICAL VALUES DEPENDING ON THE PRESENCE/ABSENCE OF AORTIC ATHEROMATOSIS

parameters with the presence of subclinical atherosclerosis assessed by various methods in a group of subjects with no evidence of CV disease.

In the current research, we have proven that the adult studied sample, free of CV diseases, is dyslipidemic, with increased values of total cholesterol and its fractions. This result is consistent with the literature data since high values of total cholesterol, LDL and non-HDL cholesterol are associated with increased CV mortality in asymptomatic individuals [20]. Moreover, in the current research, the lipid parameters correlated with all markers of subclinical atherosclerosis, especially with IMT and PWV, as well as with the presence of aortic and carotid atheromatosis. Orakzai et al. showed on more than 1600 individuals that high values of triglycerides, LDL and non-HDL cholesterol were strongly associated with increased coronary artery calcium score ($p < 0.001$), another marker of detecting subclinical atherosclerosis [21]. Another recently published study confirmed that low HDL cholesterol and low concentrations of large particles in HDL molecules were associated with increased risk of CV events, especially stroke [22]. However, in the remaining group of non-dyslipidemic asymptomatic patients who suffer acute CV diseases, older age, male gender, high blood pressure values and low HDL cholesterol proved to be accurate predictors while the presence of subclinical imagining atherosclerosis provided incremental prognostic value [23].

The inflammatory status assessed by fibrinogen levels correlated only with PWV ($p = 0.01$) and was at the statistical limit for IMT ($p = 0.06$). Nonetheless, on a 5-year follow-up, fibrinogen and C-reactive protein proved to be independent predictors of subclinical atherosclerosis and were associated with high IMT values [24].

Serum uric acid was one of the biochemical markers that correlated with most of the subclinical atherosclerosis determinants (IMT and carotid plaques, PWV, SBPao or LVMI). Riccioni et al. showed on 640 asymptomatic individuals that increased IMT values or the presence of carotid plaques were associated with high uric acid, lipid values and inflammatory markers (fibrinogen, as well) [25]. Moreover, in middle-age subjects, hyperuricemia was an independent risk factor for subclinical atherosclerosis assessed by coronary artery calcification ($p = 0.008$ after multivariate adjustment) [26]. In our research, no correlation was obtained between uric acid values and ABI as marker of peripheral target organ damage. However, a research conducted on more than 1200 patients showed that hyperuricemia correlated with peripheral artery disease detected by ABI but the association was significant only in males [27].

Patients with chronic renal failure were not included in the current study but a mild decrease in renal function within GFR normal limits was associated with carotid and aortic atherosclerosis, respectively with increased arterial stiffness. These results are consistent with the literature data since the IMT and atherosclerotic plaques correlate with lower GFR values [28]. Moreover, on a five-year follow-up, increased carotid IMT along with hypertriglyceridemia was independently associated with the development of chronic kidney disease on a cohort of almost 2000 participants [29]. As perspectives for our study

continuation, mid and long-term follow-up of the subjects will reveal which biochemical and imagistic markers could better predict the CV risk in initially asymptomatic patients.

Conclusions

The individuals free of CV diseases present high values of total cholesterol and its lipid fractions while other biochemical markers are in normal ranges. Most biological values are significantly increased in men and in aging persons. Increased values of non-HDL, LDL, total cholesterol, triglycerides, uric acid and, respectively, abnormal parameters of renal function (creatinine, urea and GFR) present the best correlations with increased markers of subclinical atherosclerosis. Thus, a closely and regular biochemical assessment is mandatory in all asymptomatic individuals for a better CV prevention.

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