

**EVALUATION OF LIPID PARAMETERS, HOMOCYSTEINE,
ADHESION MOLECULES AND CAROTID INTIMA-MEDIA
THICKNESS IN CHILDREN FROM FAMILIES WITH
CIRCULATORY SYSTEM DISEASES HISTORY**

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Abstract. Aim: The evaluation of lipid parameters, homocysteine, soluble adhesion molecules in the blood serum and the thickness of common carotid artery wall in children from families with diagnosed risk factors of atherosclerosis. **Material and methods:** The study included 18 children (8 girls and 10 boys) aged 2-3 years and their parents from families with circulatory system disease in their history. The control group was composed of 16 children (8 girls and 8 boys) aged 2-3 years from families without that risk. In all of the subjects concentrations of triglycerides, total cholesterol and its fractions: LDL, VLDL, HDL and of apolipoproteins (apo-AI, apo-B), lipoprotein (a), homocysteine as well as of sICAM-1 (intracellular adhesion molecule-1), sVCAM-1 (vascular cell adhesion molecule-1) and sE-selectin in the blood serum were determined. The intima-media thickness (IMT) measurement was made with ultrasonographic method in children and their parents from families with positive history and in children from families with negative history. **Results:** The concentration of triglycerides, VLDL cholesterol and lipoprotein (a) had the highest value in the blood serum of the children from the families with risk factors of atherosclerosis as compared to the levels of the children from families without that risk and the differences were statistically significant ($p < 0.05$). The evaluation of the relationship between the IMT and lipids, lipoproteins, apolipoproteins, homocysteine and soluble adhesion molecules in children from families with risk of cardiac ischaemia and in children from control group did not confirm any significant statistically correlations. The statistically significant positive correlation was confirmed between IMT and total cholesterol in mothers and between IMT and apo-B in fathers from families with positive history. **Conclusion:** The significant increase in the concentrations of triglycerides, VLDL and lipoprotein (a) in children with positive family history of atherosclerosis risk indicates that this group is particularly exposed to early atherosclerotic changes. We are planning to continue the research in this group of children in order to find the atherosclerosis risk factors.

Key words: lipids, lipoprotein (a), homocysteine, adhesion molecules, IMT, atherosclerosis risk factors

Rezumat. Scop: evaluarea parametrilor lipidici, homocisteina, moleculele solubile de aderență în ser și grosimea peretelui arterei carotide la copii provenind din familii prezentând factori de risc ai aterosclerozei. **Material și metode:** Studiul a inclus 18 copii (8 fete și 10 băieți) în vârstă de 2-3 ani și părinții lor din familii cu afecțiuni circulatorii în antecedente. Grupul martor a fost alcătuit din 16 copii (8 fete și 8 băieți) în vârstă de 2-3 ani provenind din familii fără antecedente cardiovasculare. La toți subiecții s-au determinat concentrațiile serice de trigliceride, colesterol total și fracțiunile sale: LDL, VLDL, HDL și apo-lipoproteine (apo-AI, apo-B), lipoproteina (a), homocisteina și moleculele solubile de aderență (ICAM-1, VCAM-

1). Grosimea intimei medii (GIM) s-a stabilit prin metoda ultrasonografică la copiii și părinții lor din familii cu antecedente pozitive și la copiii din familii cu antecedente negative.

Rezultate: Concentrațiile de trigliceride, VLDL colesterol și lipoproteina (a) au avut valorile cele mai mari în serul copiilor din familii prezentând factori de risc aterosclerotici în comparație cu nivelurile găsite la copiii provenind din familiile fără risc iar diferențele au fost statistic semnificative ($p < 0,05$). Evaluarea relației între GIM și lipide, lipoproteine, apolipoproteine, homocisteină și molecule solubile de aderență la copii provenind din familii cu risc ischemic cardiac, nu a indicat valori statistic semnificative. Asociația GIM cu valorile colesterolului total la mame și cu apo-B la tații cu istoric familial pozitiv, a indicat valori semnificative statistic. **Concluzii:** creșterea semnificativă a valorilor trigliceridelor, VLDL și lipoproteinei (a) la copiii cu antecedente familiale de risc aterosclerotic indică faptul că acest grup este expus de timpuriu modificărilor aterosclerotice. Autorii își propun continuarea cercetării la acest grup de copii pentru a defini factorii de risc ai aterosclerozei.

Cuvinte cheie: lipide, lipoproteina (a), homocisteina, molecule de adeziune, GIM, factori de risc aterosclerotici

INTRODUCTION

During the last decades, in many countries the decrease of mortality due to circulatory system diseases has been observed. Despite this fact the diseases developing on the base of atherosclerosis are still the main reason for deaths in highly industrialised countries.

Atherosclerosis is developing for many years and though its clinical symptoms are visible in older age, the onset of atherosclerotic alterations may have its origin in early childhood (1,2). Atherosclerosis is a chronic disease of medium and big arteries. The complex atherosclerotic plaques in the internal vascular wall membrane result from many complex pathological processes (3). In the development of atherosclerotic changes we can distinguish three phases: initiation, progression and complications (2). Fatty streaks are the earliest morphologically identifiable atherosclerotic alterations. They can occur in the internal membrane of big arteries even in children during their first years of life. They are reversible and therefore it is the

optimal time for starting prophylactic activities. During the next stage fibrous laminae may occur in these fatty streaks and this can cause irreversible changes. They do not cause any significant narrowing of vascular lumen and do not present any clinical symptoms yet, but they prove the existence of asymptomatic atherosclerosis.

Fully developed atherosclerotic laminae and the related complications concern the 3rd - 4th decade of life on the average (1,2). Presently it is assumed that the dysfunction of the endothelial cells is one of the main pathogenetic factors of atherosclerosis (4,5,6).

The onset and development of atherosclerotic changes depend on the presence of the favouring factors, such as genetic, constitutional and environmental ones. An early possible detection and limiting of risk factors is the basis for prevention of atherosclerosis and its complications. At present we know that only in half of the patients with symptoms of cardiac ischaemia, classical risk factors that are responsible for its

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occurrence may be identified. Detection of other risk factors than the traditional ones is the subject of intensive research. Family history including cardiac ischaemia is often considered an independent risk factor and the children coming from these families constitute a group especially predisposed for early development of atherosclerotic changes. Disorders of lipid metabolism and particularly elevated cholesterol concentration are surely the favouring factors of atherosclerotic changes. The new atherosclerosis risk factors are: lipoprotein (a), apolipoproteins A and B, homocysteine, fibrinogen, C-reactive protein (CRP). The assessment of adhesion molecules may be helpful in the diagnosis of early risk of cardiac ischaemia (7,8). The ultrasonographic evaluation of common carotid artery wall is a non-invasive method which will allow for detection early atherosclerotic changes in arteries (9).

The study aimed at the evaluation of the parameters of lipid metabolism (triglyceride, total cholesterol and its fractions: HDL, LDL, VLDL, apolipoprotein AI and B, lipoprotein (a), homocysteine, soluble adhesion molecules (sICAM-1, sVCAM-1 and sE-selectin) in the blood serum and the thickness of common carotid artery wall in children from families with early symptoms of atherosclerosis.

MATERIAL AND METHODS

The study included 18 children (8 girls and 10 boys) aged 2-3 years and their parents from families with circulatory system disease in their history. The

age of parents ranged from 21 to 46 years. The control group was composed of 16 children (8 girls and 8 boys) aged 2-3 years from families without that risk. On the basis of the family history of parents, the atherosclerosis risk factors were established in the families of the studied children: hypercholesterolemia, obesity, hypertension, diabetes, cardiac infarction and stroke. The subjects of the study were the progeny and their parents from families in which the early symptoms of cardiac ischaemia were found. The parents of the studied children are young and do not always show up any clinical symptoms of cardiac ischaemia, therefore the family history including this illness was considered. The parents agreed in writing for including their children in this study. The blood specimens for biological tests in children and their parents were sampled from the ulnar vein on fast in the morning (after at least 10-hour break from the last meal).

The concentration of triglycerides, total cholesterol and HDL cholesterol in blood serum was determined by using the Cormay reagents and the Cobas Mira S analyser. The concentration of LDL and VLDL cholesterol were calculated by the Friedewald formula. The concentration of apolipoproteins: apo-AI and apo-B were determined with immunoturbidimetric method using the "Roche" reagents. The concentration of lipoprotein (a) was determined with immunoturbidimetric method using the "Human" reagents. The concentration of homocysteine was determined with

ELISA (Enzyme-linked immunosorbent assay) method by using Axis Homocysteine EIA Package Insert by Axis-Shield AS (Germany). The concentrations of intracellular adhesion molecule-1 (sICAM-1), vascular cell adhesion molecule-1 (sVCAM-1) as well as sE-selectin were determined by a quantitative ELISA method. The assays were performed according to the procedure recommended by the manufacturer, with the application of kits: human sICAM-1 BMS201, human sVCAM-1 BMS232, human sE-selectin BMS205 (Bender Med Systems Diagnostics GmbH, Vienna, Austria).

The degree of atherosclerotic changes in the vessels was evaluated by measuring the IMT of common carotid artery wall with an ultrasonographic unit. The IMT measurement was made by ultrasonographic method in children and in their parents from families with positive history and in children from families with negative one. The IMT was evaluated both on the right and left side, and the mean value was calculated.

The test evaluating the Intima Plus Media Thickness was carried out on ATL 3500 ultrasonographic unit with a probe emitting ultrasounds of 12 MHz frequency.

The patients were in supine position with the head slightly bent backwards. We aimed at the most precise visualisation of common carotid artery walls – both left and right- from the point of beginning from aortic arch or brachio-cephalic trunk up to the point of their branching into internal and

external carotid arteries. The adopted parameter is IMT which constitutes the distance (in mm) between the first layer visible from the arterial lumen (intima) and the borderline between the second visible layer (adventiva) and the dark medium layer (media). The IMT defined by some researchers seems to be a particular important index defining the degree of early atherosclerotic changes in children and adults (10,11).

The statistic analysis of results was made with application of Statistica 5.1 PL software. For evaluation of the properties and the analysed parameters the arithmetic mean (M), standard deviation (SD) were used. The evaluation of the significance of the differences of the mean values of lipids, lipoproteins and apolipoproteins in blood serum of children related to the risk factors of atherosclerosis were tested with t-Student test for the values characterised by the normal distribution. In case of abnormal distribution of the variables the statistical analysis was performed with Mann-Whitney test. For correlation analysis, Pearson's correlation coefficient was calculated for normally distributed variables or Spearman's rank-correlation coefficient for other variables. In the statistical analyses the assumption of hypothesis was verified on the significance level $\alpha = 0.05$, so the differences for $p < 0.05$ were considered statistically significant.

RESULTS

The concentrations of the studied parameters of lipid metabolism, homocysteine and soluble adhesion

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molecule in the blood serum as well as the IMT of children from the families with circulatory system diseases in their history and in control group are presented in table 1.

The concentration of triglycerides, VLDL cholesterol and lipoprotein (a) had the highest value in the blood

serum of the children from the families with risk factors of atherosclerosis as compared to those of the children from families without that risk and the differences were statistically significant ($p < 0.05$).

Table 1. The concentrations of the parameters of lipid metabolism in the blood serum and the value of IMT

Parameters	Children from families with positive history (n = 18)	Children from families with negative history (n = 16)	p
	M ± SD	M ± SD	
Triglyceride (mg/dl)	105.44 ± 69.04	83.03 ± 29.26	p < 0.05
Total-cholesterol (mg/dl)	165.56 ± 30.99	167.83 ± 33.15	ns
HDL-cholesterol (mg/dl)	55.46 ± 14.28	47.35 ± 11.92	ns
LDL-cholesterol (mg/dl)	88.96 ± 37.14	103,87 ± 32.30	ns
VLDL-cholesterol (mg/dl)	21.09 ± 13.81	16.62 ± 5.85	p < 0.05
Apolipoprotein- AI (mg/dl)	154.22 ± 18.73	147.33 ± 19.90	ns
Apolipoprotein- B (mg/dl)	73.11 ± 18.82	77.58 ± 17.59	ns
Lipoprotein (a) (mg/dl)	18.27 ± 26.11	5.89 ± 3.65	p < 0.05
Homocysteine (µmol/L)	12.20 ± 2.46	10.67 ± 2.41	ns
sVCAM-1 ng/ml	1616.11 ± 561.92	1516.13 ± 501.75	ns
sICAM-1 ng/ml	391.15 ± 95.28	384.31 ± 57.96	ns
sE-selectin ng/ml	58.37 ± 17.17	72.57 ± 38.16	ns
IMT (mm)	0.43 ± 0.04	0.39 ± 0.06	ns

The concentrations of the studied parameters of lipid metabolism, homocysteine and soluble adhesion molecules in the blood serum of

children, mothers and fathers from the families with circulatory system disease in their history as well as the IMT are presented in table 2.

Table 2. The concentrations of the parameters of lipid metabolism in the blood serum and the value of IMT in children

Parameters	Children (n = 18)	Mothers (n = 18)	Fathers (n = 18)
	M ± SD	M ± SD	M ± SD
Triglyceride (mg/dl)	105.44 ± 69.04	74.56 ± 25.59	160.00 ± 84.40
Total-cholesterol (mg/dl)	165.56 ± 30.99	177.89 ± 34.28	180.56 ± 23.82
HDL-cholesterol (mg/dl)	55.46 ± 14.28	67.77 ± 19.94	51.07 ± 15.26
LDL-cholesterol (mg/dl)	88.96 ± 37.14	95,21 ± 26.80	97.49 ± 33.00
VLDL-cholesterol (mg/dl)	21.09 ± 13.81	14.91 ± 5.12	32.00 ± 16.88
Apolipoprotein- AI (mg/dl)	154.22 ± 18.73	189.56 ± 33.71	162.89 ± 15.73
Apolipoprotein- B (mg/dl)	73.11 ± 18.82	70.00 ± 16.24	106.89 ± 43.78
Lipoprotein (a) (mg/dl)	18.27 ± 26.11	29.60 ± 34.48	15.43 ± 14.47
Homocysteine (µmol/L)	12.20 ± 2.46	11.63 ± 4.19	15.07 ± 4.26
sVCAM-1 ng/ml	1616.11 ± 561.92	952.98 ± 249.80	930.41 ± 492.07
sICAM-1 ng/ml	391.15 ± 95.28	294.50 ± 62.13	363.93 ± 134.46
sE-selectin ng/ml	58.37 ± 17.17	29.28 ± 19.44	34.08 ± 37.51
IMT (mm)	0.43 ± 0.04	0.59 ± 0.20	0.61 ± 0.10

There was analyzed the association between blood serum values of lipids, lipoproteins, apolipoproteins, homocysteine and soluble adhesive molecules in children, mothers and fathers (table 3). Looking for the relationship between IMT and the tested parameters in the

blood serum of children with a positive family history, a negative correlation between IMT and homocysteine ($r = -0.48$) and positive correlation between IMT and sVCAM-1 ($r = 0.37$) was confirmed, but they were not statistically significant. The

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relationship between IMT and the tested parameters in the blood serum

of the children from controls group were not statistically significant.

Table 3. The correlations between the IMT and the studied parameters in 18 families (children, mothers and fathers) with circulatory system diseases in their history

Correlation	children		mothers		fathers	
	r	p	r	p	r	p
IMT vs TG	0.12	0.75	-0.25	0.52	-0.04	0.91
IMT vs T chol.	0.07	0.86	0.83	0.01	0.45	0.22
IMT vs HDL chol.	- 0.20	0.61	0.49	0.18	- 0.28	0.46
IMT vs LDL chol.	- 0.03	0.58	0.40	0.28	0.33	0.38
IMT vs VLDL chol.	0.12	0.75	-0.25	0.52	- 0.04	0.91
IMT vs Apo AI	- 0.02	0.96	0.66	0.05	0.27	0.49
IMT vs Apo B	0.26	0.50	0.10	0.79	0.69	0.04
IMT vs Lp(a)	0.10	0.80	- 0.21	0.58	0.15	0.70
IMT vs Hcy	- 0.48	0.19	- 0.30	0.43	0.47	0.20
IMT vs sVCAM-1	0.37	0.32	-0.27	0.49	0.52	0.15
IMT vs sICAM-1	-0.04	0.93	0.11	0.77	-0.11	0.78
IMT vs sE-selectin	0.16	0.68	-0.06	0.88	-0.09	0.81

When analysing the correlation coefficients between IMT and the studied parameters in the mothers serum, the statistical significance at $p < 0.05$ with correlation coefficient $r = 0.83$ was confirmed between the IMT and the total cholesterol concentration. However, the correlation coefficient between IMT and the concentration of LDL cholesterol was $r = 0.40$, between IMT and HDL cholesterol was $r = 0.49$ and between IMT and apo-AI was $r = 0.66$, but they were not statistically significant. When analysing the correlation of IMT in the fathers and the value of the studied parameters, also statistically significant relationships were confirmed. The correlation coefficient between IMT and apo-B concentration was $r = 0.69$ and the relationship was statistically significant ($p < 0.05$). However between IMT and total cholesterol $r = 0.45$, LDL cholesterol

$r = 0.33$, HDL cholesterol $r = -0.28$, homocysteine $r = 0.47$, sVCAM $r = 0.52$ the correlations were not statistically significant.

DISCUSSION

The significant increase in the concentrations of triglycerides, VLDL cholesterol and lipoproteina (a) in studied children with positive family history on atherosclerosis risk factors indicates that this group is particularly exposed to early atherosclerotic changes. Lipoprotein (a) is an independent, genetically determined atherosclerosis risk factor (7).

Children with a positive family history had higher BMI, higher lipoprotein (a) and apo-B levels. Levels of lipoprotein (a) and apo-B may be predictive of future cardiovascular disease in predisposed children (12).

The studies evaluating the relationship between the IMT and triglycerides, total cholesterol, LDL cholesterol, VLDL cholesterol and HDL cholesterol, apolipoproteins (apo-AI, apo-B), lipoprotein (a), homocysteine and soluble molecules (sVCAM-1, sICAM-1, sE-selectin) in children from the families with diagnosed risk of cardiac ischaemia did not confirm any significant statistically correlations between the studied parameters in children coming from these families. The age of the studied children was 2-3 years and it is likely that at this age no visible changes in the carotid IMT were noticed.

The accessible references included research that evaluates IMT in patients with family hyper-cholesterolemia. In these patients aged 11-27 years, with family hypercholesterolemia, a higher value of IMT was confirmed as compared with the control group and the positive correlation between IMT and total cholesterol, LDL-cholesterol and triglycerides (13). In patients with family hypercholesterolemia a positive correlation between the carotid artery wall thickness and the concentration of total cholesterol, LDL cholesterol and triglycerides and a negative correlation between IMT and the index HDL-cholesterol/total cholesterol. According to these authors ultrasonographic evaluation of changes in the common carotid artery wall may be performed in children and youth with family-based hypercholesterolemia in order to diagnose early atherosclerotic changes in the vessels (14). Tonstad et al. noticed that the concentrations of lipids,

fibrinogene and homocysteine are associated with the markers of early atherosclerotic changes in the common carotid artery but only in the second decade of life (15). Cuomo et al., when evaluating the IMT in children, youth and young adults with family history including early cardiac failure, proved that the changes in vascular structure that are characterised by a bigger IMT value, are present in this group independently from other coexisting traditional risk factors of atherosclerosis (16). Megnien et al. indicated a bigger thickness of IMT in patients with homozygotic homocystonuria and the relationship between IMT and age and homocystein concentration in blood serum (17).

The research which has been conducted within the recent years proves the positive correlation between IMT and the concentrations of soluble adhesive molecules in the blood serum. Adhesive molecules play an important role in the preliminary phase of the development of athero-sclerosis and are the exponent of the endothelium dysfunction. The positive correlation between IMT and the adhesive molecules concentration in blood serum seems to be one of the first symptoms of atherosclerosis (18).

Observation of the common carotid arterial wall thickness may be a significant index that evaluates the development and progression of atherosclerotic changes in the vessels. This is a non-invasive method and may be used even in the youngest patients (19).

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Recently while evaluating the risk of cardiac ischaemia apart from the known relationships of triglyceride, total cholesterol and LDL, VLDL, HDL cholesterol concentrations, the attention is paid to the concentration of apolipoproteins, lipoprotein (a), homocysteine and adhesive molecules as the independent risk factors. The IMT evaluation is the test that can be a good index of early atherosclerotic changes in the vessels.

On the basis of research studies carried out by various authors we may draw a conclusion that the significant element in the prevention and treatment of atherosclerosis is the evaluation of early biochemical and immunological markers. Early diagnosis of the increased risk factors of atherosclerosis and introduction of modification of environmental factors in children may, in future, significantly reduce the clinical symptoms of cardiac ischaemia (20).

CONCLUSION

The determination of lipid profile and other biochemical indices of early atherosclerotic changes should be performed in children coming from families including circulatory system diseases in their family history. Out of new atherosclerosis risk factors, lipoprotein (a) may have a real cardiovascular disease prediction value in children. Ultrasonographic evaluation of the thickness of common carotid artery wall may be useful in tracing the development of atherosclerosis of arteries in children with positive family history of atherosclerosis risk

factor. We are planning to continue the research in this group of children in order to find the atherosclerosis risk factors.

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