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PERSPECTIVES

Contribution of the atherogenic lipoprotein profile to the development of arterial hypertension

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Abstract: *Objectives:* Determination of non-atherogenic and atherogenic plasma lipoproteins, including small dense LDL, in patients with newly diagnosed arterial hypertension and identification of the phenotype of lipoprotein profile: non-atherogenic phenotype A vs. atherogenic lipoprotein phenotype B, in plasma of examined subjects.
Background: Atherogenic lipoproteins play an important role in the pathogenesis of arterial hypertension. Impaired lipoprotein metabolism results in overproduction of triglyceride-rich particles and LDL 3–7 subfractions – small dense LDL – a strongly atherogenic LDL subpopulation accelerating the development of arterial hypertension.
Methods: Total cholesterol and triglycerides were analyzed by enzymatic CHOD-PAP method, Roche Diagnostics, Germany. Lipoprotein profiles of plasma described as atherogenic lipoprotein phenotype B or a non-aterogenic lipoprotein phenotype A were examined by a new method of lipoprotein separation by means of electrophoresis on polyacrylamide gel (Lipoprint LDL system). Prostacyclin and thromboxane A_2 in plasma were analysed by ELISA method. Score of Atherogenic Risk was determined as a ratio of atherogenic and non-atherogenic plasma lipoproteins.
Results: 1) High percentage of atherogenic hypertriacylglycerolemia (93 %) and atherogenic mixed hyperlipemia (86 %) in subjects with arterial hypertension. 2) Low percentage of atherogenic hypercholesterolemia (52 %) in subjects with arterial hypertension. 3) Atherogenic normolipemia (7 %) in control group of healthy subject.
Conclusion: Contribution of this method lies in benefits as follows: A) Quantification of non-atherogenic and atherogenic plasma lipoproteins. B) Identification of high percentage of atherogenic dyslipoproteinemia (86 – 93 %) in subjects with arterial hypertension. C) Presence of small dense LDL in plasma is decisive for declaring the atherogenic lipoprotein profile in both hyperlipemia and normolipemia *(Tab. 5, Ref. 24)*. Full Text in free PDF *www.bmj.sk.*
Key words: atherogenic vs. non-atherogenic lipoprotein profile, atherogenic normolipemia.

The arterial hypertension (AH) is one of the most serious cardiovascular diseases. More than 20 % of adult population suffers from this disease. Together with the main risk factors, i.e dyslipoproteinemia and tobacco smoking, AH belongs to risk factors of developing atherosclerosis in coronary, cerebral and peripheral arteries (1).

AH is a permanent, long-lasting increase in blood pressure over 140/90 mmHg in middle-aged persons. In persons older than 70 years, AH is considered when blood pressure values exceed 160/95 mmHg. Statement of WHO/ ISH (International Society of Hypertension) on management of hypertension (2, 3).

Dyslipoproteinemia, which frequently accompanies AH and multiplies the risk of atherosclerosis development, can also be considered as one of the multiple sources contributing to the rise in AH (4, 5). Atherogenic lipoproteins in plasma cause endothelial dysfunction, increase the vessel tone and support the development of AH terminating in organ ischemia (3–8).

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The new laboratory diagnostic method of plasma lipoproteins on polyacrylamide gel- Lipoprint LDL system quantifies atherogenic lipoproteins, including small dense LDL (9, 10), and characterizes the lipoprotein profile as a) non-atherogenic lipoprotein profile, phenotype A and b) atherogenic lipoprotein profile, phenotype B (11, 12).

Atherogenic lipoprotein profile is characterized by predominant presence of atherogenic lipoproteins: very low density (VLDL), intermediate density IDL1 and IDL2, and small dense low density lipoproteins (sdLDL) while the latter represent highly atherogenic LDL subfractions forming fractions LDL 3–7. Small dense LDLs are smaller than LDL1, LDL2 with their diameter below 26.5 nm (265 Angström), floating within the density range between d = 1.048 – 1.065 g/ml, i.e. higher than those of LDL 1, LDL 2 (15–17, 24). They are detected on PAG as subtle bands on the anodic end of gel, right behind HDL migrating at the head of separated lipoproteins. Small dense LDLs are highly atherogenic particles because of their biological characteristics (Tab. 1) (13, 14).

The aim of this study was to determine the occurrence of atherogenic vs. non-atherogenic lipoprotein profiles in patients with newly diagnosed arterial hypertension and to compare this result with lipoprotein parameters of control healthy subjects.

Tab. 1. Small dense LDL are more aterogenic for (13, 14).

* low recognition by LDL-receptors (configuration alterations of Apo B) →
* enhanced aptitude for oxidation and acetylation →
* Oxid-LDL → release of pro-inflammatory cytokines → muscle cell apoptosis
* Oxid-LDL → release of metalloproteinase → collagen degradation
* Oxid-LDL → enhanced aptitude for trapping by macrophages (scavenger-receptors) → stimulation of foam cell formation
* easier penetration into the subendothelial space and formation of cholesterol deposits

Tab. 2. Stratification of hypertensive patients according to the occurrence of atherogenic vs non-atherogenic lipoprotein profile (phenotype B vs phenotype A) (n=107).

	Atherogenic profile n=84 (78.5 %)	Non-atherogenic profile n=23
Normolipidemia	9 (64 %)	5 (36 %)
Hypercholesterolemia	12 (52 %)	11 (48 %)
Hypertriglyceridemia	38 (93 %)	3 (7 %)
Mixed hyperlipidemia	25 (86 %)	4 (14 %)
78.5 % of hypertensive patients: atherogenic lipoprotein profile		
52 % atherogenic lipoprotein profile in hypercholesterolemia		
93 % atherogenic lipoprotein profile in hypertriglyceridemia		
86 % atherogenic lipoprotein profile in mixed hyperlipidemia		

Patients and methods

In our study, 107 patients with newly diagnosed arterial hypertension were examined. Repeated blood pressure (BP) examination in all groups of hypertonic patients confirmed an increase in blood pressure over 150 mmHg for systolic and over 90 mmHg for diastolic blood pressure. Average systolic blood pressure was 172±19 mmHg and average diastolic blood pressure was 101±11 mmHg. The group of hypertonic patients represented 66 men and 41 women. The average age of men was 50±17.6 years and average age of women was 51.0±13.4 years.

The control group consisted of 150 healthy normotonic and normolipemic volunteers with no signs of cardiovascular disease and no biochemical signs of lipid metabolism disorders.

Total cholesterol and triglycerides in plasma were analyzed from lipid parameters by means of enzymatic CHOD-PAP method, Roche Diagnostics, Germany.

Non-atherogenic lipoprotein fenotype A and an atherogenic lipoprotein phenotype B were determined by Lipoprint LDL System Quantimetrix, CA, USA.

The score of atherogenic risk (SAR) was calculated as a ratio of non-atherogenic to atherogenic plasma lipoproteins. Values of SAR over 10.8 characterized a non atherogenic lipoprotein profile, while values below 9.8 characterized an atherogenic lipoprotein profile. Values between 9.8 and 10.8 represented the gray zone.

Stable plasmatic forms of Prostacyclin (PGI_2) and thromboxane A_2 (TxA_2), namely 6-keto-PGF_{1alpha} and thromboxane B_2 (TxB_2), respectively, were analysed by ELISA method, DRG, USA.

The blood samples were taken from the cubital vein after 12 hour of fasting. EDTA-K_2 plasma was obtained and used for analyzing the lipid and biochemical parameters.

The statistical evaluation of measured values was performed with the Student´s t-test for unpaired observations. Values of $p < 0.05$ were accepted as statistically significant.

Results

The structuring of hypertonic patients according to the occurrence of atherogenic vs. non-atherogenic lipoprotein profiles reveals that hypertonic patients with hypertriglyceridemia had an atherogenic lipoprotein profile in 93 %, patients with mixed hyperlipidemia in 86 %, patients with normolipemia in 64 %, while patients with hypercholesterolemia had an atherogenic lipoprotein profile in only 52 % (Tab. 2). This method confirmed

Tab. 3. Plasma concentration of lipids, lipoproteins, prostanoids and Score of Atherogenic Risk (SAR) in the control group.

	Chol (mmol/l±SD)	TAG	VLDL	LDL12	LDL37	LDL	HDL	PGI2 (pg/ml±SE)	TxA2	PG/Tx	Score
control (non-atherogenic profile n=140)	4.28±0.60	1.15±0.39	0.60±0.16	1.29±0.38	0.03±0.003	2.31±0.53	1.35±0.32	5194±604	1277±07	4.1±2.7	37.8±19.7
control (atherogenic profile n=10)	4.25±0.54	1.44±0.40	0.68±0.14	1.16±0.24	0.22±0.08	2.24±0.36	1.32±0.31	2467±384	868±362	2.8±1.6	6.0±2.0
control (total number n=150)	**4.27**±0.60	**1.17**±0.39	**0.61**±0.16	**1.28**±0.37	**0.04**±0.004	**2.30**±0.52	**1.34**±0.32	**4967**± 589	**1243**± 124	**4.0**±2.6	**35.8**±18.5
non-athero vs athero		**p <0.05**			**p <0.0001**						**p <0.0001**

Tab. 4. Plasma concentration of lipids, lipoproteins, prostanoids and Score of Atherogenic Risk (SAR) in patients with arterial hypertension.

	Chol (mmol/l SD)	TAG	VLDL	LDL12	LDL37	LDL	HDL	PGI2 (pg/ml±SE)	TxA2	PG/Tx	Score
AH	5.32±0.98	1.56±0.55	0.84±0.31	1.78±0.44	0.08±0.04	3.02±0.71	1.49±0.34	5983±2497	1282±248	4.6±2.9	24.2±13.6
(non-atheroprofile n= 23)											
AH	5.15±1.14	2.48±1.34	1.01±0.35	1.47±0.58	0.42±0.31	2.99±0.96	1.18±0.34	3041±160	844±52	3.6±1.3	5.1±2.0
(atheroprofile n= 84)											
AH	**5.19**±1.10	**2.28**±1.07	**0.97**±0.34	**1.54**±0.55	**0.35**±0.25	**3.00**±0.91	**1.25**±0.34	**4116**±668	**1039**±95	**4.0**±1.6	**9.2**±4.5
(total number n=107) non-athero profile vs atheroprofile		**p <0.002**	**p <0.05**	**p <0.02**	**p <0.0001**		**p <0.001**				**p <0.0001**

Tab. 5. Plasma concentration of lipids, lipoproteins, prostanoids and Score of Atherogenic Risk (SAR)control group vs. pacients with arterial hypertension.

	Chol (mmol/l ±SD)	TAG	VLDL	LDL12	LDL37	LDL	HDL	PGI2 (pg/ml±SE)	TxA2	PG/Tx	Skore
control	4.27±0.60	1.17±0.39	0.61±0.16	1.28±0.37	0.04±0.004	2.30±0.52	1.34±0.32	4967±589	1243±124	4.4±2.6	35.8 ±18.5
(n=150)											
AH	**5.19**±1.10	**2.28**±1.07	**0.97**±0.34	**1.54**±0.55	**0.35**±0.25	**3.00**±0.91	1.25±0.34	4116±668	1243±95	4.0±1.6	**9.2**±4.5
(n=107)											
control vs AH	⟷	**p <0.001**	⟶		**p <0.0001**	**p <0.03**					**p <0.0001**

also an existence of atherogenic lipoprotein profile in 7 % of healthy normotensive and normolipemic volunteers of the control group (Tab. 3). The plasmatic concentrations of lipids and atherogenic lipoproteins, namely that of VLDL, LDL and small dense LDL are significantly higher in patients with arterial hypertension. The presence of small dense LDL in plasma is decisive for declaring the atherogenic lipoprotein profile in hypertensive patients, hyperlipidemic subjects, as well as in the normolipemic control group (Tabs 4 and 5).

Discussion

The new diagnostic method, Lipoprint LDL system, quantifies atherogenic lipoproteins and specifies the types of lipoprotein profile in examined subjects, namely as atherogenic vs. non-atherogenic types (24), which is a fundamental methodological contribution of this new analytical and diagnostic method. Hyperlipoproteinemia represents a risk factor for the development of cardiovascular diseases and plays an important role in the pathogenesis of arterial hypertension. Using this methodological diagnostic novelty it has been found out that up to 93 % of hypertonic patients with hypertriglyceridemia had an atherogenic lipoprotein profile with a high presence of strong atherogenic lipoproteins, ie. small dense LDL in their plasma lipoprotein profiles.

As opposed to the latter, hypercholesterolemia was accompanied with an atherogenic lipoprotein phenotype B in 52 % of hypertonic patients only. Based on this finding, it can be assumed that triglycerids and hypertriglyceridemia play a much more important role than it used to be generally accepted. Until now, the most important role in the pathogenesis of vascular degenerative atherosclerotic injury was attributed to cholesterol and hypercholesterolemia. Our presented results are in agreement with other authors who based on the fact that triglycerid-rich lipoproteins can generate small dense LDL in high quantity (14) attract attention to hypertriglyceridemia, as a risk factor of cardiovascular diseases (18–21).

The strong atherogenic lipoproteins – small dense LDL – have been found in the lipoprotein profile of both groups (hypertonic patients and control group) (3, 4). Their presence is decisive for declaring the atherogenic profile. This is a rule, which is valid for hyperlipidemia as well as for normolipemia. In case of normolipemia, a new conception would be installed, namely an existence of **atherogenic normolipidemia** as a risk factor for the development of cardiovascular disease. A special form of normolipemia can also be atherogenic. This is a new reality, which is to be taken as a base for a new approach in prevention and treatment of cardiovascular diseases in future clinical practice.

Appropriate endothelial secretion of prostacyclin (PGI_2) and thromboxane A_2 (TxA_2) characterizes the intactness of endothelial function. A physiological molar ratio of PGI_2/TxA_2 is = 1.5. An impaired endothelium reduces the prostanoid synthesis. Consequently, the ratio of $PGI2/TxA_2$ decreases below 1.0. In our study

group of hypertonic patients it was confirmed that their secretion of PGI_2 was suppressed, however the changes in prostanoid secretion were not statistically significant when compared to the control group. These results support the generally accepted knowledge about the role of prostanoids in the development of cardiovascular diseases; nevertheless it can be assumed that at the time of examination of our group of hypertonic patients, the endothelial synthesis of prostanoids did not reflect sufficiently the beginning of the injuring process within the endothelium due to short duration of disease. During the development of disease, the characteristics of impaired endothelium become more profound.

The score of atherogenic risk as a relation between non-atherogenic and atherogenic lipoproteins in plasma is a newly introduced score, which can help in expressing the atherogenic risk measure of tested persons. The results obtained in our study are in good correlation with the atherogenic risk of tested persons who had the diagnosis of arterial hypertension and who were at risk of developing a cardiovascular disease. The newly introduced score is going to be tested in further clinical studies. Its predictive value is high and therefore a high clinical utility of SAR is being expected (22, 23).

Conclusion

The contribution of the new method lies in benefits as follows:

- Determination of atherogenic andnon-atherogenic lipoprotein profile in patient serum.
- Introduction of a new risk measure, namely that of the score of atherogenic risk (SAR) for estimating the atherogenic risk of examined persons.
- Identification of high percentage of non-atherogenic hypercholesterolemia (48 %), atherogenic hypertriglyceridemia (93 %) and atherogenic normolipemia (64 %) in hypertonic patients in the presented clinical study.
- Presence of small dense LDL in serum is decisive for declaring the atherogenic lipoprotein profile. It is valid for both hyperlipidemic and normolipidemic patients.

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