ASSESMENT OF SMALL DENSITY LDL IN FAMILIAR CARDIOVASCULAR RISK

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Abstract
Elevated concentration of small dense LDL (sdLDL) has been shown to be closely associated with cardiovascular diseases. Situation is however more complicated by the influence of frequently occurring comorbidities, genetic predisposition and environmental influences. The aim of our study was to determine the average concentrations of sdLDL in the Slovakian population and to estimate its relationship with routinely used laboratory markers of cardiovascular diseases. Venous blood samples from 122 volunteers were tested for concentrations of sdLDL, total cholesterol, triacylglycerols, LDL-cholesterol, HDL-cholesterol, apolipoprotein A-1, apolipoprotein B and lipoprotein a. High correlation was quite expectedly found between sdLDL and LDL-cholesterol (Pearson´s R=0,70) and sdLDL with ApoB (Pearson´s R=0,86). We also performed family screening of 5 families with the individuals with elevated parameters with the signs of genetic load, but subsequent molecular biological analysis must be carried out. The sdLDL parameter seems to be a very good predictor of cardiovascular diseases, however only in the case of its complex evaluation with another factors and treatment factors, such as hyperbaric oxygenotherapy.

Key words: laboratory medicine, cardiovascular risk, sdLDL

1 Introduction

Diseases of lipid metabolism, in particular cardiovascular disease, remain the number one cause of health problems and death, especially CHD caused by atherosclerosis [1].

Low-density lipoprotein (LDL) is the largest lipoprotein fraction of plasma (40-60%). It is considered as the most atherogenic type of lipoprotein. LDL is separated into multiple subclasses, which are generated during the delipidation process from very-low-density lipoprotein (VLDL) to intermediated- low-density lipoprotein (IDL) and LDL particles [2-8]. Interestingly, atherogenicity differs among LDL subclasses [8]. The predominance of small dense LDL (sd-LDL) has been accepted as a risk factor for cardiovascular events by the National Cholesterol Education Program Adult Treatment Panel III (NCEP III) [9]. Some studies have shown that the modulation of LDL particle size by hypolipidemic agents reduces CVD risk; however, some studies have failed to confirm their effectiveness [3, 4, 10]. These discrepancies may be explained by differences in patient characteristics, lipid and non-lipid risk factors for coronary atherosclerosis, and techniques used for separating LDL subclasses [7, 8].

2 Aim

The goal of our study was to determine the sdLDL values in the population of Slovakia. Subsequently we try to investigate the relationships between sdLDL concentrations and routinely used markers of cardiac risk and to identify familial predisposition in relation to lipid parameters.

3 Material and methods

Venous blood was collected from the 122 volunteers (70 women) and (52 men) aged 15-83 years. New parameter sdLDL we tested together with parameters used routinely in assessment of cardiovascular risk: total cholesterol (CHOL), triacylglycerols (TAG), LDL-cholesterol (LDL), HDL-cholesterol (HDL), Apolipoprotein A-1 (ApoA1), Apolipoprotein B (ApoB) and lipoprotein a (LIPa). Analysis of blood samples was conducted from October 2012 to October 2013. For the serum analyses, blood was collected with the MONOWETTE (Cat. No: 04.1935, 4.9 ml) and the separation gel. The blood was centrifuged at 20 ºC., 1500×g for 10 minutes (Rotanta centrifuge 96). Serum samples were analyzed by the analyzer Olympus AU 400 and COBAS 6000. Calibration solutions and the control material was purchased from Olympus analyzer AU 400 and on the ROCHE COBAS 6000.

4 Results and discussion

The 122 volunteers (men and women), aged (48.41 ± 14.86) years, with median 50 years, were tested for cardiovascular markers. Basic statistical parameters of all tested cardiovascular markers are summarized in table 1.

Table 1 *Basic statistics of tested laboratory parameters*

Parameter	units	*n*	$\bar{x}$	*sd*	x_m	*min.*	*max.*
CHOL	mmol/l	122	5.48	1.13	5.37	3.17	8.40
TAG	mmol/l	122	1.47	1.04	1.22	0.34	8.25
HDL	mmol/l	122	1.66	0.45	1.58	0.70	2.67
LDL	mmol/l	122	3.49	1.12	3.40	1.17	6.86
sd-LDL	mmol/l	122	0.83	0.39	0.76	0.20	1.90
ApoA1	g/l	120	1.70	0.35	1.66	1.02	2.84
ApoB	g/l	122	1.04	0.29	1.00	0.51	1.77
LIPa	mg/l	121	217.80	247.04	92.01	49.87	1009

Legend: *n* – number of patients, $\bar{x}$ – arithmetical mean, *sd* – standard deviation, x_m – median, *min*. – minimal value, *max.* – maximal value

Most respondents had examined almost all parameters. Only in two of them (1.64%) was missing data of the concentration of ApoA1 and in one case (0.82%) lipoprotein levels were missed. Among all of the testing individuals we determined the degree of dependence on a given correlation coefficient (Table 2).

Table 2 *Reliance between sdLDL and other cardiovascular risk parameters*

Parameter	units	*n*	*R*	R^2	*95% C.I.*
CHOL	mmol/l	122	**0.65**	0.42	0.53 – 0.74
TAG	mmol/l	122	0.37	0.14	0.20 – 0.51
HDL	mmol/l	122	-0.11	0.01	-0.29 – 0.07
LDL	mmol/l	122	**0.70**	0.49	0.59 – 0.78
ApoA1	g/l	120	0.05	<0.01	-0.13 – 0.23
APoB	g/l	122	**0.86**	0.73	0.80 – 0.90
LIPa	mg/l	121	0.17	0.03	-0.01 – 0.34

Legend: n- number of patients, *R* – Pearson´s correlation coefficient, R^2 – coefficient of determination, 95% C.I. – 95% confidence interval of *R*

The highest correlation coefficient was found between sdLDL and ApoB (R = 0.86), which value was even higher than the correlation coefficient between total LDL and sdLDL (R = 0.70) and between total cholesterol and sd-LDL (R = 0.65). In both cases it is necessary to take into

account the correlation mediated, because the fact that sdLDL is one of the basic groups to which LDL cholesterol is divided. Measured values of lipid parameters in individual volunteers we assessed according to normal reference range. Results are summarized in Table 3.

Table 3 *Reference ranges of tested parameters*

Parameter		units	Men		Women	
			Min	Max	Min	Max
CHOL		mmol/l	3.8	5.20	3.80	5.20
TAG		mmol/l	0.6	2.20	0.60	2.20
HDL		mmol/l	0.78	1.82	0.78	1.82
LDL		mmol/l	0.00	4.00	0.00	4.00
Apo A-1		g/l	1.10	2.05	1.25	2.15
Apo B		g/l	0.55	1.44	0.55	1.25
LIPa		mg/l	100.00	200.00	100.00	200.00
sd-LDL	21 - 44 years of life	mmol/l	0.24	1.10	0.24	1.10
	45 - 54 years of life		0.28	1.26	0.24	1.10
	55 - 75 years of life		0.28	1.26	0.28	1.26

Legend: *min*. – minimal value, *max.* – maximal value

For originating and development of cardiovascular syndrome, which may gradually develop into serious cardiovascular problems, hereditary factors are of great importance. Predisposition for developing the disease is then inherited from parents and they may or may not even occur at all siblings. Genetically lipoprotein a is mainly influenced. In our tested file there were several individuals with mutual family relationships (mother, father, offspring, and siblings). Their number is small for statistical evaluation, but first data obtained point out that this issue should be paid increased attention (Tables 5-8).

In the examined family 1 eldest son had high levels of LIPa (588.80 mg/l; table 4). Predisposition was obtained from the father, in which the level of LIPa was 619.08 mg/ml, and also there has been found high levels of TAG (3.94 mmol/l). Second-born son had LIPa concentration in the low standard, but had higher serum total and LDL fractions and sdLDL, and increased levels of CHOL (6.68 mmol/l). His predisposition to higher levels was obtained probably from the mother (CHOL = 6.80 mmol/l).

Table 4 *Parameters of lipid metabolism in the family 1*

person		Age	CHOL	TAG	HDL	LDL	sdLDL	ApoA1	ApoB	LIPa
		years	mmol/l					g/l		mg/l
F	♂	71	5.73	3.94	1.78	2.56	0.44	1.96	0.76	**619.08**
M	♀	68	**6.80**	1.72	2.04	**4.01**	0.94	2.11	1.08	104.75
P1	♂	44	5.64	1.38	1.78	3.48	0.50	1.70	0.97	**588.80**
P2	♂	41	**6.68**	2.20	2.58	**4.37**	**1.38**	2.07	1.25	97.64

Legend: *F* – father, *M* – mother, *P1* – offspring 1, *P2* – offspring 2

In the family 2 with one male descendant we observed in the offspring very high levels of LIPa (816.98 mg/l), LDL (6.86 mmol/l), sdLDL (1.65 mmol/l) and the CHOL (8.40 mmol/l). Probably he received the predisposition from his father (Table 5). In the family 3 with female children we found in her father slightly increased concentration of TAG (2.40 mmol/l) and threshold levels of LIPa (294.73 mg/l). The mother, who is on oral antidiabetic treatment because of diagnosis E11.90 (diabetes mellitus without insulin-dependent diabetes without complications), she had high levels of CHOL, TAG, LDL fraction and the sdLDL (Table 6). Her child had elevated only LIPa (640.33 mg/l).

Table 5 *Parameters of lipid metabolism in the family 2*

person		Age	CHOL	TAG	HDL	LDL	sdLDL	ApoA1	ApoB	LIPa
		years	mmol/l					g/l		mg/l
F	♂	62	6.09	0.93	2.07	**4.42**	**1.79**	2.41	1.36	**567.6**
M	♀	60	5.48	0.48	1.35	**4.54**	0.77	1.25	1.27	86.34
P1	♂	33	**8.40**	1.24	1.87	**6.86**	**1.59**	1.65	1.77	**816.98**

Legend: *F* – father, *M* – mother, *P1* – offspring 1

Table 6 *Parameters of lipid metabolism in the family 3*

person		Age	CHOL	TAG	HDL	LDL	sdLDL	ApoA1	ApoB	LIPa
		years	mmol/l					g/l		mg/l
F	♂	61	5.12	**2.40**	0.93	3.48	1.06	1.31	1.08	**294.73**
M	♀	57	**8.15**	**3.38**	1.39	**5.61**	**1.65**	1.61	1.64	114.57
P1	♀	32	4.20	0.80	1.45	2.07	0.49	1.52	0.74	**640.33**

Legend: *F* – father, *M* – mother, *P1* – offspring 1

Interestingly appear sibling comparisons (Table 7), where older siblings S1 and S2, which are fraternal twins. Son S2 together with sibling S3 had high levels of LIPa. Unfortunately, we cannot verify from whom they have acquired predisposition, because their parents do not live. However, from mutual consultation we learned that the father surpassed heart attack at a young age and next heart attack he had not survived.

Table 7 *Parameters of lipid metabolism in offsprings in the family 4*

person		Age	CHOL	TAG	HDL	LDL	sdLDL	ApoA1	ApoB	LIPa
		year	mmol/l					g/l		mg/l
S1	♂	55	**6.90**	1.49	1.38	**4.83**	1.07	1.57	1.48	69.90
S2	♂	55	**5.73**	2.05	1.17	**4.05**	**1.32**	1.25	1.36	**904.85**
S3	♂	44	5.05	1.38	1.30	3.38	0.67	1.28	1.09	**897.91**

Legend: *S1*- sibling 1, *S2*- sibling 2, *S3*- sibling 3

In the family 5 that was represented by 4 generations we can observe hereditary dependence of LIPa. The offspring obtained predisposition for increased LIPa levels from both parents, and the father of the mother (table 8). In male offspring no. 3 we observed high levels of CHOL (7.44 mmol/l), LDL (5.67 mmol/l), sdLDL (1.31 mmol/l), ApoB (1.69 g/l) and LIPa (816.98 mg/l). This boy have acquired predisposition from his father. High levels of CHOL, Apo B and sdLDL we also found in the youngest member of the family, young girl - daughter of first offspring. She acquired predisposition probably from grandfather, but also from his own father, with whom we had to lay down the lipid spectrum.

Table 8 *Parameters of lipid metabolism in the family 5*

person		Age	CHOL	TAG	HDL	LDL	sdLDL	ApoA1	ApoB	LIPa
		year	mmol/l					g/l		mg/l
FM	♂	83	**5.84**	1.26	2.14	3.90	1.21	1.90	0.95	**543.84**
MM	♀	81	**5.57**	0.83	1.70	3.16	0.89	1.83	0.99	202.52
M	♀	62	4.72	1.83	1.24	2.99	0.82	1.41	0.87	**520.16**
F	♂	62	**7.90**	1.56	1.75	**5.22**	**1.37**	1.71	**1.59**	**792.62**
P1	♀	39	5.17	0.63	1.68	3.43	0.85	1.82	1.04	**303.44**
P2	♀	34	4.06	0.74	1.96	1.95	0.68	1.73	0.58	174.88
P3	♂	32	**7.44**	1.77	1.89	**5.67**	**1.31**	1.64	**1.69**	**816.98**
PP1	♀	16	**6.85**	0.89	2.11	**4.06**	**1.28**	2.04	**1.32**	129.60

Legend: *FM* – mother´s father, *MM* – mother´s mother, *F* – father, *M* – mother, *P1* – first offspring, *P2* – second offspring, *P3* – third offspring, *PP1* – daughter of first offspring

5 Conclusion

In the experimental part of the work we investigate together 122 volunteers in three cycles of measurement. We tested the lipid parameters with emphasis on sdLDL cholesterol, which is in Slovakia still used in routine practice. In view of the fact that increased concentration sd LDL-points to the increased risk of cardiovascular disease, it can be said that, from our volunteers on the basis of the measurement results confirmed that the males have a higher risk of their occurrence, especially since the men are found above levels compared to women. Determination of sdLDL cholesterol better predicts of cardiovascular disease, compared to commonly determined lipid parameters (CHOL, TAG, HDL, LDL, ApoB). By analyzing real samples, we found a relatively high correlation between sdLDL and ApoB (R = 0.86), less the total LDL and sdLDL (R = 0.70). In our reference file was located several individuals with mutual family relationships. Their number was small for statistical evaluation, but first data obtained point out that the issue of cardiac markers in terms of familial relationships is attention increasingly.

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