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ORIGINAL ARTICLE

Small dense LDL – an important part of the atherogenic lipoprotein profile in individuals with impaired metabolism of lipoproteins. Comparison of two analytical procedures

Stanislav Oravec[1], Vlasta Jediná[2], Ingrid Žitňanová[3], Elisabeth Dostal[4], Ferdinand Devínsky[5]

1 1st Department of Internal Medicine, Faculty of Medicine, Comenius University, Bratislava, Slovak Republic.
2 Medirex a.s., Bratislava, Slovak Republic.
3 Institute of Medical Chemistry, Biochemistry and Clinical Biochemistry, Medical faculty, Comenius University, Bratislava, Slovak Republic.
4 Krankenanstalten Labor Dr. Dostal, Vienna, Austria.
5 Faculty of Pharmacy, Comenius University, Bratislava, Slovak Republic.

Correspondence to: Stanislav Oravec, Professor, MD, PhD
1st Department of Internal Medicine, Faculty of Medicine, Comenius University, Mickiewiczova 13, 813 69 Bratislava, Slovak Republic

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Abstract

OBJECTIVES: To compare two different analytical methods for determination of small dense LDL and to determine a share of corresponding and non-corresponding (inconsistent) results

METHODS: In the group of 104 hyperlipidemic patients and 20 healthy individuals of the control group we analysed the total cholesterol and triglycerides by enzymatic CHOD PAP method (Roche Diagnostics, Germany) in EDTA-K_2 plasma. Small dense LDL (sdLDL) were quantified by the electrophoretic method for lipoprotein analysis on polyacrylamide gel (PAG) (Lipoprint LDL System, Quantimetrix, CA, USA) and simultaneously, the small dense LDL concentrations in the indentical samples were analysed by an enzymatic method LDL–EX ´Seiken´(Randox, England).

RESULTS: In 31 patients we found the discrepancy in the sdLDL levels using the two different procedures. Out of them, 24 patients tested by enzymatic method ´SEIKEN´ had higher sdLDL values (more than 0.9 mmol/l) compared to the Lipoprint LDL results, which identified normal sdLDL values in the same samples (in 23% of tested patients). In 7 patients out of the 31 tested patients with discrepant sdLDL values, the Lipoprint LDL identified increased values of plasma sdLDL (more than 0.155 mmol/l), while the enzymatic LDL-EX Seiken did not find an increased concentration of sdLDL (in 7% of tested patients). In the control group a discrepancy in the sdLDL results between the two tested analytical methods was not found.

CONCLUSION: The concentration of sdLDL in plasma lipoprotein spectrum obtained by two different laboratory procedures was analysed, compared, evaluated and 70% identical corresponding results have been confirmed.

Abbreviations and units:

LDL	- low density lipoproteins
sdLDL	- small dense LDL
T-chol	- total cholesterol
TG	- triglycerides
PAG	- polyacrylamide gel

INTRODUCTION

Small dense LDL (sdLDL) represent the strong atherogenic lipoproteins and are present as a regular part of lipoprotein spectrum in individuals with hyperlipoproteinemia (Koba *et al.* 2002; Vakkilainen *et al.* 2003; Krauss 2010; Hoogeveen *et al.* 2014). Their high predictive value in diagnostics of degenerative cardiovascular diseases was confirmed (Ai *et al.* 2010; Hirayma & Miida 2012; Zhao *et al.* 2009; Oravec *et al.* 2015). Several clinical studies confirmed sdLDL to be a predictor of stenotic occlusion of coronary arteries manifested as a coronary heart disease (angina pectoris), which can proceed into the origin of sudden cardio-vascular (myocardial infarction) (Koba *et al.* 2002; Oravec *et al.* 2011a; Oravec *et al.* 2011c; Oravec *et al.* 2014) or a cerebro-vascular event (Zhao *et al.* 2009; Oravec *et al.* 2011b). There is assumed a causal correlation between an increased triglyceride concentration in blood, localised in triglyceride rich lipoprotein families on one hand, and an increased creation of sdLDL on the other hand (Berneis & Krauss 2002; Ai *et al.* 2010; Hoogeveen *et al.* 2014; Wu & Parhofer 2014; Toth 2016).

Aim of the study was to determine a share of corresponding and inconsistent results in sdLDL analysis, which were analysed by two different analytical methods:

a) by electrophoretic determination of plasma lipoproteins including sdLDL (Lipoprint LDL System and
b) by enzymatic LDL-EX Seiken in a group of patients with hyperlipoproteinemia

PATIENTS AND METHODS

A group of 104 patients with impaired metabolism of lipoproteins consisted of 64 females (average age 55 ± 20 years) and 40 males (average age 50 ± 20 years). Patients were treated with hypolipemics: 1) by statins: atorvastatin 20 mg daily, fluvastatin with a prolonged efect 80 mg daily, or by 2) fibrates: phenofibrat 215 mg daily with a prolonged effect. A part of patients (14 patients) because of light intolerance of pharmacological treatment changed later the treatment (however, after finishing the study (myalgie)) for dietethic recommendations. The others 90 patients tolerated the treatment with hypolipemics during the study very well.

Control group consisted of 20 normolipidemic healthy probands, 14 females and 6 males (average age 42 ± 20 years), without clinical and laboratory signs of cardio-vascular disease. All tested individuals had normal blood pressure and were nonsmokers. Blood of tested individuals (patients and controll group probands) was collected in the morning after 12 hour fasting time from cubital vein into EDTA-K_2 vacutainers. The blood samples were centrifuged at 2000 × g for 15 minutes at 4°C. Obtained plasma samples of tested individuals were stored at –20°C until the time of analysis.

In the group of 104 hyperlipidemic patients and 20 healthy individuals of the control group we analysed total cholesterol and triglycerides by enzymatic CHOD PAP method (Roche Diagnostics, Germany) in EDTA-K_2 plasma. Small dense LDL were identified and quantified by electrophoretic method for the lipoprotein analysis on polyacrylamide gel (PAG) (Lipoprint LDL System, Quantimetrix, CA, USA) (Hoefner *et al.* 2001) and at the same time small dense LDL concentrations in the indentical samples were analysed by an enzymatic method LDL–EX ´Seiken´(Randox, England) (Hirano *et al.* 2005).

Lipoprint LDL method declares normal values of sdLDL up to 6 mg LDL cholesterol/dl, i.e. 0.155 mmol cholesterol/l.

Enzymatic method LDL-EX, Seiken recommends ´cut off´ limit for sdLDL 0.9 mmol/l, i.e. 34.8 mg/dl.

Normal concentration of sdLDL in plasma, analysed by enzymatic LDL-EX Seiken method is approx. 6 times (5.8 times) higher than normal concentration of sdLDL, declared by the Lipoprint LDL System (Table 1).

RESULTS

In the group of 104 tested patients in the clinical study, comparing the results of analysis of small dense LDL particles in plasma by two different analytical procedures, a discrepancy in the results was found in 31 patients (29.8%). The sdLDL were analyzed by the Lipoprint LDL system (Quantimetrix USA) and simultaneously by the enzymatic method LDL-EX Seiken. 70.2% identical corresponding results have been confirmed.

Out of 31 patients with discrepant results, in 24 tested patients the enzymatic method 'SEIKEN' identified increased sdLDL values over the 'cut off' concentration declared by this method - i.e. 0.9 mmol/l. Lipoprint LDL System in these patients found normal concentrations of sdLDL i.e. lower than 0.155 mmol/l (6 mg/dl). 24 patients represent 23.1% from the whole group of tested patients (Table 1, 2).

In 7 patients Lipoprint LDL found increased, abnormal plasma values of sdLDL, more than declared 0.155 mmol/l (6 mg/dl) as the 'cut off' values for this analytical procedure, while the enzymatic method LDL-EX Seiken did not confirm an increased concentration of sdLDL in their plasma. 7 patients represent 6.7% from the whole group of tested patients (Table 2).

In the control group of 20 healthy probands there were not found any discrepancies in the results

Tab. 1. Concentration of lipids and sdLDL in the control group vs. tested hyperlipidemic patients, analysed by the LDL-EX Seiken method and Lipoprint LDL System

	T-cholesterol	TG	sdLDL-Seiken		sdLDL-LipoprintLDL	
	mmol/l	mmol/l	mmol/l	mg/dl	mg/dl	mmol/l
Norm:	< 5.2	< 1.7	< 0.90	<34.8	< 6.0	< 0.155
Control n = 20	4.51± 0.1	0.73± 0.06	0.525± 0.06	20.3	2.05± 0.16	0.053
Hyperlipidemic group n= 104	**6.03**±0.15	1.38± 0.12	**0.943**±0.08	36.5	**6.41**± 0,15	**0.166**

of sdLDL analysis between the two different analytical procedures.

DISCUSSION

Small dense low density lipoproteins (sdLDL) represent an atherogenic part of the lipoprotein spectrum. In individuals with hyperlipoproteinemia sdLDL are a regular part of hyperlipoproteinemia (Koba *et al.* 2002). Hypertriglyceridemia participates in acceleration of creation of abnormal, strong atherogenic sdLDL (Berneis & Krauss 2002; Packard 2003; Diffenderfer & Schaefer 2014; Wu & Parhofer 2014; Toth 2016). They have a high predictive value in the diagnostics of cardio-vascular diseses, caused by an atherosclerotic process (Oravec *et al.* 2011b; Oravec *et al.* 2014). Recently, there are tested optimal clinical-laboratory procedures, which are able to identify and analyse sdLDL easily and which are convenient for a routine clinical practice.

In the present laboratory practice there are available two analytical methods: 1) an enzymatic method LDL-EX (Seiken Randox, GB) for identification and quantification of sdLDL in plasma (Hirano *et al.* 2005), and 2) an electrophoretic method for identification and quantification of plasma lipoproteins, including sdLDL, on polyacrylamide gel (PAG) = Lipoprint LDL System (Quantimetrix, CA, USA) (Hoefner *et al.* 2001).

These two analytical procedures for sdLDL analysis, declare two different reference intervals, i.e. normal values of sdLDL concentration in plasma. The enzymatic LDL-EX SEIKEN method however, tolerates as normal values of sdLDL the concentrations, which are 5.8 times higher than normal values of sdLDL analyzed by the Lipoprint LDL System, an electrophoretic method for lipoprotein analysis on the polyacrylamide gel (PAG). This fact is strange, as these both analytical procedures identify and quantify very important risk factor for premature development of cardiovascular diseases, as well as an independent predictor of the sudden cardiovascular event, which has a high predictive value. Which concentrations of sdLDL obtained from these two different analytical procedures have a higher clinical prediction? This question has not been answered yet.

In our study a 70% agreement in results of sdLDL concentrations, analysed by two different analytical procedures has been confirmed, as in 31 tested patient a discrepancy in the results was found. 1) In 7 tested patients the Lipoprint LDL method identified an increased concentration of sdLDL (in 6.7% from the total number 104 of examined patients), however, the enzymatic method SEIKEN identified in these patients normal values. The explanation of this fact could be found probably in very intensive hypolipemic treatment with following normalisation of metabolic parameters, where approx. six times more sensitive Lipoprint LDL analysis identified still increased sdLDL values.

In 24 tested patients (in 23.1% from the total number 104 of examined patients) SEIKEN enzymatic method found an increased concentration of sdLDL, where the Lipoprint-LDL analysis did not confirm an increased concentration of sdLDL. This number of tested patients is not irrelevant, however this discrepancy in the analysis by the two different analytical procedures can not be

Tab. 2. Plasma lipid values and sdLDL concentration, discrepant results in sdLDL, analysed by different analytical methods: LDL-Seiken and Lipoprint LDL

	sdLDL-Seiken		sdLDL-LipoprintLDL	
	mmol/l	mg/dl	mg/dl	mmol/l
Control:	< 0.90	< 34.8	< 6.0	< 0.155
Seiken sdLDL values increased, Lipoprint sdLDL values in the normal range				
n = 24	**1.28**±0.14	49.6±1.4	2.54±0.16	0.066±0.006
Seiken sdLDL values in the normal range, Lipoprint sdLDL values increased				
n= 7	0.63±0.12	24.4±1.3	**8.9**±1.24	**0.23**±0.06

unambiguously explained. The changes of physical and chemical charcteristics of sdLDL, as well as electromigration characteristics of lipoprotein particles in electric field under the effect of special form of treatment, could explain this discrepancy in results (of sdLDL concentration), obtained by the two analytical procedures.

Further sudies however are needed to investigate the useful clinical indices and to determine the benefit for patients with coronary sclerosis and cerebro-vascular damages.

Large number of tested patients, hyperlipemic individuals before a hypolipemic treatment was started, as well as a larger number of normolipemic individuals, could better explain a discrepancy in results obtained by the two different analytical procedures in the analysis of sdLDL. Also, the other novel analytical procedures for determination of the atherogenic lipoproteins including sdLDL could be followed (Tsai et al. 2014, Tani et al. 2014, Tani et al. 2017, Hayashi et al. 2017).

In the control group of 20 normolipemic probands a discrepancy in results between these two tested methods was not found.

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AUTHORS CONTRIBUTION

All of the authors made important intellectual contributions to the manuscript and all authors approved the final version before submission.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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