Lipoprotein Lp(a) in lipoprotein spectrum indentified by Lipoprint LDL system

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Abstract

OBJECTIVE: Identification of lipoprotein subfractions in lipoprotein profile by Lipoprint LDL system, where a lipoprotein(a), an independent risk factor for the development of cardiovascular disease, migrates with. The concentration of lipoprotein(a) in serum over 0.3 g/l increases the risk of athero-thrombosis and a brain stroke. The persons with increased levels of lipoprotein(a) and contemporarily increased cholesterol level in serum, are at increased risk of the inception of cardiovascular or cerebrovascular event even 3-times.

PATIENTS AND METHODS: In a general group of subjects with increased serum concentration of lipoprotein(a) a lipoprotein profile analysis was performed. The general group of subjects was divided into two groups: subgroup with the lipoprotein(a) concentration in the range between 0.3–0.8 g/l and a subgroup with the lipoprotein(a) concentration over 0.8 g/l, to learn if the lipoprotein(a) particles of different serum concentration and different size do not migrate in different positions of the lipoprotein spectrum. For the analysis of serum lipoproteins an innovated electrophoresis method on polyacrylamide gel (PAG) - Lipoprint LDL system USA, was used. Lipids: a total cholesterol and triglycerides in serum were analysed by an enzymatic method CHOD PAP (Roche Diagnostics, FRG), lipoprotein(a) was analysed by an immuno-nephelometric method (Roche Diagnostics, FRG).

RESULTS: In the Lipoprint LDL system using a polyacrylamide gel (PAG) for the lipoprotein separation, lipoprotein(a) migrates in the position IDL2-IDL3. In the band of IDL2 a high Lp(a) values can be identified, when the increment of IDL2 subfraction is over the value of 0.015 g/l, i.e. 15 mg/dl (reference range for IDL2) and when the increment of IDL3 subfraction is over the value of 25 mg/dl, i.e. 0.025 g/l (reference range for IDL3).

CONCLUSIONS: A clear contribution of new method is:

- identification of the lipoprotein subpopulations where the lipoprotein(a) migrates with
- different migration position for the mild increased lipoprotein(a) concentration and high lipoprotein(a) concentration in serum was not confirmed.

ORIGINAL ARTICLE

Abbreviations:
HDL - high density lipoproteins
IDL - intermediate lipoproteins
LDL - low density lipoproteins
VLDL - very low density lipoproteins
Lp(a) - lipoprotein(a)
PAG - polyacrylamide gel

INTRODUCTION

Lipopoprotein (a) (Lp(a)) represents beside hypercholesterolemia, arterial hypertension and nicotine abuse the next independent risk factor for a development of cardiovascular diseases. When Lp(a) is increased contemporarily with an increased cholesterol level in serum, it can be assumed increased risk of the inception of cardiovascular event even 3-times (Danesh *et al.* 2000; Dahlen & Ekstedt 2001; Berglund & Ramakrishnan 2004). Lp(a) for the first time was identified, characterized and ascribed by Berg in 1963 (Berg 1963). Lp(a) is synthesised by liver (Rader *et al.* 1993), its biological half life in the circulation is 3–4 days and will be catabolised in the kidney (Kostner *et al.*1986, Albers *et al.*2007). Regarding to its structural resemblance with LDL (Lundstam *et al.*1999) it was assumed, that Lp(a) is a special isoform of LDL. That is why it was expected its metabolic degradation pathway by LDL-receptors. However this metabolic pathway for Lp(a) katabolism (degradation) was not confirmed (Rader *et al.* 1995, Oravec *et al.* 1998) (Figure 3).

Increased serum concentration of Lp(a) is associated with an increase incidence of the inception of phlebothrombosis (Caplice *et al.* 2001), as this lipoprotein interacts with the coagulation system in the human body. The competition with the plasminogen was confirmed, as the plasminogen has the chemical structure near to Lp(a) (McLean *et al.* 1987). It is an important finding, because the increased concentration of Lp(a) correlates with the arise of thrombosis in the veins and of the arise of cardiovascular diseases too. The Lp(a) serum level in general group of subjects varies in a broad reference range from 0.2 mg/l – 2 g/l, (0.02–200 mg/dl) and the physiological concentration of Lp(a) in serum is regarded the value, which is lower than 30 mg/dl i.e. 0.3 g/l (Ryane & Torelli 2005).

Approximately 20 years ago was Lp(a) in the focus of research workers as a newly declared independent risk for development of cardiovascular events and for the arise of phlebo-thrombosis of peripheral veins. Now, after longer recession time comes Lp(a) again into the focus of interest of the neurologists. Lp(a) recently was postulated as a risk factor for an arise of acute cerebral ischemia and stroke (Smolders *et al.* 2007; Wilde 2003). In the routine laboratory practice is Lp(a) generally quantified by an immuno-nephelometric method.

A newly introduced electrophoretic method for identifying a serum lipoprotein spectrum Lipoprint system does not analyse specifically the increased Lp(a), however it would be a contribution of this method, to identify lipoprotein subfractions where the Lp(a) migrates with.

The aim of the work was to identify the migration position of Lp(a) in Lipoprint LDL system.

PATIENTS AND METHODS

In the general group of subjects the 175 individuals with increased serum Lp(a) levels were identified. The cut of value was the concentration 0.3 g/l i.e. 30 mg/dl (Marcovina *et al.*2000) (Figure 1). This tested group consisted from 81 males, average age of 58 years and 94 females with average age of 60 years.

The subjects with increased Lp(a) levels were divided into the subgroup with middle increased Lp(a) up to 0.8 g/l and the group with the Lp(a) levels higher than 0.8 g/l in order to learn if smaller and larger lipoprotein(a) particles migrate in the same position in lipoprotein electrophoretic analysis (Figure 2).

The control group created also from general group of subjects consisted from 128 individuals who had the serum Lp(a) concentration lower than 0.3 g/l (Marcovina *et al.* 2000).

In both groups was analysed total cholesterol and triglycerides by enzymatic CHOD PAP method (Roche, FRG), the concentration of lipoprotein (a) was analysed by an immuno-nephelometric method (Roche, FRG).

Lipoprotein spectrum with lipoprotein subpopulations were analysed and quantitatively evaluated by the Lipoprint LDL system (Quantimetrix, California,USA) (Hoeffner *et al.*2001).

The results were evaluated statistically by an impair Students' test.

RESULTS

The Lp(a) in both subgroups i.e. a subgroup of lower Lp(a) concentration (0.3–0.8 g/l) and a subgroup of higher concentration (more than 0.8 g/l) migrate in the same electrophoretic distance; in the IDL2 and IDL3 lipoprotein fractions. These two subfractions have significantly higher concentration than individuals, who have normal Lp(a) concentration under 0.3 g/l and who create the control group. Other lipoprotein subfractions do not show the differences in the concentrations between control values and individuals with increased Lp(a) values. That is why, it can be supposed that significantly increased concentrations of ILD2 and IDL3 in individuals with higher Lp(a) serum concentrations can be caused by the presence of Lp(a) in these two lipoprotein subfractions.

The differences in the concentrations of IDL2 between control and Lp(a) and also between control and IDL3 are significant; at IDL2 $p<0.005$ and at IDL3 $p<0.0001$. Statistical significance is higher in IDL3 subfraction ($p<0.0001$).

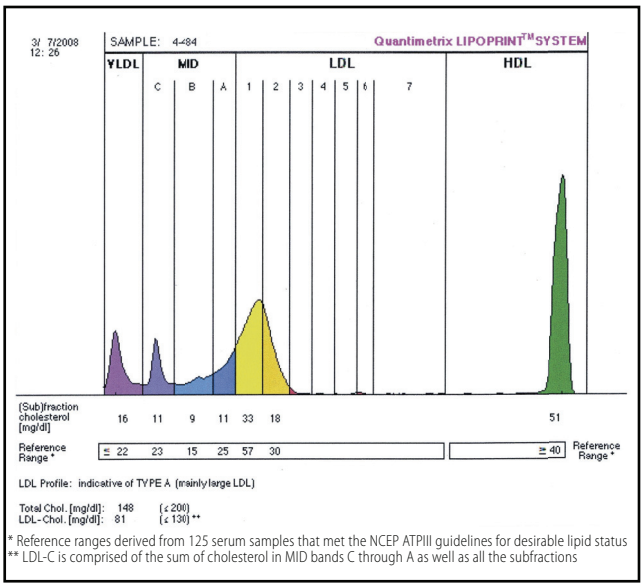

Fig. 1. Lipoprotein spectrum of normolipemics with low concentration of lipoprotein(a) in serum (Lp(a) lower than 0.3 g/l).

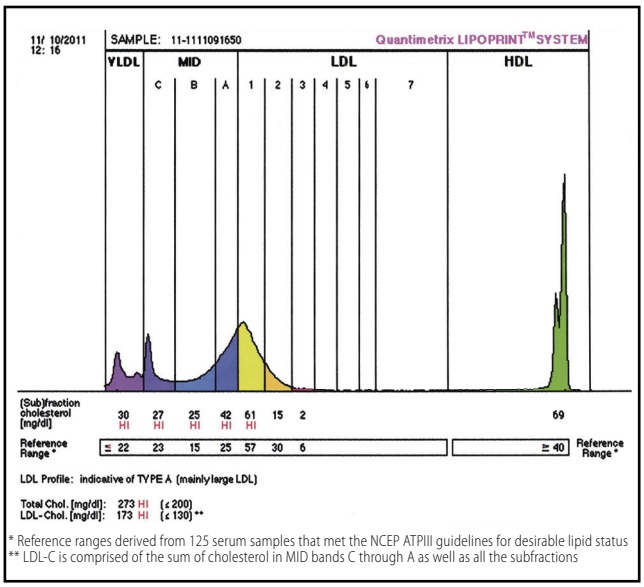

Fig. 2. Lipoprotein spectrum of individuals with high concentration of lipoprotein(a) in serum (Lp(a) higher than 0.3 g/l).

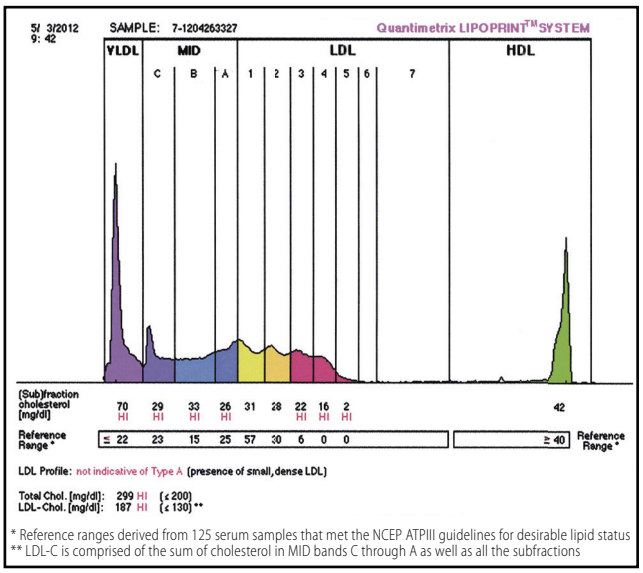

Fig. 3. General hypolipemic treatment does not reduce the lipoprotein(a) concentration in serum.

DISCUSSION

Lipoprotein(a) represents an independent risk factor for the development of diseases of cardiovascular system (Berglund & Ramakrishnan 2004). Later was determined its role in the pathogenesis of the inception of thrombo-embolic diseases after to learn a close structural likeness with plasminogen and by this way a possibility of Lp(a) to interfere with the process of coagulation on the level of fibrinolysis (McLean *et al.* 1987; Caplice *et al.* 2001). Increased Lp(a) serum concentration over 0.3 g/l (30 mg/dl), at contemporarily increased lipid levels in serum (cholesterol, triglycerides), multiplies the risk of the vascular event inception in risk person three times (Danesh *et al.* 2000).

Answering the question about a migrating place of lipoprotein(a) in spectrum of migrating lipoproteins using the Lipoprint system can not be irrelevant. Lipoprotein (a) is a risk factor of the inception of vascular event (Smolders *et al.* 2007) and every early information or suspicion about its increased level in serum/plasma helps us to find an optimal therapeutic approach to the patient with contemporarily increased lipid levels in blood. In our study on the general group of subjects with 175 individuals with increased Lp(a) levels it was found that lipoprotein (a) migrates within IDL2 and IDL3 lipoprotein subfractions, comparing to the control group with normal Lp(a) level (Tables 1–3).

Our analysis also answered the question about the possible migration place of Lp(a) in the migration position of small dense LDL (LDL3–7). In the LDL3–7 lipoprotein subfractions is the migration of lipoprotein(a) particles very unprobable, in spite of a comparable size of lipoprotein(a) and particle size of lipoproteins migrating in LDL3–7 subfractions. The serum values of LDL3–7 even in the subgroup with very high lipoprotein(a) concentration are lower than in our control group with low lipoprotein(a) values.

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Tab. 1. Lipoprotein and lipid values: Control group vs. Subjects with Lp(a) concentrations in the interval between 0.301–0.80 g/l.

Lp(a) g/l	VLDL mmol/l	IDL1 x ± SD	IDL2	IDL3	LDL1	LDL2	LDL37	HDL	TAG	T-Chol
Control group (n=128)										
0.13±0.06	0.83±0.37	0.58±0.19	0.43±0.15	0.49±0.19	1.21±0.44	0.79±0.36	0.26±0.24	1.63±0.46	1.43±0.82	6.31±1.34
Subgroup of subjects with Lp(a) concentrations in the interval between 0.301–0.80 g/l (n=98)										
0.54±0.14	0.83±0.34	0.55±0.19	**0.48±0.14**	**0.60±0.18**	1.25±0.45	0.73±0.37	0.21±0.20	1.65±0.50	1.42±0.84	6.29±1.30
$p<0.0001$			$p<0.02$	$p<0.0001$						

Tab. 2. Lipoprotein and lipid values: Control group vs. Subjects with Lp(a) concentrations higher than 0.80 g/l.

Lp(a) g/l	VLDL mmol/l	IDL1 x ± SD	IDL2	IDL3	LDL1	LDL2	LDL37	HDL	TAG	T-Chol
Control group (n=128)										
0.13±0.06	0.83±0.37	0.58±0.19	0.43±0.15	0.49±0.19	1.21±0.44	0.79±0.36	0.26±0.24	1.63±0.46	1.43±0.82	6.31±1.34
Subgroup of subjects with Lp(a) concentrations higher than 0.80 g/l (n=77)										
1.07±0.20	0.93±0.40	0.93±0.17	**0.49±0.15**	**0.64±0.23**	1.27±0.45	0.79±0.35	0.20±0.20	1.67±0.44	1.55±1.0	6.44±1.22
$p<0.0001$			$p<0.005$	$p<0.0001$						

Tab. 3. Lipoprotein and lipid values: Control group vs. All subjects with increased Lp(a) concentration in serum.

Lp(a) g/l	VLDL mmol/l	IDL1 x ± SD	IDL2	IDL3	LDL1	LDL2	LDL37	HDL	TAG	T-Chol
Control group (n=128)										
0.13±0.06	0.83±0.37	0.58±0.19	0.43±0.15	0.49±0.19	1.21±0.44	0.79±0.36	0.26±0.24	1.63±0.46	1.43±0.82	6.31±1.34
All subjects with increased Lp(a) concentrations in serum (n=175)										
0.77±0.32	0.87±0.37	0.55±0.18	**0.49±0.15**	**0.62±0.20**	1.26±0.45	0.76±0.36	0.20±0.20	1.66±0.47	1.48±0.93	6.36±1.26
$p<0.0001$			$p<0.005$	$p<0.0001$						

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