Low density lipoprotein size in relation to carotid intima-media thickness in coronary artery disease

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Abstract: Objective: With increasing interest in the role of non-traditional lipid risk factors in coronary artery disease, we undertook this study to relate LDL subclass size and carotid intima-media thickness of the common carotid artery in coronary artery disease patients.

Methods: The study was conducted in 106 patients during their first visit (study group I) and after 12 months (study group II). Intima-media thickness of the common carotid artery was determined using B-mode ultrasound. Separation of LDL subclasses was performed by 3–31 % polyacrylamide gradient gel electrophoresis. Results: LDL was the dominant subclass in both study groups, but there was statistically significant difference in the distribution of dominant LDL subclasses (p<0.01). The mean carotid intima-media thickness was significantly increased (p<0.001) in coronary artery disease patients after 12 months period. There was significant negative correlation between intima-media thickness and LDL size in both study groups (p<0.05). Intima-media thickness was not significantly correlated with plasma lipid concentrations. Multiple regression analyses show that strongest independent predictor of the intima-media thickness variation was diastolic blood pressure, followed by LDL size and age, and accounted for 29 % of the observed variability in intima-media thickness.

Conclusion: LDL particle size is independently associated with carotid intima-media thickness in coronary artery disease patients with normal levels of traditional lipid risk. These results imply that small, dense LDL subclasses are an important indicator for assessing atherosclerosis and its progression (Tab. 4, Ref. 39).

Key words: LDL size, gradient gel electrophoresis, intima-media thickness, CAD.
Recently, it has been shown that small low-density lipoprotein and LDL particle size are associated with carotid IMT independent of total cholesterol or LDL cholesterol (25, 26).

In this study we investigated the relationship of LDL size and other risk factors with intima-media thickness (IMT) of the common carotid artery (CCA) in CAD patients with clinically apparent atherosclerosis at their first visit and 12 months after.

Material and methods

Subjects and blood samples collection

Study group I. One hundred and five patients (75 men and 30 women, aged 36 to 74 years) with a history of coronary artery disease were recruited at the time of their first visit at the University Cardiology Clinic, Medical Faculty, Skopje.

The diagnosis of CAD was based on the subjects medical history, clinical signs and symptoms, characteristic electrocardiogram changes, increased concentrations of cardiac enzymes and echocardiography assessment of left ventricular systolic and diastolic function. Hypertension was defined as systolic blood pressure \( \geq 140 \) mmHg, diastolic blood pressure \( \geq 90 \) mmHg, antihypertensive medications, or any combination of these. Individuals with diabetes mellitus, renal diseases, neoplastic disorders, and those treated with lipid-lowering drugs were excluded.

Study group II. Ninety five patients from study group I (68 men and 27 women, aged 41 to 74 years) were analyzed 12 months after their first visit of the Clinic.

The height and weight of each patient was used to calculate body mass index (BMI; kg/m\(^2\)). The study was performed according to the Helsinki declaration and was approved by the Ethics Committee of the Macedonian Medical Chamber. All subjects signed informed consent forms prior to examination.

Sample preparation

Venous blood for the analysis was obtained after a 12 hour overnight fast and collected into K\(_3\)EDTA containing tubes. After centrifugation at 3000 rpm for 10 minutes, plasma samples were stored at +4°C within 48 hours. One portion of each sample for LDL subclass separation was stored at -80°C until analysis within 2 months.

Lipid and apoprotein measurements

All lipid measurements were performed in fresh plasma samples, within 48 hours, kept at +4°C. Plasma total cholesterol and triglyceride concentrations were examined using enzymatic methods (Randox, Crumlin, UK). Determination of plasma HDL cholesterol concentrations with dextran sulfate-magnesium precipitation was followed by enzymatic determination of cholesterol. The Friedewald formula was used to calculate LDL cholesterol concentrations (27). ApoA-1, ApoB and Lp(a) concentrations were measured by the immunonephelometry method (DADE Behring, Marburg, Germany).

Non-denaturing polyacrylamide gradient gel electrophoresis

Non-denaturing polyacrylamide 3–31 % gradient gel electrophoresis (PAGE) was performed to separate LDL subclasses and estimates their size. Since sources of specialized Pharmacia GE-2/4 electrophoresis chambers and commercial gels have become uncertain, we used an alternative, Mini-Protein II Electrophoresis Apparatus (BioRad 165-2941, Hercules, CA, USA). Therefore, a new casting protocol was developed and glass cassettes fitting the BioRad electrophoresis chamber were made in our laboratory. The gradient gel characteristics and all details of the method have been presented in our previous publication (28). This new gel format allowed LDL and HDL subclasses separation on the same gel and thus duplication of work was avoided.

Plasma samples and human standard were prestained for 18 hours with Sudan Black B for analyses of cholesterol-stained lipoproteins. Ten samples were loaded to each gel. Human plasma standard, high molecular weight protein standard (HMW; 17-0445-02, Pharmacia Biotech, Uppsala, Sweden) and carboxilated polystyrene microspheres (beads; Duke Scientific, Palo Alto, CA) were loaded to calibrate for particle size. Beads were prestained with Sudan Black B, six hours before loading, and were loaded in the same line as HMW standard, 2 hours after beginning of electrophoresis to avoid mixing. HMW protein standard was stained after separation with Coomassie brilliant blue G-250. The gels were sealed in plastic bags and could be stored for several years with no loss of stain.

Lipoprotein profiles were analyzed using a laser densitometer at 632 nm with Image Master Software (version 1.0; 1993; Pharmacia). LDL peak particle sizes were calculated from the calibration curve based on the inverse relationship between the log of the known sizes of the standards on the y-axis and their migration distances from the start of the gel (Rf) on the x-axis. LDL peak particle sizes in the plasma sample absorbance profiles were calculated using Gels Scan software (56-1131-38, Pharmacia). LDL subclasses were classified as phenotype A (diameter <25.5 nm) and phenotype B (diameter >25.5 nm).

Measurement of intima-media thickness

The ultrasonographic scanning of the common carotid arteries (CCA) was performed using high resolution B-mode ultrasonography (SSA-770A; Toshiba, Tokyo, Japan) with a linear array probe (7.5 MHz), according to a standardized protocol. The IMT was measured manually by using a special vernier caliper after taking a picture. The far walls of right and left common carotid artery were scanned. IMT was measured by measuring the linear distance, perpendicular to the luminal axis, between 2 points defined by the ultrasonic interface, 1 cm distal to the carotid bifurcation. CCA IMT was calculated as the mean of the right and left CCA.

Statistical analysis

The data are presented as mean ± standard deviation (SD). Comparison of mean LDL particle sizes, age, plasma lipid and apoprotein concentrations between groups was performed with the two-sample unpaired Student’s t-test. The differences in LDL subclass distribution between two groups were evaluated with \( \chi^2 \)-test. Pearson correlation and multiple regression analysis were performed to investigate association between IMT and other clinical and laboratory parameters. The value of \( p<0.05 \) was considered.
Table 1. Clinical and laboratory variables of CAD patients (study group I and study group II).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CAD (I)</th>
<th>CAD (II)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>27.5±3.6</td>
<td>27.9±3.5</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.58±1.35</td>
<td>4.76±1.37</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.02±0.02</td>
<td>1.03±0.02</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>2.89±1.34</td>
<td>3.03±1.28</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.50±0.01</td>
<td>1.6±0.3</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>ApoB (g/L)</td>
<td>1.05±0.60</td>
<td>1.15±0.50</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>ApoA-1 (g/L)</td>
<td>1.31±0.05</td>
<td>1.32±0.25</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Lp(a) (g/L)</td>
<td>0.35±0.02</td>
<td>0.33±0.19</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>LDL diameter. size (nm)</td>
<td>25.71±0.57</td>
<td>25.02±0.86</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>HDL diameter. size (nm)</td>
<td>9.49±0.82</td>
<td>9.34±0.74</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>IMT (mm)</td>
<td>0.83±0.02</td>
<td>0.91±0.026</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>80.9±12</td>
<td>82±11</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>145±17</td>
<td>140±16</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

Data are presented as mean±S.D.

Table 2. Distribution of dominant LDL subclasses in patients with CAD (%).

<table>
<thead>
<tr>
<th>Predominant LDL subclass</th>
<th>Phenotype (%) I</th>
<th>Phenotype (%) II</th>
<th>Phenotype (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL 1</td>
<td>A 2.2</td>
<td>39.8</td>
<td>0 74</td>
</tr>
<tr>
<td>LDL 2</td>
<td>37.6</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>LDL 3</td>
<td>59</td>
<td>66</td>
<td></td>
</tr>
<tr>
<td>LDL 4</td>
<td>B 2.2</td>
<td>61.2</td>
<td>10 76</td>
</tr>
</tbody>
</table>

Significant difference for all analyses in the study. All statistical analyses were done using STATWIN software (version 5.0 A, Statsoft Inc. 1984-95; Tulsa, OK 74104, USA).

Results

Clinical and laboratory variables of participants in study group I and study group II are shown in Table 1.

There were no statistical differences in plasma HDL cholesterol, ApoA, ApoB, Lp(a), BMI, systolic and diastolic blood pressure between the groups.

Patients from study group II had significantly higher mean total cholesterol (p<0.005), LDL cholesterol (p<0.001) and triglycerides (p<0.005) compared to study group I.

Although LDL particle size was not statistically different between two study groups, LDL size was slightly decreased (25.71±0.57 nm vs 25.02±0.86; N.S.) in CAD patients after 12 months period. The mean IMT in all participants was 0.89±0.13 mm and was significantly increased 12 months after the first measurement (0.91±0.026 mm; p<0.001).

Table 2 displays the LDL subclasses distribution in two study groups. In most patients from study group I (61.2 %), atherogenic phenotype B was observed due to the presence of smaller LDL subclasses as dominant subclass (LDL3 and LDL4). In 39.8 % of the patients LDL1 and LDL2 subclasses were dominant and they belonged to phenotype A. In study group II, 76 % of the patients belonged to the phenotype B, while 24 % of the patients had phenotype A.

LDL3 was the dominant subclass in both study groups, but there was statistically significant difference in the distribution of dominant LDL subclasses with shift towards smaller subclasses in study group II (χ²=4.79; p<0.01).

Univariate linear regression analysis was used to determine the association of clinical and laboratory variables to CCA IMT (Tab. 3).

Correlation between the IMT and respective variables in patients from study group I (n=105) and study group II (n=95).

Of the laboratory variables, LDL size of the dominant subclass showed the strongest negative correlation with CCA IMT in both study groups. In contrast, other lipid and apoprotein plasma concentrations were not significantly associated with CCA IMT in both study groups, although plasma lipid concentrations were increased in study group II. Major LDL size was strongly inversely related to the IMT of the CCA, indicating that a predominance of small LDL particles is associated with an increased IMT.

Of the clinical parameters, diastolic blood pressure (r=0.5, p<0.05; r=0.48, p<0.05, for both study group respectively); and systolic blood pressure (r=0.49, p<0.05; r=0.51, p<0.05, for both study groups respectively) were also significantly related to the CCA IMT. Age was also significantly correlated with IMT in both study groups (r=0.26, p<0.05; r=0.25, p<0.05 for both study groups respectively).

Multiple regression analysis was performed to identify independent determinants of CCA IMT. Variables that were significant in univariate analysis were used as independent variables in multiple regression analysis (Tab. 4).

Age, systolic, diastolic blood pressure and LDL size accounted for 29 % of the observed variability in IMT. Other variables were not independent factors for IMT. In both study groups, multiple regression analysis show that strongest independent predictor of the IMT variation was diastolic blood pressure (0.322, p=0.016; 0.342,
p=0.018, respectively), followed by LDL size (0.260, p=0.04; 0.269, p=0.039, respectively) and age (0.192, p=0.037, 0.206, p=0.035, respectively).

Discussion

A number of studies have examined the relationship between LDL heterogeneity and CAD. In the first population-based case-control study (29) a threefold increased risk of myocardial infarction was found in subjects with a predominance of small dense LDL particles (phenotype B). The cross-sectional studies consistently suggest an association between small dense LDL particles and CAD. A plasma concentration of small dense LDL, (above 100 mg/dL) was associated with a 4.5-fold increased risk of CAD (30).

We used high resolution polyacrylamide gel electrophoresis to measure LDL size of the dominant subclass in CAD patients. LDL3 was dominant subclass in both study groups, but there was statistically significant difference in the distribution of dominant LDL subclasses with shift towards smaller subclasses in CAD patients after 12 months period ($\chi^2$=4.79; p=0.01). Although LDL particle size was not statistically different between two study groups, mean LDL size was slightly decreased in CAD patients after 12 months period.

Our results were in agreement with other case-control studies reporting an increased prevalence of small LDL particles (phenotype B) in patients with coronary artery disease (26, 29, 30, 31), and confirmed the association of small LDL particles and coronary artery disease.

An increased IMT is considered a reliable marker of early atherosclerosis (25). Increased carotid IMT and its progression are associated with cardiovascular risk factors (32). Therefore, we measured IMT using high resolution B-mode ultrasonography to estimate atherosclerosis and its progression in CAD patients at their first visit and after 12 months. The mean IMT in all participants was 0.89±0.13 mm and was significantly increased 12 months after the first measurement (0.91±0.026 mm; p<0.001).

In our study, we also demonstrated that LDL size is strongly associated with IMT of the carotid arteries and multivariate analysis revealed that LDL size was the second strongest predictor of IMT, after diastolic blood pressure. The association is shown to be independent of plasma LDL concentration and together with LDL particle size distribution with a high-resolution polyacrylamide gel electrophoresis may be a valuable approach to estimate individual cardiovascular risk and predictor of atherosclerosis progression in patients with established atherosclerosis as well as in asymptomatic individuals. Such follow-up may facilitate the directed development of preventive strategies.

Several pathophysiological mechanisms for the increased atherogenicity of small LDL have been suggested. Compared with larger and more buoyant LDL particles small LDL more readily infiltrate the arterial wall, bind more tightly to arterial wall proteoglycans and are more susceptible to oxidative modification (35, 36, 37).

Furthermore, small dense LDL particles have a prolonged plasma residence time most probably due to a decreased affinity for the LDL receptor and in effect an extended opportunity to exert atherogenic effect (36).

Although the metabolic origin of small dense LDL is not fully understood, the distribution of LDL particle size in human plasma is believed to be the result of the coordinated actions of hepatic and endothelial lipases as well as mediators of neutral lipid exchange (38, 39).

In conclusion, our results suggest that LDL particle size is significantly associated with carotid IMT in CAD patients independent of traditional lipid and established risk factors. Small dense LDL particles play an important role in causing vascular change, leading to atherosclerosis. Our results indicate that the evaluation of LDL particle size distribution with a high-resolution polyacrylamide gel electrophoresis may be a valuable approach to estimate individual cardiovascular risk and predictor of atherosclerosis progression in patients with established atherosclerosis as well as in asymptomatic individuals. Such follow-up may facilitate the directed development of preventive strategies.

References


