Association between oxidized low-density lipoprotein cholesterol concentration and atherosclerosis

Asocierea dintre concentrația de lipoproteine cu densitate joasă oxidate şi aterosclerază

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Abstract

Oxidized LDL (ox-LDL) is thought to play a key role in the inflammatory response in the arterial vessel wall. This study aimed to investigate the association between oxidized low density lipoprotein cholesterol and carotid intima-media thickness (IMT), a surrogate measure of atherosclerosis. Four groups of subjects were included in the study: a control group that included 12 normocholesterolemic healthy subjects (68% males, 32% females), 32 subjects with clinical signs of coronary artery disease (71% males, 29% females), 12 patients with arterial hypertension (60% males, 40% females), and 12 dyslipidemic patients (68% males, 32% females). Lipid profiles of the patients were measured by enzymatic methods. Ox-LDL was measured by a commercially available sandwich ELISA (Mercodia AB, Uppsala, Sweden) and carotid IMT by high-resolution B-mode ultrasound. Serum ox-LDL levels were higher in coronary artery disease patients (92.8 ± 8.12 U/L) and dyslipidemic patients (70 ± 16.24 U/L) compared with hypertensive (55.8 ± 10.84 U/L) and control subjects (53.7 ± 7.11 U/L). A positive, moderate correlation between ox-LDL and carotid IMT was found only in coronary artery disease group (R^2 = 0.58, $p<0.001$). We did not found significant correlation between ox-LDL and carotid IMT hypertensive, dyslipidemic and control groups. These results indicate that ox-LDL can be considered a marker of carotid atherosclerosis, and suggest that measurement of ox-LDL-C gives useful information in the risk assessment for atherosclerotic disease.

Key words: *oxidized low-density lipoprotein, carotid intima-media thickness, atherosclerosis.*

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Rezumat

LDL (lipoproteine cu densitate joasa) oxidate par a avea un rol cheie în răspunsul inflamator din peretele arterial. Scopul acestui studiu a fost de a investiga asocierea dintre LDL oxidate şi grosimea intima-media carotidiană (IMT) considerata un marker surogat al aterosclerozei. Au fost incluşi în studiu 4 grupuri: un grup control alcătuit din 12 subiecţi sănătoşi (68% bărbaţi, 32% femei), 32 subiecţi cu semne clinice de boala arterială coronariană (71% bărbaţi, 29% femei), 12 pacienţi cu hipertensiune (60% bărbaţi, 40% femei) şi 12 subiecţi cu dislipidemie (68% bărbaţi, 32% femei). Profilul lipidic al pacienţilor a fost măsurat prin metode enzimatice. LDL oxidate au fost determinate prin metoda sandwich ELISA (Mercodia AB, Uppsala, Sweden) iar grosimea intima-media carotidiană (IMT) prin ultrasonografie B-mode de înaltă rezoluţie. Nivelul seric de LDL oxidate a fost mai mare în grupul de pacienţi cu boală arterială coronariană (92.8 ± 8.12 U/L) şi în grupul de pacienţi dislipidemici (70 ± 16.24 U/L) comparativ cu subiecţii cu hipertensiune (55.8 ± 10.84 U/L) şi grupul de control (53.7 ± 7.11 U/L). Doar la grupul de pacienţi cu boală coronariană arterială am găsit o corelaţie moderată, pozitivă între LDL oxidate şi grosimea intima-media carotidiană (R^2=0.58, p<0.001). Între LDL oxidate şi grosimea intima-media carotidiană la subiecţii cu hipertensiune, dislipidemie şi la grupul de control nu am observat corelaţii semnificative. Aceste rezultate indică faptul că, nivelul seric de LDL oxidate, poate fi considerat un marker al aterosclerozei şi sugerează că, măsurarea acestora ne oferă informaţii utile în stratificarea riscului de boală aterosclerotică.

Cuvinte cheie: *LDL oxidate, grosime intima-media carotidiană, ateroscleroză*

Introduction

Hypercholesterolemia is one of the most important risk factors for atherosclerosis and coronary heart disease (1). Plasma total cholesterol distributes among three major lipoprotein classes including low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), and high-density lipoprotein (HDL) (1). An increase in plasma LDL levels leads to an increase in the adherence of circulating monocytes to arterial endothelial cells and at the same time to an increased rate of entry of LDL into the intima, resulting in a higher steady state concentration of LDL in the intima (2). Interestingly, high plasma and plaque levels of ox-LDL are associated with the vulnerability of the plaques (3). It appears likely that LDL is oxidized in microdomains in the arterial wall, sequestered by proteoglycans and other extracellular matrix constituents, where it is protected from plasma antioxidants (4). Many cell types are capable of oxidizing LDL, including monocytes, macrophages, neutrophils, endothelial cells, smooth muscle cells and fibroblasts (5). Ox-LDL has many characteristics that potentially promote atherogenesis, in addition to the ability to be taken up rapidly by macrophages to form foam cells (5). It is a chemoattractant for circulating monocytes, both directly and also via stimulation of the release of monocyte chemoattractant protein-1 from endothelial cells. Plasma ox-LDL has also been associated with subclinical atherosclerosis in clinically healthy population (6).

Carotid artery intima-media thickness (IMT), measured noninvasively by high-resolution B-mode ultrasonography, has been associated with the risk of coronary artery disease, stroke, and myocardial infarction, and it predicts the progression of coronary artery disease (7).

The present study was conducted to investigate the determinants of plasma ox-LDL and the association between ox-LDL and carotid IMT.

Material and methods

In a 24 months period, sixty-eight subjects (66% males and 34% females) from the IVth Medical Clinic of the University of Medi-

Table 1. Baseline characteristics, lipid levels, ox-LDL and IMT in the study groups (mean ± SD)

Characteristics	Control group	Coronary artery disease group	Hypertensive group	Dyslipidemic group
Patients (n)	12	32	12	12
Age (y)	56 ± 4.25	58 ± 3.15	55 ± 4.75	53 ± 5.68
Male sex (%)	68	71	60	68
Systolic BP (mmHg)	114 ± 6.08	149 ± 15.96	147 ± 10.10	120 ± 11.17
Diastolic BP (mmHg)	68 ± 5.84	90 ± 10.23	88 ± 7.78	72 ± 8.88
TC (mg/dL)	180 ±11.28	253 ± 19.67	200 ± 12.79	244 ± 21.53
LDL cholesterol (mg/dL)	115 ± 5.82	177 ± 13.62	122 ± 22.81	132 ± 20.94
HDL cholesterol (mg/dL)	50 ± 6.40	27 ± 2.05	49 ± 10.74	33 ± 8.90
Triglycerides (mg/dL)	98 ± 27.09	242 ± 69.35	176 ± 74.13	295 ± 92.79
ox-LDL (U/L)	53.7 ± 7.11	92.8 ± 8.12	56 ± 10.84	70 ± 16.24
IMT (mm)	0.79 ± 0.24	1.60 ±0.18	0.89 ± 0.26	0.94 ± 0.25

cine and Pharmacy Victor Babes Timisoara were included in the study. The subjects included 32 persons with coronary artery disease, 12 with hypertension, 12 with dyslipidemia, and 12 normocholesterolemic healthy subjects, aged between 50-60 years old and sex ratio 45 men (66%) and 23 women (34%). The characteristics of the subjects are summarized in *Table 1*.

Blood samples for serum cholesterol, serum triglycerides, and lipoprotein fractions were drawn after a fasting period of 10 to 12 hours. Cholesterol and triglyceride levels were determined by fully enzymatic techniques (8, 9). LDL cholesterol was calculated as described by Friedewald et al (10).

Hypertension was diagnosed if the subject was on anti-hypertensive medication or blood pressure was 140/90mmHg or higher (11). Dyslipidemia was diagnosed according to the criteria of the Japan Atherosclerosis Society, if a subject had one or more of the following: LDL-C > 140 mg/dL, TG > 150 mg/dL and HDL-C < 40 mg/dL (12).

Ox-LDL was measured by a commercially available sandwich ELISA (Mercodia AB, Uppsala, Sweden) (normal values 40 – 75 U/L)

Carotid-IMT was measured by high-resolution B-mode ultrasonography with an ultrasonographic apparatus with 7-MHz in-line sectascanner (Model ProSound SSD-4000, Aloka Co., Ltd., Tokyo, Japan).We analyzed the maximum thickness of intima-media complex as carotid-IMT as we previously reported (normal values < 1,3 mm).

Continuous variables are expressed as means ± SD. Means were compared using analysis of variance or the Student t-test. Pearson's correlation was used to test bivariate correlations and results were verified using the non-parametric Spearmans's rank correlation test. Multivariate linear regression analysis was used to test the relationship between IMT and oxidized LDL, in models including classical risk factors. The relationship between carotid IMT and oxidized LDL was tested for all four groups, defined by traditional risk factors (Pearsons's and Spearman's rank bivariate correlation tests were performed). Statistical significance was defined as two–sided $p < 0.05$. All statistical analyses were performed using Excel Microsoft Office 2003.

The procedures followed were in accordance with the ethical standards of the Hospital Ethics Committee and with the Helsinki Declaration of 1975, as revised in 2000.

Results

The most elevated concentration of oxidized lipoproteins was found in coronary artery disease group (p<0.01), comparative with control, hypertensive and dyslipidemic groups.

A significant positive correlation was found between ox-LDL and LDL in coronary artery disease group (R^2 = 0.74, p < 0.001) (*Figure 1*) as well as in hypertensive group (R^2= 0.73, p < 0.001) (*Figure 2*), dyslipidemic group (R^2 = 0.79, p < 0.001) (*Figure 3*), and control group (R^2=0.79, p < 0.001) (*Figure 4*).

A positive moderate correlation between ox-LDL and carotid IMT was found only in coronary artery disease group (R^2 = 0.58, p<0.001) (*Figure 5*). We did not find significant correlation between ox-LDL and carotid IMT hypertensive R^2 = 0.03, p<0.53), dyslipidemic (R^2 =0.04, p=0.50), and in control groups (R^2 = 0.09, p<0.32).

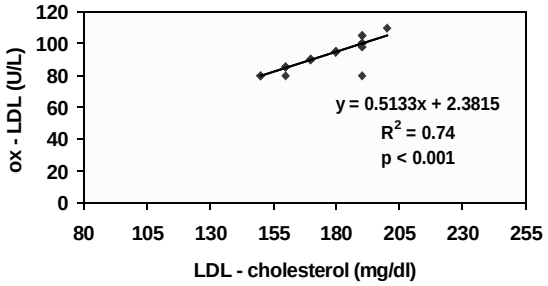

Figure 1. Correlation between ox-LDL and LDL-cholesterol in coronary artery disease group

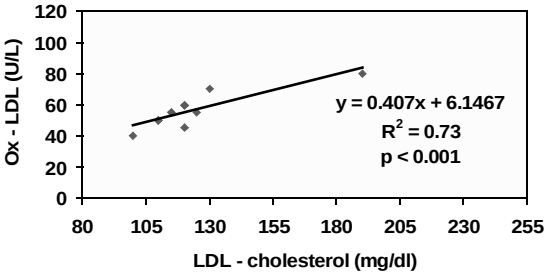

Figure 2. Correlation between ox-LDL and LDL-cholesterol in hypertensive group

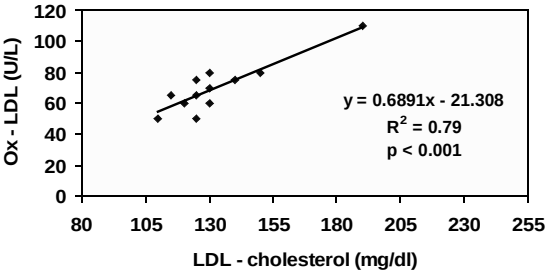

Figure 3. Correlation between ox-LDL and LDL-cholesterol in dyslipidemic group

Discussions

We compared the associations of carotid IMT with ox-LDL-C, LDL-C and other lipid parameters in 68 consecutive subjects having various risk factors for cardiovascular disease. In univariate analysis, carotid IMT was most closely associated with ox-LDL among the lipid variables tested in coronary artery disease patients. These results suggest that ox-LDL is a

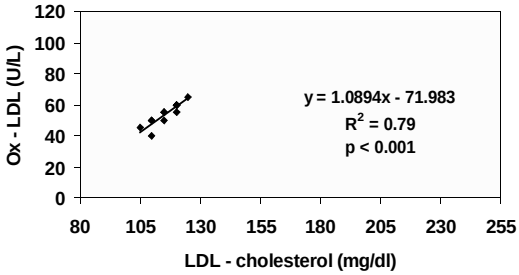

Figure 4. Correlation between ox-LDL and LDL-cholesterol in control group

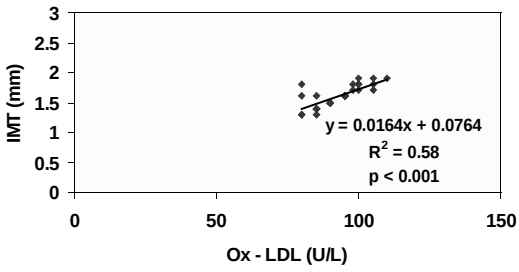

Figure 5.Correlation between ox-LDL and IMT in coronary artery disease group

quantitative risk marker of atherosclerosis that is more closely associated with carotid-IMT than standard lipid parameters. The present study confirmed the relationship of hypercholesterolemia with ox-LDL (6) in coronary artery disease patients and hypertensive patients.

The characteristics of ox-LDL isolated from plasma of cardiovascular disease patients are comparable to those of Ox-LDL isolated from atherosclerotic lesions (13). In the present study, ox-LDL was measured by a specific enzyme-linked immunosorbent assay method. (Mercodia AB, Uppsala, Sweden). The potential origin of circulating ox-LDL may be a direct release of modified LDL from ruptured or permeable plaques, or ischemic injury (3). The association between ox-LDL and mean IMT in coronary artery disease patients are in concordance with previous studies in which ox-LDL was associated with the extent of cardiovascular disease (14). Several recent publications have tested the correlation between the IMT of carotid arteries and LDL oxidation measured by concentrations of circulating ox-LDL or autoantibodies against ox-LDL. A high titer of autoantibodies against ox-LDL has been found to be an independent predictor of the progression of carotid atherosclerosis (15). However, in other studies an inverse correlation has been found between carotid arterial IMT and autoantibodies against ox-LDL (16). Discrepancy among previous reports concerning the association between autoantibodies against ox-LDL and carotid atherosclerosis may result from the enormous heterogeneity of ox-LDL, which is a complicated particle with many different modifications in the phospholipid and apolipoprotein B components of LDL (17).

Proinflammatory oxidized low-density lipoprotein may be a unifying link between lipid accumulation and inflammation in the vessel wall. If large studies show that such ox-LDL measurements do have predictive value, then their measurement along with the lipid profile and markers of inflammation may improve our ability to provide a more accurate atherosclerotic risk analysis, particularly of acute clinical events. Our results suggest that the increase in circulating ox-LDL might be partly due to a backdiffusion of ox-LDL from an atherosclerotic arterial wall into the blood in the early phase of atherosclerosis.

Conclusion

In conclusion, the present study provides the evidence that ox-LDL is a quantitative risk marker of carotid atherosclerosis in the mixed population. We need prospective cohort studies or randomized controlled trials to prove whether ox-LDL is a better predictor of the development of atherosclerotic disease than other lipid measurements.

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