ORIGINAL REPORT

Serum ox-LDL Level is Reduced with the Extent of Stenosis in Coronary Arteries

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Abstract- Oxidized LDL (ox-LDL) lipoproteins are proposed as important modified particles triggering pro-inflammatory events through receptor-mediated pathways. We evaluated the circulating ox-LDL level on the concept that the chronic immune events may affect ox-LDL clearance as the vessel stenosis develops in coronary arteries. One hundred sixty five subjects underwent coronary angiography and then, subdivided into four subgroups controls (n=85); SVD, 2VD and 3VD (n=80). The serum ox-LDL level and other biochemical parameters were measured using ELISA method and routine laboratory techniques, respectively. The serum ox-LDL level in the control group (4.81±1.41 mU/mg) was significantly higher than patients (4.28±1.73 mU/mg, $P<0.05$). The ox-LDL/LDL ratio was conversely reduced with the extent of stenosis as compared with the controls ($P<0.05$). Furthermore, no difference was observed in the ox-LDL/LDL ratio between the 2VD and 3VD patients. We suggested the atherosclerosis process increases the total clearing capacities of the circulating ox-LDL particles.

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Keywords: CAD; Ox-LDL/LDL; Stenosis

Introduction

Based on the concepts suggested in the development of atherosclerosis process, “response to injury” (1) and “oxidative modification” (2) hypotheses may describe the oxidized low density lipoprotein (ox-LDL) and remnant lipoprotein (RLP) roles in the progression of pro-inflammatory events through the so-called “scavenger receptor” pathways (3-5).

The oxidative modification hypothesis is focused on factors involved in LDL oxidation, cellular uptake, and secretion of cellular chemoattractants in subendothelial space of vessels (6). The oxidized LDL lipids such as malondialdehyde and 4-hydroxynonenal derivatives can chemically modify the apo B-100 lysine residues (7). Although the LDL particles may be oxidized minimally (mm-LDL) or fully (ox-LDL), but up to 90%, could be cleared with the macrophage CD 36 (8) and class A scavenger (SR-A) receptors (9). These events trigger the release of inflammatory factors and develop endothelial dysfunction, macrophage migration, smooth muscle cell stimulation and finally, production of atherosclerotic plaques (10).

The fraction of oxidized LDL particles (less than 10%) releases into the circulation (6). Some reports have been suggested that the serum ox-LDL antibody level is a biomarker for the development of atherosclerosis. However, the IgG and IgM-LDL titers are showed to be equivocal (11,12).

Some studies also showed an association between the ox-LDL level and coronary artery disease (CAD) (13-15). The results were controversial, probably owing to differences in oxidized derivatives and techniques used for the measurement of modified LDL particles (16-18). In this study, we investigated the relationship between serum ox-LDL-4E6 level and the progression of atherosclerosis process in the coronary arteries.

Materials and Methods

Subjects

One hundred sixty five subjects (Controls and Patients) were recruited from who underwent coronary angiography between September 2010 and February 2011. The clinical data was obtained through cardiologist records. Participants had no renal failure, acute myocardial infarction within the last three months

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and diabetes. Furthermore, patients had at least 50% stenosis in one of major epicardial arteries (LAD, LC and RC) and were divided into three subgroups: single vessel disease (SVD), two vessel disease (2VD) and three vessel disease (3VD). Controls also had normal coronary arteries with stenosis less than 5%. University ethics committee was approved the study and, informed consent was obtained from participants.

Biochemical parameters

Whole blood samples were collected in EDTA-containing vacutainers and, were immediately stored at -80 °C. Lipid profile and other biochemical parameters were measured with routine laboratory methods. The LDL-cholesterol level was calculated by Friedewald formula.

Ox-LDL assay

Enzyme-linked immunoabsorbent (ELISA) methods for the serum oxidized-LDL measurement are based on specific monoclonal antibodies such as DLH3, 4E6, and E06 (19). In this study, the serum oxidized LDL level was measured using a sandwich technique (Mercodia, Uppsala Sweden) developed with capture antibody mAb-4E6 (20).

Statistical analysis

Data analysis was obtained using SPSS software (Ver. 16). The normal distribution was evaluated by Kolomogorov-Smirno test. The differences between groups were evaluated by the student's t test, chi-square test and analysis of variance (ANOVA), followed by post hoc testing with Tukey's test. P value less than 0.05 was considered to be significant.

Table 1. Characteristics of the study population.

Parameter	Control (n=85)	Case (n=80)	*P*-Value
Sex(male/female)	29/56	54/26	0.02
Age (years)	55.80±13.260	60.66±11.880	0.01
Body Mass Index (kg/m^2)	26.57±6.54	24.68±4.43	0.06
Smoking(yes/no)	14/60	21/59	0.134
LDL-Cholesterol (mg/dl)	88.31±34.63	107.76±41.91	0.002
HDL-Cholesterol (mg/dl)	39.89±12.64	36.48±15.88	0.14
Triglyceride (mg/dl)	159.12±71.43	179.29±91.57	0.13
Total Cholesterol (mg/dl)	132.80±69.65	146.84±75.51	0.23
Systolic Blood Pressure (mmHg)	128.85±27.69	134.16±23.52	0.2
Diastolic Blood Pressure (mmHg)	79.23±17.65	79.33±18.09	0.97
OX-LDL (mU/dl)	4.81±1.41	4.28±1.73	0.033

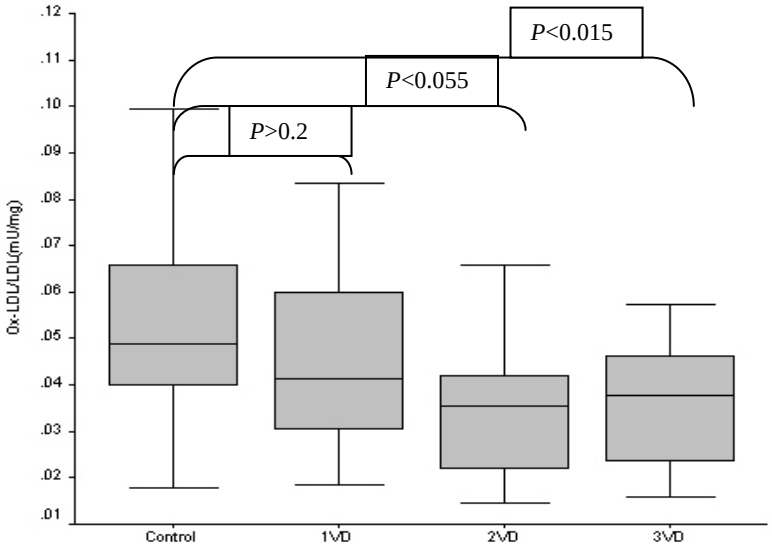

Figure 1. Ox-LDL/LDL ratio in the study population.

Table 2. Characteristics of the study patients.

Parameter	Single vessel disease (SVD) N=20	Two vessel disease (2VD) N=21	Three vessel disease (3VD) N=39
Left Anterior Descending (LAD) artery			
Proximal of LAD (n)	14	19	39
Medial of LAD (n)	1	17	31
Distal of LAD (n)	0	3	13
Left Coronary Artery (LC) artery			
Proximal of LC (n)	2	14	37
Medial of LC (n)	1	7	24
Distal of LC (n)	0	0	5
Right Coronary Artery (RC) artery			
Proximal of RC (n)	1	5	34
Medial of RC (n)	1	2	10
Distal of RC (n)	0	0	1
Age (years)	58.45±8.73	61.81±9.30	61.18±14.35
Body Mass Index (kg/m2)	25.45±3.12	23.15±6.26	25.16±3.59
LDL-Cholesterol (mg/dl)	99.20±48.59	98.57±42.43	117.10±36.72
HDL-Cholesterol (mg/dl)	37.40±18.21	35.62±17.08	36.46±14.27
Triglyceride (mg/dl)	156.15±76.26	197.66±108.68	180.13±88.77
Total Cholesterol (mg/dl)	142.30 ±87.28	151.52±58.99	146.84 ± 78.67
OX-LDL (mU/dl)	4.655±2.29	3.82±1.21	4.33±1.64
OX-LDL/HDL (mU/mg)	0. 11±0.05	0.12±0. 11	0.11±0.06

Results

The results showed that the serum LDL and ox-LDL levels are significantly different between patients and controls (P<0.05). Furthermore, ox-LDL level decreased among patient subgroups (Table 2) so that we found a significant difference between the ox-LDL/LDL ratio and the severity of stenosis in coronary arteries (Figure 1). Based on the characteristics of patients, the stenosis of left anterior descending (LAD) artery was prevalent. Furthermore, these results showed no differences in the BMI (P=0.19), LDL level (P=0.15), Age (P=0.62), ox-LDL level (P=0.31), ox-LDL/LDL ratio (P=0.24) and ox-LDL/HDL ratio (P=0.92) between the patient subgroups. We showed that with the development of stenosis in coronary arteries, the ox-LDL level is conversely related to LDL level (Figure 2).

Our results also showed no significant correlation (R^2 = 0.02, P>0.05) between age and ox-LDL/LDL ratio in the study population (Figure 3).

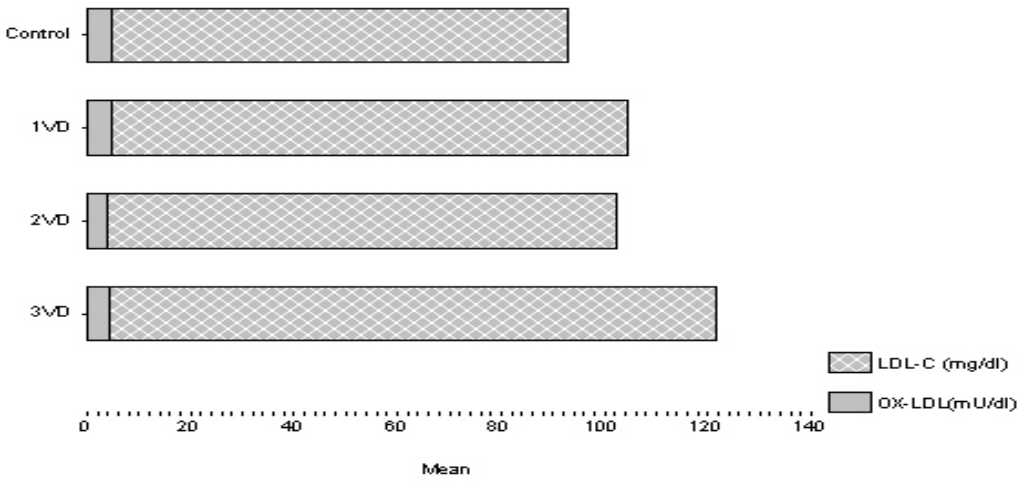

Figure 2. LDL and ox-LDL means in the study population.

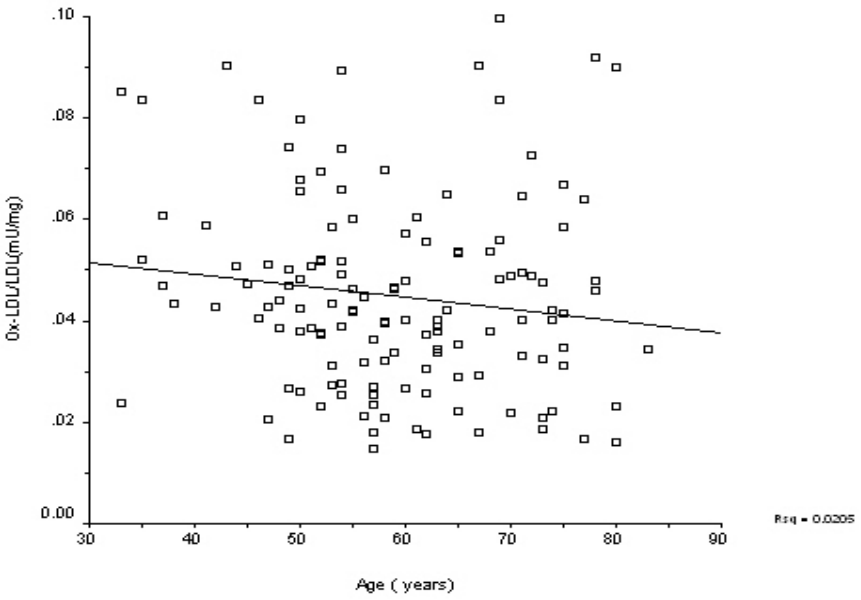

Figure 3. Correlation between the ox-LDL/LDL ratio and age (P>0.05).

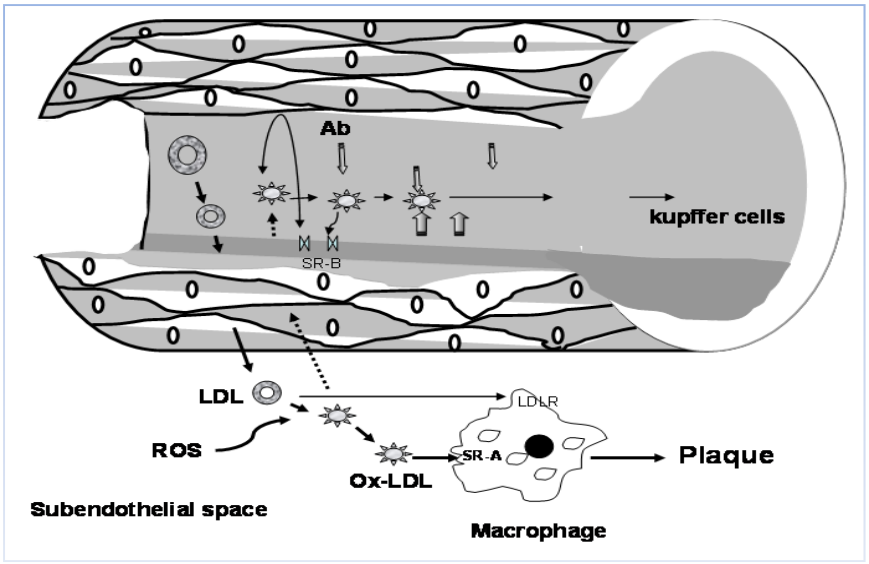

Figure 4. Ox-LDL trafficking hypothesis. LDL particles are modified by ROS (Reactive oxygen system) in subendothelial space. The fraction of ox-LDL particles are transferred through endothelium and, are subject to be cleared by SR-B receptor and Kupffer cells. Ab; Antibody: SR-A, SR-B and LDLR; scavenging ox-LDL receptors.

Discussion

The oxidative modification hypothesis is based on the concept that oxidized particles can develop the atherosclerosis process (2). Numerous studies have been supported the role of ox-LDL as a major factor involved in the initiation of atherosclerotic events (13-15). Some reports have also shown the association of unstable, ruptured plaques on the increase of the plasma ox-LDL level (21).

Since immune responses are continued during the atherosclerosis process (22) thus, one may think the immune complexes produced during the development of inflammatory events help to clear the circulating ox-LDL particles via hepatic kupffer cells (23,24). Furthermore, with the progression of inflammatory reactions and the extent of stenosis in coronary arteries, the capacity of ox-LDL scavenger receptors elevates due

to the increase of activated immune cells and also, the expression of other scavenger receptors (25). In addition, some studies have failed to inhibit the coronary stenosis by antioxidants (26). These findings, in agreement with our results, support the hypothesis that progression of stenosis in coronary arteries may be independent of the serum ox-LDL level (27-29).

Moreover, it is well known that the different monoclonal antibodies such as DLH3, 4E6 and E06 are used for the ox-LDL assay (20), thus it is obvious that the sensitivity and specificity of methods may be related to the different results.

Our patients were taken lipid lowering medications. Some studies have reported that the administration of statins diminishes the plasma LDL level resulting in the reduction of circulating ox-LDL particles (30-32). The medications didn't reduce the serum LDL level in patients as compared to controls resulting that the reduced ox-LDL is not related with drugs used by patients.

In conclusion, our results showed that the serum ox-LDL level is conversely related to the extent of stenosis in coronary arteries. We proposed that the increased immune complexes, proliferation of immune cells and the increased expression of scavenger receptors as described above, may facilitate the clearance of circulating ox-LDL particles (Figure 4). Hence, we thought it is not a suitable biomarker to determine the severity of damage in arteries.

Acknowledgements

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