ACTA MEDICA SALINIANA

ISSN 0350-364X

Acta Med Sal 2010; 39 (1): 26-30

ORIGINAL PAPER

ELEVATED ANTIBODIES TO OXIDIZED LOW DENSITY LIPOPROTEIN ARE POSITIVELY RELATED WITH A SEVERITY OF CORONARY ARTERY DISEASE

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Received: 03.11.2009
Accepted: 17.02.2010

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ABSTRACT

Background: The prognostic value of circulating antibodies to oxidized low-density lipoprotein (anti-oxLDL) in patients with coronary heart disease is not completely clear.
Aim: We aimed to investigate the association between levels of anti-oxLDL in three groups of patients with different grades of severity of coronary heart disease.
Patients and methods: The study included 101 patients classified into three groups: one (N=35) with acute myocardial infarction (AMI), a group (N=35) with angiographicallly proven coronary artery disease (APCAD), and a group without angiographicallly proven coronary artery disease (N=31) designated as a control group. Levels of IgG anti-oxLDL antibodies were meausured by enzyme-linked immunosorbent assay.
Results: Mean anti-oxLDL value was significantly higher in patients with AMI than in patients with APCAS (1342.1±581.5 mIU/ml vs. 553.0±183.3 mIU/ml, p<0.001), as well as compared with control group (1342.1±581.5 mIU/ml vs. 246.5±114.3, p<0.001). Similarly, significant difference in anti-oxLDL levels was found between the patients with APCAS and control group (p<0.001).
Conclusions: The present study showed that elevated levels of anti-oxLDL are positively related with a severity of coronary artery disease. Hence, elevated levels of anti-oxLDL may identify patients with unstable coronary heart disease. Oxidized LDL in circulating plasma could serve as a marker of cardiovascular events.

Keywords: *antibodies to oxidized low density lipoprotein; coronary artery disease; myocardial infarction; coronarography*

INTRODUCTION

Although coronary disease is a leading cause of death in individuals with coronary risk factors, the majority of these individuals do not actually develop coronary symptoms before the onset of acute myocardial infarction (AMI) or sudden death. In fact, AMI or sudden death is frequently the first symptom of coronary disease, hence the importance of the screening of patients with unstable plaques at the onset of AMI or sudden cardiac death.[1] The role of inflammation in acute coronary syndrome is crucial and supported by findings of coronary plaque instability and clinical evidence of the circulating reactant levels in acute phase of inflammation.[2] Atherosclerosis of coronary arteries is a main cause of coronary heart disease. Oxidized low density lipoprotein (ox LDL) is considered as a key factor in the genesis of inflammatory processes in atherosclerotic lesion.[3] Detection of oxLDL in human plasma has opened new avenues in the study of relationships in

Table 1. *Average values of oxLDL (mU/ml) in groups*

OxLDL	N	Mean value	Standard deviation (SD)	Minimal value	Maximal value
AMI	35	1342.1	581.5	685.0	3000.0
APCAD	35	1553.0	183.3	220.0	1250.0
Controls	31	246.5	114.3	85.0	585.0

AMI- acute myocardial infarction, APCAD- angiographically proven coronary artery disease, ox LDL- oxidized low density lipoprotein

atherosclerotic process and prediction of the onset of coronary heart disease.[4]

Oxidized low density lipoprotein occurs after endothelial dysfunction and after penetration of LDL particles in the intima of blood vessel. Oxidized LDL is product of oxidation of LDL caused by free radicals, substances with unpaired electrons which tend to be highly reactive. Unlike ordinary LDL, oxidized LDL causes accumulation of cholesterol esters in macrophages.[5] Oxidized LDL implies some mechanisms which are important for the development of atherosclerosis, such as cytotoxicity, inhibition of motility of macrophages and monocyte hemotactic action.[6,7]

Research on animals and humans revealed that oxidized LDL is a better predictor of atherosclerosis and is a more powerful pro-atherosclerotic stimulator than "normal" LDL.[7] Elevated levels of oxidized LDL are associated with increased occurence of plaque rupture. [8] There is a close association between oxidized LDL and coronary artery disease, which suggests that the risk of plaque progression is more important antioxidant status than the level of LDL.[9] Elevated levels of oxidized LDL could play a role in the transition from a stable to a vulnerable, unstable plaque. It means that oxidized LDL may not only contribute to the development and progression of atherosclerosis, but can also directly improve plaque rupture.[8] However, the role of ox LDL as a marker of cardiovascular events is not completely clear.

The aim of this study was to investigate relation of ox LDL plasma levels with the severity of coronary artery disease and the possible role of ox LDL as a marker at the onset of coronary heart disease.

MATERIALS AND METHODS

We prospectively analyzed 101 patients of both gender, aging from 39 to 82 years. The study was conducted on a University Clinical Center Tuzla. We divided patients into three distinctive groups.

First group were patients with AMI (N = 35). The diagnosis of AMI was based on a history of prolonged ischemic chest pain, characteristic ECG changes (ST segment elevation in two or more close leads), and elevated troponin-I (over 0.15 ng/ml) and creatine kinase (>2 times above normal range within 24 hours after onset of pain). Second group were patients with APCAD with occlusion of coronary arteries more than 50% (N=35). Third group were patients without angiograpfically proven coronary artery disease in other words, patients with regular angiographycal findings (N=31). This group was designated as a control group. Second and third group of patients underwent coronary angiography which is currently considered as "gold standard" for estimation of coronary anatomy and represent the definitive diagnostic test for evaluation of anatomical location and severity of coronary artery disease. The second group (APCAD positive) consisted of patients presenting with various signs and symptoms of coronary syndrome and also with a positive stress test. Patients from third group underwent coronary angiography due to undefined chest pain, since we were unable to either prove or exclude coronary artery disease with other methods and diagnostic procedures. The main excluding criterion was treatment with statins.

Direct measurement of oxLDL in serum or plasma is extremely complicated. For this reason we measured antibodies to ox LDL (anti-oxLDL) in order to provide stability and accuracy of data. Anti-oxLDL can be considered a marker of LDL oxidation in the tissues or cells. In the group of patients with AMI, blood samples were taken within 24 hours of onset of chest pain and after AMI was diagnosed beyond reasonable doubt. Blood sample was taken immediately before coronary angiography in patients of second and third group. Blood samples from all of the three groups were collected, frozen at -80 ° C and analyzed within one month. As oxidative modification of LDL is a nonenzymatic posttranslational reaction, genetic techniques cannot be applied to detect oxLDL, but immunological

Table 2. *Comparison of oxLDL means values between groups (Tukey post-hoc test)*

(I) Group	(J) Group	Mean difference (I – J)	p-value	95% Confidence interval	
				Lower limit	Upper limit
AMI	APCAD	789.1	<0.001	-581.7	996.6
	Controls	1095.7	<0.001	881.7	1309.7
APCAD	AMI	-789.1	<0.001	-996.6	-581.7
	Controls	306.5	0.003	92.5	520.6
Controls	APCAD	-306.5	0.003	-520.6	-92.5
	AMI	-1095.7	<0.001	-1309.7	-881.7

AMI- acute myocardial infarction, APCAD- angiographically proven coronary artery disease, oxLDL- oxidized low-density lipoprotein

techniques turned out to be very useful. There are several methods for immunological measurement of ox-LDL. We measured anti oxLDL performing by enzyme-linked immunosorbent assay (ELISA) commercial kit (Biomedica BI-20032, OLAB IgG) manually, using HRP colorimeter. At the surface of this commercial kit there is an antigen-malondialdehyde modified human apolipoprotein B-100 (MDA-Apo B-100) which interact with anti-oxLDL antibodies in human serum. In order to check out this test mouse anti-human IgG-HRPO antibody (whose antigen is TMB) was used.

Statistical analysis

Statistical analysis was performed in SPSS 12.0 statistical software package (SPSS Inc, Chichago, IL, USA). All variables were presented with their corresponding measures of central tendency and dispersion, by using standard tests of descriptive statistics.

Qualitative variables were compared by using Chi-square test. Quantitative variables were tested for normal distribution by using Kolmogorov-Smirnov test. We used Student's t-test for comparison of 2 variables, while for comparison of 3 variables we used Analysis of Variance (ANOVA) test with Tukey's post-hoc analysis.

Significant correlation between variables was tested by using Pearson's parametric correlation. All tests were performed with 95-percent statistical significance (p<0,05).

RESULTS

The total sample included 64/101 (63.4%) males and 37/101 (36.6%) females. In the AMI group there were 24/35 (68.6%) males and 11/35 (31.4%) females. In the group of patients with APCAD were 25/35 (71.4%) males and 10/35 (28.6%) females, while in the control group were 15/31 (48.4%) males and 16/31 (51.6%) females. There was no significant difference in gender between three groups (X2=4.39; df=2; p=0.11).

The median age in the full sample was 60 years (interquartile range: 53-69 years) with a minimum of 39 and maximum of 82 years. There was no significant difference in the age between 3 groups (F = 0.56, df = 98, p = 0.58).

Values were normally distributed so we used parametric ANOVA test with a post-hoc Tukey test. We found significant difference in the values of oxLDL between groups (p <0.001).

The level of oxLDL in patients with AMI was significantly higher than in patients with APCAD (p<0,001) or in control subject (p<0,001). Ox LDL levels in patients with APCAD were significantly higher than those in control subjects (p<0,001). Details of this comparison is presented in Table 2.

The average value of ox LDL did not differ between males (785 ± 608) and females (640 ± 545) subjects (t = 1.19, df = 99, p = 0.24). We found a very low (r = 0.2) and borderline significant (p = 0.046) correlation between age and the value of ox LDL.

DISCUSION

Oxidative modification of LDL is extremely complex, because it involves the oxidation of all classes of lipids (sterols, fatty acids in phospholipids, cholesterol esters and triglycerides) and protein particles.[5] Oxidative modification of lipoproteins is widely accepted as a key event in the genesis of atherosclerosis. Previous studies have suggested that ox LDL may also play a role in triggering thrombosis by inducing platelet adhesion and by decreasing the fibrinolytic capacitates of endothelial cells.[13] Oxidized LDL decreases production on nitric oxide (NO) and accelerates atherosclerotic lesion formation in the coronary arteries and in the aorta.[8] Hence, ox LDL can be considered a marker of LDL oxidation in the tissues or cells; it means that ox LDL can be considered as a marker of cardiovascular events.[12.]

We determined the usefulness of circulating antibodies to oxidized LDL levels for identifying of patients with coronary heart disease. Our study showed a positive relation of increased levels of ox LDL with severity of coronary heart disease. We found significant difference of ox LDL levels ($p < 0.001$) between patients with AMI and control groups. Significant difference between ox LDL levels ($p < 0.001$) was found between subjects with APCAD and control group and between subjects with acute myocardial infarction and APCAD ($p < 0.001$). Hence, this finding demonstrates that ox LDL levels relate directly to the severity of coronary heart disease. It suggests that raised ox LDL levels may have a destabilizing effect on plaque composition, most likely by enhancing the inflammatory processes and surface thrombosis. Furthermore, elevated levels of oxidized LDL can be considered as indirect indication of coronary plaque instability in acute coronary syndrome. This observation suggests that oxidized LDL in circulating plasma could serve as a marker of cardiovascular events.

Similar results were reported by Ehara et al.[10] They examined the level of anti - ox LDL in patients with acute myocardial infarction, unstable angina pectoris, stable angina pectoris and in patients without coronary artery disease as a control group. Research conducted by Yamashita et al.[2] aimed to investigate whether there is a relation between the levels of oxidized LDL in patients with certain classes of unstable angina pectoris, according to Braunwalds classification. The level of oxidized LDL in patients with Braunwald class III unstable angina pectoris was significantly higher than in patients with class I or control subjects. This study showed that in each class of unstable angina pectoris according to Braunwalds classification, elevated level of oxidized LDL is closely correlated with the presence angiographically detect complex and morphology of thrombotic lesions. Our research demonstrated that there is no significant difference in the level of oxidized LDL by age of respondents in any of the control group.

Previous studies clearly indicate the extremely important role in oxidized LDL in the development and progression of coronary artery disease and unstable coronary plaque.[13] Prediction of the worst forms of coronary disease, such as acute myocardial infarction and unstable angina pectoris, is of great importance for clinical practice. Predict acute coronary event means, to a large extent, reduce its severity and consequences and this raises the need for determining a reliable marker of coronary plaque instability and coronary events. So far, in clinical practice several markers of acute coronary events (high sensitive C reactive protein-hsCRP, interleukin-6) are used, but their use is more academic than practical. Our study, as well as some others, suggests that measuring of anti-oxLDL can get reliable information on acute coronary events better than other markers used in common practice.

CONCLUSIONS

The present study showed that elevated levels of anti-oxidized low density lipoprotein levels are positively related with a severity of coronary artery disease. Hence, elevated levels of anti-oxLDL may identify patients with unstable coronary heart disease. Oxidized LDL in circulating plasma could serve as a marker of cardiovascular events.

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