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SERUM ANTI-OXLDL ANTIBODIES IN PATIENTS WITH ANGIOGRAPHICALLY CONFIRMED CORONARY ARTERY DISEASE

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Abstract

Cardiovascular disease (CVD) remains the leading cause of death worldwide. Oxidized low density lipoprotein (oxLDL) is believed to be central to the atherosclerotic cascade. Oxidative modification of LDL induces immunogenic epitopes in the LDL molecule, and the presence of antibodies against oxidized LDL (anti-oxLDL) has been demonstrated in human sera. Anti-oxLDL titer not only can predict a presence of atherosclerotic CAD but may also be a marker of plaque instability.

The primary aim of this study was measurement of serum anti ox-LDL concentrations in CAD patients confirmed with coronary angiography. The secondary aim of the study was to evaluate if there is an association between anti-ox-LDL concentration and conventional risk factors for CAD.

Ninety patients with coronary artery disease and 90 controls were included in the study. Patients were selected according to the positive result of coronary angiography.

Total cholesterol, triglycerides, HDL-c and LDL-c were determined on Roche C311 Cobas Analyzer and OxLDLD and anti-oxLDL were determined with sandwich ELISA technique.

We find significant difference between CAD patients and controls regarding OxLDL ($p< 0.001$). Serum anti OxLDL antibodies were correlated with LDL-c and we found significant correlation ($p<0.05$) in the CAD group. The regression model showed that the variables *Age, BMI, DBP, SBP, Total cholesterol, HDL-c LDL-c and TG* explained 30.39% of the variance from the variable *anti-ox-LDL*.

Our results show that serum levels of ox LDL are associated with presence of CAD and with the lipid parameters.

Keywords:cardiovascular disease, coronary artery disease, anti oxLDL, LDL.

Introduction

Cardiovascular disease (CVD) remains the leading cause of death worldwide [1]. Dyslipidemia, especially hypercholesterolemia, hypertension, obesity, insulin resistance, diabetes mellitus, a high level of C-reactive protein (CRP), stress, alcohol consumption, and smoking are well known atherosclerotic risk factors [2].

Approximately, 75 % of deaths of CVD could be prevented by changes in lifestyle habits [3].

Low density lipopoprotein (LDL) has to go through several structural changes to reach atherogenic characteristics [4].

Oxidative stress is an important activator of lipid oxidation [5]. Oxidized low density lipoprotein (oxLDL) is believed to be central to the atherosclerotic cascade. An oxidative stress is a result of imbalance between reactive oxygen production and the antioxidant defense system [6].

OxLDL can activate the expression of different adhesion molecules on the cell surface and trigger the activation of endothelial cells [7].

This process is followed by releasing of proinflammatory cytokines, synthesis of reactive oxygen species (ROS), and production of proteolytic enzymes. All of them contribute to plaque destabilization [8].

Post mortem studies have suggested that lesions with greater oxLDL deposition may be at increased risk of plaque rupture. Oxidative modification of LDL induces immunogenic epitopes in the LDL molecule, and the presence of antibodies (IgG) against oxidized LDL (anti-oxLDL) has been demonstrated in human sera. Anti-oxLDL titer not only can predict a presence of atherosclerotic CAD but may also be a marker of plaque instability.

Results of several studies demonstrated the increased titer of anti-oxLDL in patients with atherosclerotic coronary artery disease (CAD) as well as cerebral or peripheral artery disease [9-11]. Conversely, other studies reported that no positive relationship was observed between anti-oxLDL titers and the extent of atherosclerosis [12,13].

The primary aim of this study was measurement of serum anti ox-LDL concentrations in CAD patients confirmed with coronary angiography. The secondary aim of the study was to evaluate if there is an association between anti-ox-LDL concentration and conventional risk factors for CAD.

Material and methods

Subjects

Ninety patients with acute myocardial infarction (AMI) and ninety control subjects without a history of cardiovascular disease or cardiovascular risk factors were included in the study. Patients were selected according to the positive result of coronary angiography.

Informed consent was obtained from all study participants. The serum was separated, stored at -20°C prior determination of lipid parameters and anti oxLDL. Total cholesterol, triglycerides, HDL-cholesterol, and LDL-cholesterol were measured in patients as well as in control subjects by enzymatic methods on Roche C311 Cobas Analyzer. Anti-oxLDL antibodies were measured by ELISA using a commercially available kit provided by Immundiagnostik AG (Bensheim, Germany). Intra-assay variability is < 7%.

Statistical analysis

Statistical analysis was carried out using the statistical software SPSS (version 23.0; IBM, SPSS, USA).

Statistical significance was assumed if p values were below 0.05.

Results

Demographic and clinical characteristics

Demographic and clinical characteristics of study group are summarized in Table 1. CAD and control group were matched for gender and age. There was no significant difference in BMI. Mean systolic blood pressure was significantly higher in CAD group compared with the control group ($p<0.001$). No significant difference was observed in diastolic blood pressure. No significant difference in total cholesterol, triglycerides, HDL-c, and LDL-c was observed among the two groups (Table 1).

Table 1. Demographic and clinical characteristics of CAD and control subjects

	Control	**CAD**
Number of subjects	90	89
Gender (M/F)	45/45	40/50
Age	56.5±5.46	57.64±6.84
BMI	26.66±2.57	27.1±3.63
Systolic Blood Pressure	121.13±10.12	144.94±17.88*
Diastolic Blood Pressure	79.12 ±8.18	81.04±12.67
Total cholesterol	5.08±0.58	4.55±1.08
LDL-c (mmol/L)	3.62 ± 0.98	3.53±0.98
HDL (mmol/L)	1.36 ± 0.32	1.03±0.28
TG	1.4 ± 0.68	1.48±0.84

Values are presented as mean±SD. BMI:Body Mass Index; LDL-c Low density lipoprotein cholesterol, HDL-c-High density lipoprotein cholesterol, TG: triglycerides.
*p<0.001

Anti-ox-LDL concentration

There was significant difference (p<0.001) between CAD and control group regarding anti-ox-LDL concentrations (Table 2, Figure 1).

Table 2. anti- ox LDL values in CAD and control group

	CAD	Control
Median	6800	4870
Minimum	3800	3800
Maximum	9920	6800
Interquartile Range	2790	700
Mean ± Std.	6715.45 ± 1713.86	4971.85 ± 608.77

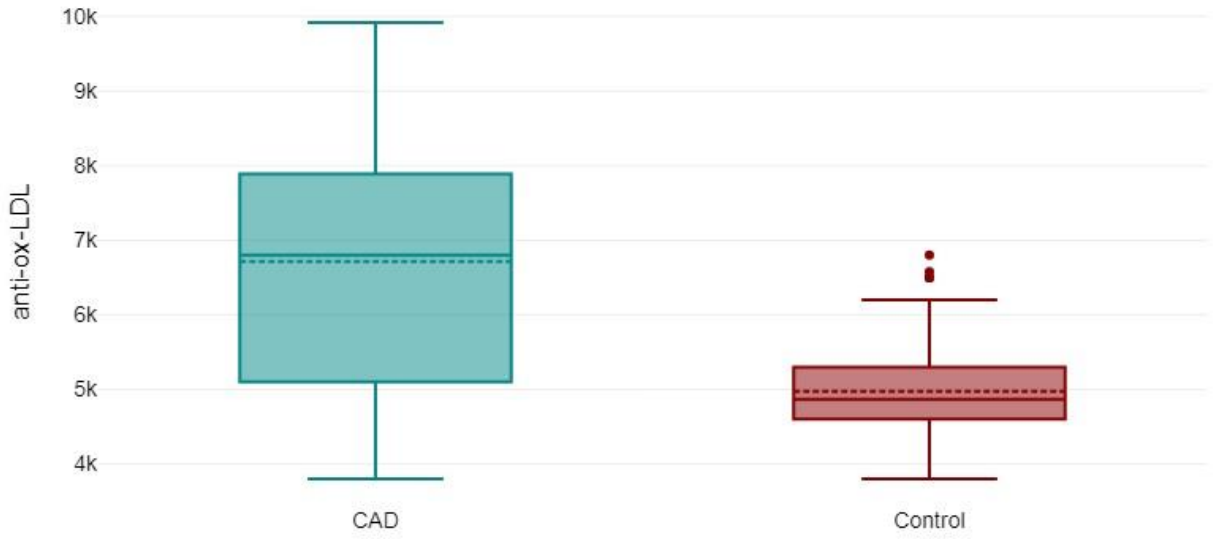

Figure 1. Boxplot of anti-ox-LDL concentration in CAD and control group

Correlation between anti-ox-LDL concentration and cardiovascular risk factors

In the control group, no significant correlation was found between serum anti-ox-LDL concentration and risk factors. In the CAD group, serum anti-ox-LDL concentration was significantly correlated with LDL-C (Table 2 and Figure 2).

Table 3. Correlation between anti-ox-LDL concentration and cardiovascular risk factors

	Control		**CAD**	
	R	**P**	**R**	**P**
Age*	-0.08	0.62	0.13	0.24
BMI*	0.27	0.09	-0.05	0.62
SBP*	0.06	0.80	-0.01	0.93
DBP*	0.17	0.22	0	0.98
Total-c (mmol/L)**	0.25	0.12	0.16	0.15
LDL-c (mmol/L)**	-0.02	0.89	**0.53**	**<0.001**
HDL (mmol/L)**	-0.1	0.53	0.05	0.65
TG (mmol/L)*	0.03	0.84	0.2	0.07

SBP: Systolic blood pressure; DBP: Diastolic blood pressure
*Pearson's correlation analysis
** Spearman's correlation analysis

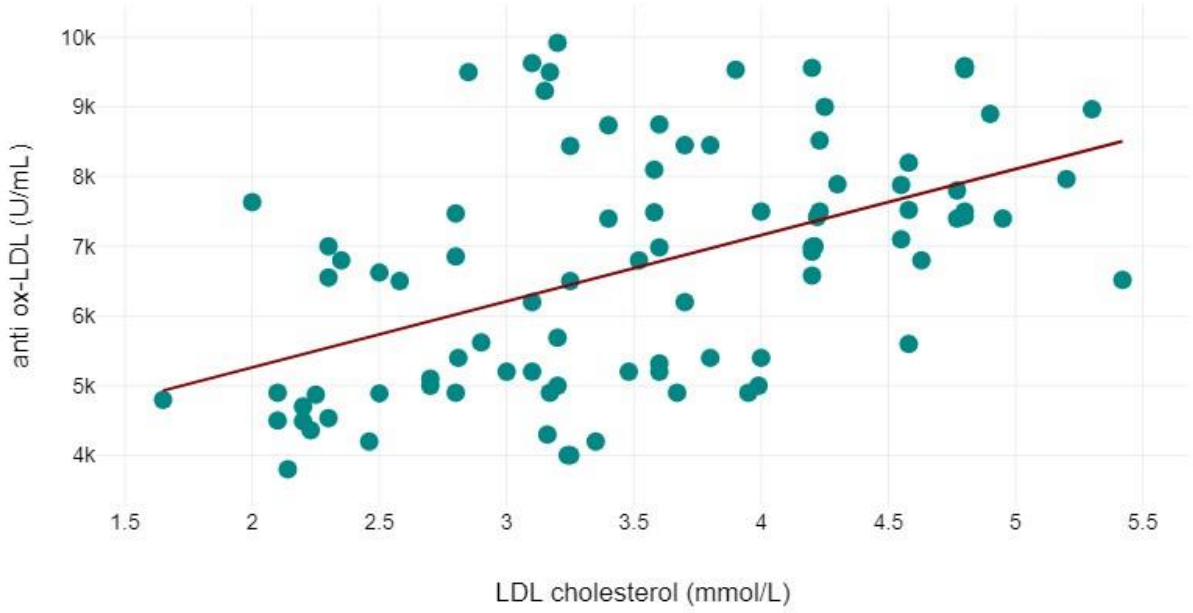

Figure 2. Scatter plot depicting significant correlation between serum anti-ox-LDL concentration and LDL-c in CAD group

Multiple linear regression analysis

A multiple linear regression analysis was performed to examine the influence of the variables *Age, BMI, DBP, SBP, Total cholesterol, HDL-c, LDL-c and TG* on the variable *anti-ox-LDL*. For LDL-c the p-value is <0.001 which means that we have evidence that LDL-c (mmol/L) impacts the dependent variable (anti ox-LDL).

The regression model showed that the variables *Age, BMI, DBP, SBP, Total cholesterol HDL-c LDL-c and TG* explained 30.39% of the variance from the variable *anti-ox-LDL*. An ANOVA was used to test whether this value was significantly different from zero. Using the present sample, it was found that the effect was significantly different from zero, F=4.37, p = <.001, R^2 = 0.3.

Table 4. Multiple linear regression analysis

	Standardized Coefficients		95% confidence interval	
Model	Beta	P	lower bound	upper bound
Age	0.12	.243	-20.14	78.32
BMI	-0.08	.386	-131.33	51.37
DBP	0.12	.405	-21.44	52.64
SBP	-0.1	.49	-35.73	17.26
Total cholesterol (mmol/L)	-0.05	.595	-409.59	236.3
HDL-c (mmol/L)	0.07	.469	-763.31	1642.75
LDL-c (mmol/L)	0.5	**<.001**	578.37	1323.88
TG (mmol/L)	0.15	.164	-124.52	722.52

Discussion

The primary goal of this study was to determine serum anti ox-LDL concentrations in CAD patients confirmed with coronary angiography. The secondary aim of the study was to evaluate if there is an association between anti-ox-LDL concentration and conventional risk factors for CAD. We find significant difference between CAD and control groups regarding serum anti ox-LDL concentrations ($p<0.001$).

The significant correlation was found between serum anti-ox-LDL IgG levels and concentration of LDL cholesterol in CAD group ($p<0.001$).

Literature data have been controversial. Erkkilä et al. [14] concluded that anti ox-LDL antibodies are associated with AMI in men but not in women because male patients with AMI had anti ox-LDL antibodies significantly higher compared to women. Inoue et al. [15] found higher levels of anti ox-LDL antibodies in patients with multivessel CAD.

In the study of Laczik et al. [16] serum ox-LDL IgG concentrations in 54 patients with acute coronary syndrome (ACS) compared to 41 matched healthy controls were higher. Tsimikas et al. [17] analyzed the association between ox-LDL IgG autoantibodies and cardiovascular events in 504 patients examined by coronary angiography. The results showed that anti-ox-LDL concentrations are positively associated with the presence of angiographically determined CAD.

Our study showed the same results. The regression model showed that the variables *Age, BMI, DBP, SBP, Total cholesterol, HDL-c, LDL-c and TG* explained 30.39% of the variance from the variable *anti-ox-LDL*.

Contrary to previous results, there are other studies with findings that there is no association between anti-ox-LDL levels and CAD: Rossi et al. [18] measured the levels of ox-LDL IgG antibodies in 529 patients undergoing coronary angiography for suspected CAD.

They found no significant association between anti-ox-LDL levels and CAD severity. Contrary to our results but consistent with Rossi et al, Virella et al. [12] found that anti-ox-LDL concentrations are neither associated with lipid levels in healthy individual nor with CAD severity. Finding of Che et al. [19] showed that anti-ox-LDL IgG antibodies in patients undergoing coronary angiography are lower in CHD patients compared to controls.

Our results showed that anti ox-LDL antibodies are elevated following an acute coronary event, for example, AMI. It can be a result of plaque rupture which leads to the release of ox-LDL in the circulation, thereby stimulating production of antibodies by immune system.

Conclusion

Measurement of ox-LDL antibodies should be conducted in patients with acute coronary syndrome because higher levels of ox-LDL and corresponding antibodies are found in vulnerable plaques in these patients.

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