Antibodies Against OxldI and Acute Coronary Syndrome

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Abstract

Background: The oxidation of low-density lipoprotein (oxLDL) induces the formation of immunogenic epitopes in molecules. The presence of autoantibodies against oxLDL has been demonstrated in the serum of patients with coronary artery disease (CAD). However, the role of these autoantibodies in the pathophysiology of acute coronary syndromes (ACS) and their clinical significance remain undefined.

Objective: To evaluate the association between antibodies against oxLDL and ACS.

Methods: Titers of IgG autoantibodies against oxLDL by copper (anti-oxLDL) and anti-D synthetic peptide derived from apolipoprotein B (antipeptD) were determined by Enzyme-linked immunosorbent assay (ELISA) in 90 patients, in the first 12 hours of ACS (cases) and in 90 patients with chronic CAD (controls).

Results: The results showed that the titers of anti-oxLDL were significantly higher ($p = 0.017$) in cases (0.40 ± 0.22) than in controls (0.33 ± 0.23). On the other hand, the titers of antipeptD were significantly lower ($p < 0.01$) in cases (0.28 ± 0.23) than in controls (0.45 ± 0.30). The difference in the titers of both antibodies between the two groups was independent of age, sex, hypertension, diabetes mellitus, dyslipidemia, body mass index, smoking, lipid profile, statin use and family history of CAD.

Conclusion: The results showed that the titers of anti-oxLDL were significantly higher in patients with acute coronary syndrome as compared to patients with coronary artery disease and may be associated with atherosclerotic plaque instability. (Arq Bras Cardiol. 2010; [online]. ahead print, PP.0-0)

Key words: Lipoproteins, LDL; autoantibodies; acute coronary syndrome.

Introduction

The low-density lipoprotein (LDL) is the main carrier of cholesterol from human plasma. LDL has a lipophilic core that consists of esters of cholesterol and triglycerides and a polar surface composed of non-esterified cholesterol and phospholipids. Phospholipids are shrouded by apolipoprotein B-100 (apoB). The LDL molecule also contains fat-soluble antioxidants such as vitamin E, α and β carotene, ubiquinol, among others[1]. However, under conditions of oxidative stress, the LDL particles, which contain polyunsaturated fatty acids (PUFA), are easily oxidized and, consequently, apoB is modified by lipid oxidation[2].

Because of its proinflammatory and pro-atherogenic properties, the oxidized low density lipoprotein (oxLDL) is a marker of risk for cardiovascular disease (CVD). It may act in the subendotelial space[3,4]. Although attractive, relevant evidence that the hypothesis of oxLDL contributes to human atherosclerosis are still not definitively established and deserves further well-designed studies that may establish its contribution in the pathophysiology of atherosclerosis[5].

Some evidence suggests that IgG autoantibodies for oxLDL are associated with proatherogenic properties and IgM autoantibodies for oxLDL play an atheroprotective role[6]. The IgM class, which recognizes specific epitopes to oxidation, may be involved in the uptake and detoxification of pro-inflammatory oxidized lipids[3]. Thus, IgG and IgM autoantibodies may have antagonistic actions.

Recent studies showed that patients with acute coronary syndrome (ACS) including acute myocardial infarction (AMI) and unstable angina (UA), had higher levels of anti-oxLDL than patients with stable angina (SA) and controls ($p < 0.05$)[7]. Other studies have questioned the actual contribution of anti-oxLDL in atherogenesis, and had no significant differences between the titers of these antibodies in normal controls and in patients with chronic or acute coronary artery disease (CAD)[8].

Within this context, the objective of this study was to evaluate the association between autoantibodies against oxLDL and ACS.

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Methods

We conducted a controlled cross-sectional study, which included individuals of both sexes, older than 40, diagnosed with CAD, acute or chronic, treated at *Hospital São Lucas da PUCRS*, in a consecutive manner, from Mar/2001 to Dec/2003.

It also included patients with clinical diagnosis of ACS without persistent ST segment elevation (ACSWSTE). The diagnosis of ACSWSTE was defined as: two episodes of angina pectoris, or one episode of angina pectoris at rest with more than 20 minutes duration in the 24 hours preceding admission, no persistent ST segment elevation above 0.05 mV; presence or absence of elevated serum markers of myocardial necrosis or micro-embolization (creatine kinase with MB fraction above 20 U/l and/or troponin I above 0.05 ng/ml). The clinical picture upon admission was stratified according to the Braunwald classification[9]. The control group consisted of patients with established CAD, asymptomatic or with stable angina pectoris class I, according to the Canadian Cardiovascular Society's classification[10].

Ineligible patients were defined as those with: presence of left bundle branch block; ST-segment elevation above 0.05 mV at resting ECG, surgery performed less than three months before, including coronary artery bypass grafting, percutaneous coronary angioplasty less three months before; neoplasms; previously known hematological and immunological disorders; connective tissue diseases or inflammatory diseases associated with elevation of serum markers. The study also excluded patients on immunosuppressive medication, immunologically active diseases, neoplasms, AIDS, thyroid diseases and debilitating gastrointestinal disorders and individuals who refused to sign the informed consent.

Clinical variables of concern

Sex, age, body mass index (BMI), hypertension (HBP), diabetes mellitus (DM), dyslipidemia, chronic exposure to smoking (active smoking), family history of CAD, use of statins and serum high-sensitivity C-reactive protein (hs-CRP).

BMI was calculated by the formula: weight in kilograms divided by squared height in meters. Obese individuals were considered those with index ≥ 30 kg/m^2. Acute hypertension patients were considered those with previous diagnosis and/or using antihypertensive drugs. DM patients were considered to be those with prior diagnosis and/or using hypoglycemic therapy (diet, insulin treatment or with oral hypoglycemic drugs). Dyslipidemia patients were considered to be those with diagnosis of changes in serum lipid profile and/or using lipid-lowering drugs.

The smokers were stratified into: current smokers, those with a current smoking habit and who had smoked in the last year, former smokers, individuals who had quit smoking for over a year, and nonsmokers.

Family history of CAD was considered positive in those individuals whose first-degree relatives had presented early cardiovascular mortality (< 55 years in men and < 65 years in women).

Laboratory variables

IgG antibodies against oxLDL and anti-peptD and hs-CRP were measured in plasma from cases and controls by Enzyme-linked immunosorbent assay (ELISA).

The lipid and protein composition of LDL were determined by Lowry method[11]. The contents of total cholesterol and triacetilglicerol was measured by the enzymatic method[12]. The detection of IgG antibodies against oxLDL generated in the serum of cases and controls, as well as IgG antibodies against synthetic peptide D derived from apoB (antipeptD IgG) and the dosage of total IgG was done by ELISA technique (Enzyme-linked immunosorbent assay)[13].

Laboratory tests were determined by enzyme-linked immunosorbent assay in the first 12 hours after defining the clinical picture of ACS, stored in a freezer at -70ºC and subsequently sent for analysis at *Instituto de Imunofisiopatologia da Universidade de São Paulo*.

Calculation of sample size

The sample size was calculated first. To detect a difference of at least 70 units of optical density under average serum IgG anti-oxLDL titer between cases and controls, we estimated a sample size of 86 patients per cohort, considering standard deviation for IgG anti-oxLDL of 140 units, $\alpha = 0.05$ and statistical power of 90% ($\beta = 0.10$).

Statistical analysis

Adherence to the normal distribution (Gaussian) of distribution of anti-oxLDL and antipeptD values in the two cohorts was assessed by Kolmogorov-Smirnov test (KS). The Mann-Whitney test was used to compare the results of determination of average serum of IgG antibodies against oxLDL, IgG against peptide D and total IgG between cases and controls.

The significance of associations between categorical variables was analyzed by Fisher exact test and, between continuous variables, Mann-Whitney test. The intervening variables were controlled by multiple linear regression. Analyses of sensitivity and specificity of cutoff values of anti-oxLDL for diagnosis of ACS were made by ROC (receiver operating characteristic).

The results were expressed as means plus or minus the standard deviation. Differences were considered significant when the p value < 0.05.

Ethical aspects

The study was approved by the Ethics Committee of *Hospital São Lucas da PUCRS* (ID: 03/01533, approved on June 13, 2003).

Results

Table 1 shows the demographic characteristics, prevalence of known risk factors and the prognostic determinants selected between the two cohorts, and the significance between the differences in these variables between groups. There was no statistically significant difference in terms of

age of cases and controls, 62 ± 12 years and 64 ± 9 years, respectively (p = 0.18).

The sex distribution between groups was also comparable and 49.0% of cases and 53.0% of controls were male (p = 0.65). The two groups did not differ in relation to obesity and one quarter of cases and one fifth of controls had body mass index > 30 kg/m² (p = 0.59). Likewise, there was no statistically significant difference between groups regarding current smoking habit (12.0% of active smokers in cases and 12.0% also in controls) nor conercing family history of CAD (p = 0.17).

The previous diagnosis of dyslipidemia (p = 0.12), mean blood cholesterol (p = 0.09), triglyceridemia (p = 0.76) and use of cholesterol-lowering drugs (statins) (p = 0, 11) also did not differ significantly between cases and controls.

The frequency of hypertension was significantly higher in cases than in controls, 80.0% and 45.0% respectively (p < 0.01) and prior diagnosis of DM, present in 35.0% of cases and for 18.0% of controls (p = 0.01). More than half of the control group and one-fifth group of cases were former smokers (p < 0.01). Serum values of hs-CRP were significantly higher in cases than in controls (p < 0.01).

Total IgG concentration did not differ significantly between groups (p = 0.74), as shown in Figure 1 and Table 2.

Mean IgG anti-oxLDL titers was significantly higher in cases (0.40 ± 0.22) than in controls (0.33 ± 0.22), (p = 0.017) (Figure 2), and this difference was independent of the distribution of other coronary risk factors in all samples (p = 0.032) (Table 2). Mean IgG antipeptD titers was significantly lower in cases (0.28 ± 0.23) than in controls (0.45 ± 0.30) (p < 0.01) (Figure 3), and this difference was independent of the distribution of other coronary risk factors in all samples (p < 0.01) (Table 2).

Table 3 presents the significance of variables in multiple linear regression model adopted in the analysis, where anti-oxLDL and antipeptD, independently, were used. Hypertension, diabetes, dyslipidemia, the condition of former smoker, obesity, high cholesterol and family history of CAD were considered as explanatory variables.

Table 1 - Characteristics of group

Variable	ACS (N = 90)	Chronic CAD (N = 90)	p
Age (yrs)	62 ± 12	64 ± 9	0.18
Male (n)	44	48	0.65
BMI> 30 kg/m² (n)	22	18	0.59
Hypertension (n)	72	40	<0.01
Diabetes mellitus (n)	32	16	0.01
Dyslipidemia (n)	48	59	0.12
Current smoker (n)	11	11	1
Former smoker (n)	17	50	<0.01
Family history CAD (n)	32	42	0.17
Hs-CRP (mg/l)	1.7 ± 3.4	0.6 ± 0.9	<0.01
Total cholesterol (mg/dl)	238 ± 80	258 ± 78	0.09
Triglycerides (mg/dl)	175 ± 103	167 ± 106	0.76
Use of statins (n)	36	47	0.11

Results expressed in absolute numbers or mean ± standard deviation of the mean. ACS - acute coronary syndrome; CAD - coronary artery disease; BMI - body mass index; hs-CRP - high-sensitive C-reactive Protein.

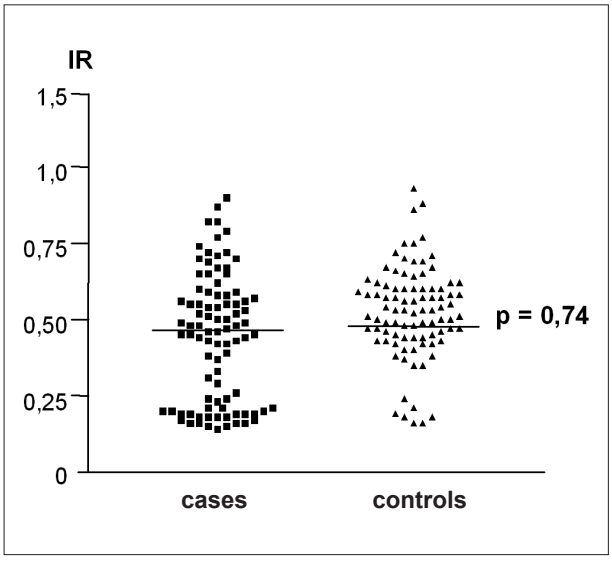

***Figure 1** - Distribution of serum titers of total IgG expressed in IR in cases and controls. IR - index of reactivity.*

Table 2 - IgG anti-oxLDL and antipeptD antibodies expressed in IR

	ACS (cases)	Chronic CAD (controls)	df **	CI 95%**	p
n	90	90			
Total IgG (IR)	0.44 ± 0.21	0.45 ± 0.30			0.74
anti-oxLDL (IR)	0.40 ± 0.22	0.33 ± 0.22			0.017*
anti-oxLDL (IR)	0.40 ± 0.22	0.33 ± 0.22	0.08**	0.04 - 0.12**	0.032**
AntipeptD (IR)	0.28 ± 0.23	0.45 ± 0.30			<0.01*
AntipeptD (IR)	0.28 ± 0.23	0.45 ± 0.30			<0.01**

** Values obtained by Mann-Whitney test. ** values determined through the multiple linear regression model, adjusted for the following variables: hypertension, dyslipidemia, diabetes, former smoker, obesity, family history and serum cholesterol. Means ± standard deviation of the mean; df - estimate of the difference between mean serum IgG anti-oxLDL titers in both cohorts; CI - 95.0% confidence interval 95.0%; IgG - immunoglobulin G; IR - index of reactivity.*

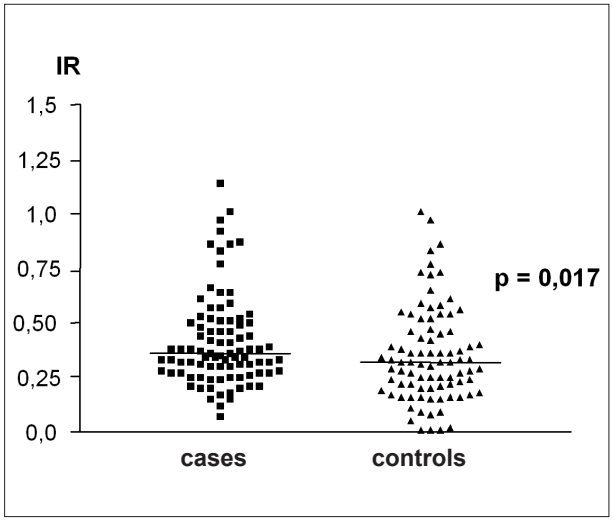

Figure 2 - Distribution of serum titers of IgG anti-oxLDL expressed in IR in cases and controls. IR - index of reactivity.

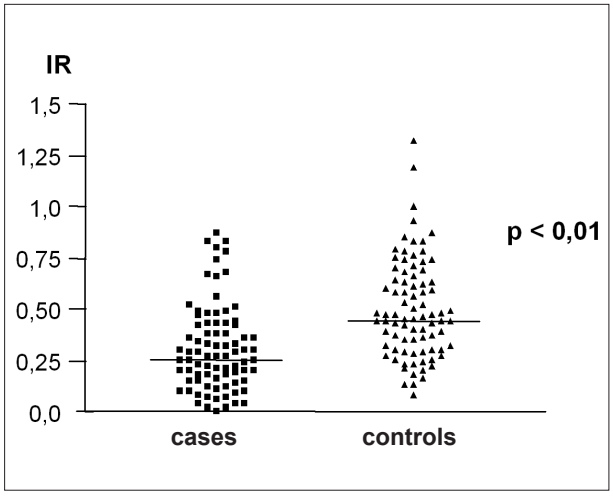

Figure 3 - Distribution of serum titers of IgG antipeptD expressed in IR in cases and controls. IR - index of reactivity.

Table 3 - Significance of variables included in multiple linear regression model to analyze the relationship between anti-oxLDL, antipeptD, acute and chronic coronary artery disease

Model	Anti-oxLDL p	AntipeptD p
Group (ACS *versus* chronic CAD)	0.032	0.001
Hypertension	0.931	0.216
Dyslipidemia *	0.282	0.520
Diabetes	0.079	0.218
Former smoker	0.255	0.517
Obesity	0.942	0.295
Family history of CAD	0.491	0.906
Cholesterol **	0.889	0.656

*Dependent variables: anti-oxLDL and antipeptD. ACS - acute coronary syndrome; CAD - coronary artery disease. * Family history of dyslipidemia. ** Total cholesterol increased.*

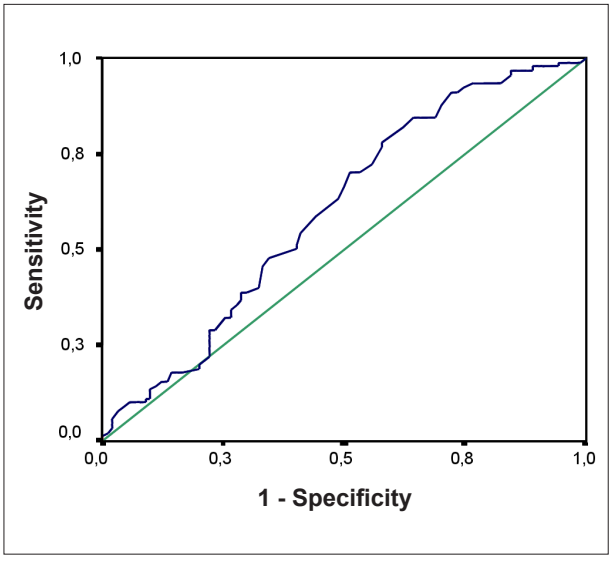

Figure 4 - ROC curve (receiver operating characteristic) for analysis of sensitivity and specificity of cutoff values of anti-oxLDL and diagnosis of ACS.

The difference between the average values of serum titers of anti-oxLDL in both groups (Table 2), although statistically significant, was small (p = 0.08, 95% CI = 0.04 to 0.12). In addition, the standardized effect size of approximately 0.35, is considered low.

The ROC curve analysis (Figure 4) does not suggest any specific cutoff point that has notable advantages over others (higher sensitivity and specificity) for diagnosis of ACS from the measurement of anti-oxLDL titers in individuals with CAD and the area under the curve was equal to 0.603 (95% CI = 0.520 - 0.686).

Discussion

Contemporary concepts challenge and suggest new paradigms on the pathophysiology and natural history of atherosclerosis and its clinical complications. Considering evidence from studies of cellular and molecular biology and clinical and experimental research, atherosclerosis understood as a process resulting from the chronic, passive and focal deposit of lipids on the arterial wall, gave rise to the notion of an, immune-mediated and systemic response[3,6,14].

The current concept of vulnerable plaque is associated with the characterization of vulnerable patient, with high probability of having a future cardiovascular event, and whose identification is a major clinical challenge[15].

One third of cases of sudden death and AMI occurs in previously asymptomatic individuals who have silent disease and/or associated risk factors. Epidemiological, observational and intervention studies highlighted the contribution of factors such as hypertension, smoking, DM and dyslipidemia for the risk of vascular disease, especially CAD. However, 20% of

individuals with CAD do not present any of the classic risk factors[16]. In addition, about half of all cases of AMI occur in people with plasma cholesterol levels within normal limits[17].

In the study of natural history of atherosclerosis and its determinants, several inflammatory markers have been identified, and in selected population samples, many of these substances are found to be capable of playing a modulating role in the risk of the disease and its complications. These include hs-CRP, CD40 ligand, V-CAM-1, MCP-1, IL-6 and IL-7[18].

Recently, a growing body of evidence suggests that local and systemic activation of the immune system may be related to the process of atherosclerotic plaque instability and its clinical manifestations. Although there is a chronic elevation of circulating immunoglobulins in patients with chronic stable CAD, a transient increase in T cell activation has been described by several authors in patients with ACS, leading them to believe that this may be a determining factor in the pathophysiology of acute complications[19].

Ammirati et al[19] observed that patients with ACS had a limited number of gene segments in the β chain of T cell receptors (RCT) activated, compared to controls with chronic CAD.

In the control group, the RCT were highly polymorphic. Furthermore, only T lymphocytes from patients with ACS proliferated in response to autologous proteins present in culprit lesions by acute event and/or in response to oxLDL. Lymphocyte proliferation in response to oxLDL was more intense in patients with ACS with higher morbidity than those with favorable outcome.

The authors suggest that in the acute phase of lesion instability, the polyclonal antibody response, characteristic of chronic CAD, lead to the activation mediated by a limited number of immunogenic molecules found in the plaque, and different from those related to lymphocyte activation observed in the chronic phase of the disease. They also draw attention to the fact that 68.0% of monotypic or oligotipic gene segments found in the acute phase have not been detected three months after the coronary event, stressing the idea that an antigen-specific population of T cells had been transiently activated during this period.

Using monoclonal antibodies against epitopes of oxLDL derived from genetically modified mice, several authors observed that oxLDL titers were significantly higher in ACS patients than in patients with chronic CAD[7,20].

Tsimikas et al[21] found an increase of 60.0% in the titers of autoantibodies against a number of epitopes generated in LDL oxidation models in patients during the acute phase and within one month after AMI. It is assumed that, in acute coronary event, the antigenic substrate originates from the direct release of modified lipoproteins, either by breakage or increased permeability of the plaque, or by disruption of cell membranes resulting from acute ischemic injury or reperfusion[22].

The oxLDL plays a critical role in the development of atherosclerosis and experimental data support that it is associated with multiple cardiometabolic risk factors[23]. These authors reported that high concentrations of oxLDL were associated with increased incidence of metabolic syndrome and its components, abdominal obesity, hyperglycemia and hypertriglyceridemia.

The oxidative modification of LDL induces the formation of immunogenic epitopes in the LDL molecule, which leads to the formation of antibodies against oxLDL, which can be detected in serum[24]. Studies have shown that the anti-oxLDL molecule can block the uptake of oxLDL by macrophages in atherosclerotic lesions, suggesting a possible protective role for the formation of foam cells[25].

In this study, we found that average serum titers of anti-oxLDL were significantly higher in patients with ACS compared to individuals with chronic CAD. The difference found was significant and independent on cardiovascular risk factors established, such as hypertension, smoking, diabetes and dyslipidemia. This difference was specific for IgG anti-oxLDL antibodies, once the concentration of total IgG did not differ significantly between cases and controls.

On the other hand, average serum titers of antipeptD were significantly lower in patients with ACS than in those with chronic CAD, and the difference was also independent on cardiovascular risk factors and the concentration of total IgG between the two groups.

Considering the study design, and because the anti-oxLDL and antipeptD values are unknown, before the onset of acute ischemia, we cannot state that these antibodies contribute to the instability of coronary lesions, or even if their increase represents an epiphenomenon resulting from plaque rupture.

However, some hypotheses are generated to interpret the behavior of the antibody response observed. It is possible to occur, during acute coronary event, in vivo, greater and quicker formation of antibodies from specific clones of B lymphocytes, pre-existing during the chronic phase of CAD in response to increased antigens. The immune response could play a protective role and contribute to the development of the acute ischemic event. The protective role of anti-oxLDL is evaluated from experimental evidence.

Shoenfeld et al[26] immunized knock out mice with homologous oxLDL, and observed that the procedure determined an increase in anti-oxLDL titers, and reduced the rate of progression of atherosclerotic lesions in these animals. Researchers propose the involvement of antibodies against oxLDL in clearing oxidized lipoproteins, both those from circulation and from the plaque, from the formation of immune complexes, which were eliminated by the monocyte-phagocytic system, via Fc receptor.

It is possible that the monoclonal response against some antigenic epitopes be more specific[27], which would explain why, in this study, there was evidence of elevated oxLDL titers (polyclonal response) and reduction of antipeptD titers (monoclonal response) in patients with ACS compared to controls with chronic CAD. It is assumed that the reduction of antipeptD titers associated with acute ischemic events reflects the higher speed with which these antigens are complexed.

On the other hand, it is conceivable that the increase in the formation of immune complexes is directly related to the process of plaque unstabilization. Virella et al[28] suggest that in humans, anti-oxLDL/oxLDL complexes would be eagerly digested by macrophages, creating a massive accumulation of intracellular cholesterol esters, responsible for plaque instability.

Mangueira et al[29] evaluated the antipeptD and anti-oxLDL titers in 66 patients with rheumatoid arthritis, a chronic inflammatory disease whose main cause of mortality is the CAD. The authors observed a significant correlation between the titers of both antibodies, but only the titlers of anti-oxLDL were higher in patients compared to controls.

Studies that analyze elevations in anti-oxLDL titers relate the increase of such titers with plaque rupture, and its reduction with the phase of stabilization of such lesions[30].

In a recent study, Doo et al[31] evaluated the prognostic value of anti-oxLDL as predictors of coronary events in a cohort of 60 patients hospitalized for unstable angina. They observed that individuals whose antibody titers were higher at admission had a significantly greater number of new coronary events during the observation period of 16 months, concluding that the determination of anti-oxLDL can be a useful way of identifying groups at higher risk.

Most studies compare the immune response to oxLDL in patients with ACS compared to controls without clinical evidence of coronary artery disease[7], However, even asymptomatic patients may have subclinical forms of the disease with severe and diffuse vascular involvement[32]. We chose to compare the antibody titers among individuals with ACS and chronic, stable and well-defined CAD, once the goal is not diagnosis, but the role of autoimmune response in characterizing different clinical pictures of the disease.

Another difference is that some studies compare acute and chronic CAD by determining titers of circulating oxLDL. This method uses monoclonal antibodies against specific epitopes in the oxLDL molecule, which is not available in our field and presents foreseeable difficulties.

The direct determination of oxLDL in plasma or serum is complicated by the possibility of in vivo modifications of samples and the variety of particles at different oxidation stages, which can be identified and whose significance in terms of predictive disease is also potentially variable. Thus, it is explained why the reproducibility of the reference preparation of oxLDL, used as standard in the test, delivers inconsistent results, even in the same population. Circulating levels of oxLDL are very low due to high concentration of antioxidants in the blood. Furthermore, they may not correlate with the amount of oxidized lipoprotein found in the atherosclerotic plaque, protected within the subendothelium.

In healthy individuals, it is estimated that the average plasma concentration of oxLDL is approximately 0.1 ng/μg of the protein portion of LDL[33]. Thus, the primary location of the analysis concerned could not be the circulation.

The samples analyzed seem to represent the population of patients with severe ischemic heart disease enrolled in tertiary care services. However, certain differences between the two groups should be considered. Exposure to some risk factors for CAD differed significantly between cases and controls. It is known that anti-oxLDL titers are higher in diabetics[34] and hypertensive patients[35] than in individuals without such diagnoses. Patients with these diseases were more prevalent in the group with ACS. Hence, average anti-oxLDL titers, significantly higher in cases, could be associated or not with the acute event, but to the greater frequency of risk factors in this group. Likewise, it is suggested that anti-oxLDL titers are higher in individuals with metabolic syndrome[36].

Although in the present study BMI did not differ significantly between cases and controls, it is conceivable that differences in insulin resistance between the two groups may have contributed to these results. HMG-CoA reductase inhibitors (statin) have immunomodulatory properties and reduce levels of circulating anti-oxLDL.

Since the use of statins did not differ between groups, it is assumed that the difference found in the average anti-oxLDL titers does not derive from the use of this therapy, although no data are available regarding the formulation, dosage and duration of use of these drugs in the samples studied, neither the average cholesterol differed between cases and controls, which confirms the results of previous investigations that found no positive correlation between serum lipoproteins and evidence of anti-oxLDL.

Average hs-CRP values were, as expected, higher in cases than in controls. Hs-CRP is considered an inflammatory marker and its serum levels may have clinical applicability in the detection and prevention of cardiovascular disease[37]. The JUPITER study, recently completed, showed that patients with cardiovascular risk, but with normal levels of LDL (< 130 mg/dl) and high hs-CRP (> 2 mg/dl), who made use of rosuvastatin, compared with placebo, had significantly fewer cardiovascular events[38].

Anti-oxLDL would be markers of autoimmunity and studies correlating hs-CRP values with anti-oxLDL titers, in ACS, shows that both are mutually independent. However, when both are high, they increase the predictive value for diagnosis of acute coronary event[39].

We tried to control the different biases of confusion in the statistical analysis. The regression model included variables that differed or not significantly different between groups, and showed that both anti-oxLDL titers and those of antipeptD were independently associated with acute coronary events in the sample.

Better knowledge of inflammation and atherothrombosis, as well as evidence of oxLDL autoantibodies, may establish new preventive strategies, including the feasibility of vaccination for primary prevention in individuals with cardiovascular risk factors.

Constraints

In this sample, we did not establish a cutoff value of anti-oxLDL titers, above which we could discriminate ACS cases with sufficient sensitivity and specificity. The immunoassay method for determination of anti-oxLDL is difficult to standardize and implement, which may lead to conflicting results, as seen in different studies. The clinical applicability of its determination will depend on further investigations, as well as cost-benefit evaluation.

Conclusion

The results showed that anti-oxLDL titers were significantly higher in ACS patients compared with patients with CAD and may be associated with atherosclerotic plaque instability.

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Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

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References

1. Itabe H. Oxidative modification of LDL: its pathological role in atherosclerosis. Clin Rev Allergy Immunol. 2009; 37 (1): 4-11.
2. Steinberg D, Witztum JL. Lipoproteins and atherogenesis: current concepts. JAMA. 1990; 264: 3047-52.
3. Gounopoulos P, Merki E, Hansen LF, Choi SH, Tsimikas S. Antibodies to oxidized low density lipoprotein: epidemiological studies and potential clinical applications in cardiovascular disease. Minerva Cardioangiol. 2007; 55 (6): 821-37.
4. Xavier HT, Abdalla DSP, Martinez TLR, Ramires JAF, Gagliardi ART. Efeitos da lipoproteína LDL-oxidada sobre a proliferação e a motilidade espontânea in vitro de células endoteliais de artérias coronárias humanas. Arq Bras Cardiol. 2004; 63: 488-92.
5. Sugiyama S, Okada Y, Sukhova GK, Virmani R, Heinecke JW, Libby P. Macrophage myeloperoxidase regulation by granulocyte macrophage colony – stimulating factor in human atherosclerosis and implications in acute coronary syndromes. Am J Pathol. 2001; 158 (3): 879-91.
6. Chou MY, Hartvigsen K, Hansen LF, Fogelstrand L, Shaw PX, Boullier A, et al. Oxidation-specific epitopes are important targets of innate immunity. J Intern Med. 2008; 263 (5): 479-88.
7. Soltesz P, Veres K, Laczik R, Der H, Csipo I, Timar O, et al. Evaluation of antibodies to oxidized low-density lipoprotein and assessment of C-reactive protein in acute coronary syndrome and stable coronary artery disease. Thromb Haemost. 2007; 98 (2): 413-9.
8. Rossi GP, Cesari M, De Toni R, Zanchetta M, Maiolino G, Pedon L, et al. Antibodies to oxidized low-density lipoproteins and angiographically assessed coronary artery disease in White patients. Circulation. 2003; 108: 2467-72.
9. Braunwald E. Unstable angina: a classification. Circulation. 1989; 80: 410-4.
10. Campeau L. Grading of angina pectoris [letter]. Circulation. 1976; 54: 522-3.
11. Lowry OH, Rosebbough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem. 1951; 193 (1): 265-75.
12. Harada LM, Carvalho MD, Passarelli M, Quintão EC. Lipoprotein desialylation simoltaneously enhances the cell cholesterol uptake and impairs the reverse cholesterol transport system: in vitro evidences utilizing neuraminidase-treated lipoproteins and mouse peritoneal macrophages. Atherosclerosis. 1998; 139: 65-75.
13. Gidlund MA, Damasceno NR, Lindoso JA, Abdalla DS, Goto H. Monoclonal antibodies against low density lipoprotein with various degrees of oxidative modifications. Braz J Med Biol Res. 1996; 29: 1625-8.
14. Magalhães CK, Siqueira Filho AG, Amino JG0C, Nolasco M. Correlação entre marcadores bioquímicos e a cineangiocoronariografia em portadores de síndromes isquêmicas agudas sem supradesnivelamento de segmento ST. Arq Bras Cardiol. 2006; 86: 223-31.
15. Naghavi M, Libby P, Falk E, Casscells SW, Litovsky S, Rumberger J, et al. From vulnerable plaque to vulnerable patients: a call for new definitions and risk assessment strategies: part I. Circulation. 2003; 108: 1664-72.
16. Khot UN, Khot MB, Bajzer CT, Sapp SK, Ohman EM, Brener SJ, et al. Prevalence of conventional risk factors in patients with coronary heart disease, JAMA. 2003; 290: 898-904.
17. Castelli WP. Lipids, risk factors and ischaemic heart disease. Atherosclerosis. 1996; 124: S1-S9.
18. Ridker PM, Rifai N, Rose L, Buring JE, Cook NR. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. N Engl J Med. 2002; 347: 1557-65.
19. Ammirati E, Vermi AC, Cianflone D, Banfi M, Foglieni C, Godino C, et al. Expansion of T-Cell receptor zeta dim effector T cells in acute coronary syndromes. Arterioscler Thromb Vasc Biol. 2008; 28 (12): 2305-11.
20. Ehara S, Ueda M, Nakuro T, Haze K, Itoh A, Otsuka M, et al. Elevated levels of oxidized low density lipoprotein show a positive relationship with the severity of acute coronary syndromes. Circulation. 2001; 103: 1955-60.
21. Tsimikas S, Beyer RW, Patel R, Miller E, Juliano J, Wilztum JL. Prospective evaluation of the role of oxidized LDL in acute coronary syndromes treated with percutaneous intervention. Circulation. 2000; 102: II-13.
22. Tsimikas S, Witztum JL. Measuring circulating oxidized low-density lipoprotein to evaluate coronary risk. Circulation. 2001; 103: 1930-2.
23. Holvoet P, Lee DH, Steffes M, Gross M, Jacobs DR Jr. Association between circulating oxidized low-density lipoprotein and incidence of the metabolic syndrome. JAMA. 2008; 299 (19): 2287-93.
24. Palinski W, Witztum JL. Immune responses to oxidative neoepitopes on LDL and phospholipids modulate the development of atherosclerosis. J Intern Med. 2000; 247: 371-80.
25. Shaw PX, Horkko S, Tsimikas S, Chang M, Palinski W, Silverman G, et al. Human-derived anti-oxidized LDL autoantibody blocks uptake of oxidized LDL by macrophages and localizes to atherosclerotic lesion in vivo. Arterioscler Thromb Vasc Biol. 2001; 21: 1333-9.
26. Shoenfeld Y, Harats D, George J. Heat shock protein 60/65, beta 2-glycoprotein I and oxidized LDL as players in murine atherosclerosis. J Autoimmun. 2000; 15: 199-202.
27. Napoli C, Glass CK, Witztum JL, Deutsch R, D´Armiento FP, Palinski W. Influence of maternal hypercholesterolemia during pregnancy on progression of early atherosclerotic lesions in childhood: Fate of Early Lesions in Children (FELIC) study. Lancet. 1999; 354: 1234-41.
28. Virella G, Lopes-Virella MF. Lipoprotein autoantibodies: measurement and significance. Clin Diagn Lab Immunol. 2003; 10: 499-505.
29. Mangueira CLP. Autoantibodies against copper-oxidized low density lipoprotein, apolipoprotein-B derived peptides and cardiolipin in rheumatoid arthritis patients: correlation with disease activity and other clinical parameters [tese]. São Paulo (SP): Faculdade de Medicina . Universidade de São Paulo; 2003.
30. Tornvall P, Waeg G, Nilsson J, Hamsten A, Regnström J. Autoantibodies against low-density lipoproteins in coronary artery disease. Atherosclerosis. 2003; 167: 347-53.
31. Doo YC, Han SJ, Lee JH, Cho GY, Hong KS, Han KR, et al. Associations among oxidized low-density lipoprotein antibody, C-reactive protein, interleukin-6, and circulating cell adhesion molecules in patients with unstable angina pectoris. Am J Cardiol. 2004; 93: 554-8.
32. Libby P. Coronary artery injury and the biology of atherosclerosis: inflammation, thrombosis and stabilization. Am J Cardiol. 2000; 86: 3-8.

33. Itabe H, Yamamoto H, Imanaka T, Shimamura k, Uchiyama H, Kimura J, et al. Sensitive detection of oxidatively modified low density lipoprotein using a monoclonal antibody. J Lipid Res. 1996; 37: 45-53.

34. Dotevall A, Hulthe J, Rosengren A, Wiklund O, Wilhelmsen L. Autoantibodies against low-density lipoprotein and C-reactive protein are associated with diabetes and myocardial infarction in women. Clin Sci. 2001; 101: 523-31.

35. Awadallah SM, Hamad M, Jbarah I, Salem NM, Mubarak MS. Autoantibodies against oxidized LDL correlate with serum concentrations of ceruloplasmin in patients with cardiovascular disease. Clin Chim Acta. 2006; 365 (1-2): 330-6.

36. Fagerberg B, Bokemark L, Hulthe J. The metabolic syndrome, smoking, and antibodies to oxidized LDL in 58-year-old clinically heath men. Nutr Metab Cardiovasc Dis. 2001; 4: 217-20.

37. Ridker PM. Clinical application of C-reactive protein for cardiovascular disease detection and prevention. Circulation. 2003; 107: 363-9.

38. Ridker PM, Danielson E, Fonseca F, Genest J, Gotto AM Jr, Kastelein JJ, et al. JUPITER Study Group. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. N Engl J Med. 2008; 359 (21): 2195-207.

39. Holvoet P, Collen D, Van de Wert F. Malondialdehyde-modified LDL as a marker of acute coronary syndromes. JAMA. 1999; 281: 1718-21.