Biochemical significance of autoantibody titers against oxidized low-density lipoprotein in patients with coronary heart disease

MAHMOUD, I. NASR; SHADEN, M. HANAFY; MOSSAN, M.; Ehab M. ABDEL-FATTAH; DALIA A. AND HASSAN M.

1. Department of Molecular Biology, Genetic Engineering and Biotechnology Research Institute (GEBRI), Sadat City, Menoufiya University. EGYPT.
2. Department of Bioinformatics, Genetic Engineering and Biotechnology Research Institute (GEBRI), Sadat City, Menoufiya University. EGYPT.
3. Department of Cardiology, Faculty of Medicine, Shebin El-Kom, Menoufiya University. EGYPT.
4. Department of public health, Faculty of Medicine, Cairo University. EGYPT.

Abstract: Oxidative modification of LDL, which plays a key role in the development of atherosclerosis, induces immunogenic epitopes in LDL molecule, and the presence of anti-OxLDL (aOxLDL) has been demonstrated in human sera. Although its biological significance in atherogenesis is unclear and not well established. The purposes of this study were to assess whether aOxLDL is related to other inflammatory markers of possible interest in atherosclerotic development, such as C-reactive protein (CRP). Moreover, to search for any correlation with lipid profile, or creatine kinase (CK) and isoenzyme (CK-MB). Fifteen patients (10 men and 5 women; mean age 41-64 years) with unstable angina (UA). Another fifteen patients (11 men and 4 women; mean age 40-65 years) with acute myocardial infarction (AMI). Ten healthy volunteers with matched age and sex were included in this study. Laboratory investigations included: Full lipid profile, C-reactive protein (CRP), creatine kinase (CK) and isoenzyme (CK-MB) and also troponineI as well as serum titers of auto antibodies against Oxidized LDL (aOxLDL) were assessed by ELISA. Elevated aOxLDL (antibody titers) were observed in AMI and UA than controls (418.40 ± 202.05, 287.13 ± 101.75, 182.90 ± 51.02 mU/ml respectively). The higher levels of aOxLDL and CRP were statistically significant in AMI than UA than controls (F = 10.606, P = 0.000 and F = 27.519, P = 0.000, respectively). On the other hand, there was no significant correlation observed between these values (r = -.160, p = 0.568). The elevated levels of aOxLDL may be useful for identifying patients of higher risk for cardiac events. It may also be a marker of plaque instability to be considered in the therapeutic strategy in acute coronary syndrome.

Key words: Oxidised LDL, Antioxidised LDL, Acute myocardial infarction, ROC curve.

1 Introduction

Oxidized LDL (OLDL) has been shown to play an important role in the pathogenesis of atherosclerosis (1). Holvoet et al (2) and others (3,4) have demonstrated an association between cardiovascular disease (CVD) and oxidation of LDL. They have also found circulating OLDL to be a prognostic marker of CVD in cardiac transplant patients (5). In middle-aged people, obesity and dyslipidemia are the strongest predictors of levels of OLDL (6). The association between dyslipidemia and oxidation of LDL has been demonstrated in individuals in the pre-diabetic state (7). Finally, Holvoet et al (8) have shown that in the Health, Aging, and Body Composition (Health ABC) cohort a high coronary heart disease (CHD) risk status before CHD events is associated with high levels of circulating OLDL, even after adjustment for LDL cholesterol.

Oxidation modification of LDL (OLDL) is postulated to be one of the earliest events in the initiation of atherogenesis (8). OLDL is present in aortas of human fetuses of hypercholesterolemic mothers (10) even prior to macrophage foam cell formation, in progressing atherosclerotic lesions of animal models (11) and within human vulnerable plaques (12). Acute myocardial infarction is a type of
acute coronary syndrome, which is most frequently (but not always) a manifestation of coronary artery disease. The most common triggering event is the disruption of an atherosclerotic plaque in an epicardial coronary artery, which leads to a clotting cascade, sometimes resulting in total occlusion of the artery. Atherosclerosis is the gradual buildup of cholesterol and fibrous tissue in plaques in the wall of arteries (in this case, the coronary arteries), typically over decades. Blood stream column irregularities visible on angiographies reflect artery lumen narrowing as a result of decades of advancing atherosclerosis. Plaques can become unstable, rupture and additionally promote a thrombus (blood clot) that occludes the artery. When a severe enough plaque rupture occurs in the coronary vasculature, it leads to myocardial infarction (necrosis of downstream myocardium). Antibodies to low density lipoproteins are detectable in practically every human being of all ages. It has been speculated that these antibodies are involved in a protective process taking place when low density lipoprotein is oxidized to OLDL. Antibodies to OLDL (aOxLDL) have been described in various disorders. Cardiac markers or cardiac enzymes are proteins from cardiac tissue found in the blood. These proteins are released into the blood stream when damage to the heart occurs, as in the case of a myocardial infarction.

The present study aimed to assess the level of aOxLDL in patients with recent acute myocardial infarction (AMI) and unstable angina (UA) compared to normal subjects. Search for any correlation between the level of aOxLDL and other inflammatory markers of possible interest in atherosclerosis.

2 Materials and Methods

2.1 Study population

The population of our study included 40 individuals. The first group of unstable angina (group I, n = 15, 10 males and 5 females, mean age 53.7± 6.8 years). The second group of acute myocardial infarction (group II; n = 15, 11 males and 4 females, mean age 53.9 ± 7.3 years). The third group of healthy volunteers as a control group (group III; n = 10, 5 males and 5 females, mean age 55.2 ± 5.6 years). Informed consents were obtained from all participants. The patients were admitted to the cardiology department in main Menufiya University. They applied to thorough clinical examination, electrocardiogram (ECG and echocardiography). Exclusion criteria included; known valvular heart disease or previous arterial fibrillation or congenital heart diseases. Diabetic patients and patients with age less than 25 years old were also excluded.

2.2 Biochemical Assays

Venous blood samples were drawn from all individuals of our study after 12 hours fasting and centrifuged at 4000 xg for 8 minutes. The separated serum was then stored and frozen at -80°C, until analysis was performed. Full lipid profile, C-reactive protein (CRP), creatine kinase (CK) and iso enzyme (CK-MB) and also troponine as well as serum titers of auto antibodies against Oxidized LDL (aOx LDL) were assessed in a later time. Total cholesterol, triglycerides, HDL and LDL cholesterol were measured with an automatic assay system using an enzymatic colorimetric method, while HDL and LDL – cholesterol serum levels were evaluated using direct method not by fried wald’s formula. Serum C-reactive protein (CRP) was assayed by turbidimetry unstable angina entry (Bio Systems, Barcelona, Spain). Both total creatine kinase (CK) and the iso enzyme creatine kinase -MB (CK-MB) were quantitatively determined using IFCC methods for the measurement of catalytic concentration of enzymes. The catalytic concentration is determined from the rate of NADPH formation, measured at 340 nm, by means of the hexokinase (HK) and glucose-6-phosphate dehydrogenase coupled reaction. The ADVIA centaur cTUI assay measures troponine I concentrations up to 50 ng/ml (mg/ L) with a mininmum detectable concentrations analytical sensitivity of below 0.10 ng/ml (mg /L ). The assay was a two – site sandwich immunoassay using direct chemiluminometric technology , which used constant amounts of polyclonal and monoclonal antibodies. The first antibody, in the lite Reagent was a polyclonal goat anti-tropn I antibody with acridinium ester. The second antibody in the solid phase was a combination of monoclonal mouse anti-troponin antibodies covalently coupled to paramagnetic particles.

Serum titers of IgG–auto antibodies against Oxidized LDL were determined with a sandwich enzyme – linked immunosorbent assay (ELISA). The kit purchased from Biomedical Gesellschaft, Vienna, Austria. Accordingly, Cu++ Oxidized LDL was coated onto micro titer strips as antigen. IgG auto antibodies against Ox LDL if present in the pre
diluted serum, were bound specifically to the antigen. After a washing step, a specific peroxides conjugated anti human IgG antibodies detected the presence of bound antibodies. Tetramethylbenzidine (TMB) was added as a non toxic chromogenic substrate. Subsequently, the concentration of IgG auto antibodies in the sample was quantified by an enzyme catalyzed color change detectable on a standard ELISA reader in mU/ml the whole assay was standardized with defined increased titer of auto antibodies against Oxidized LDL. (22)

2.3 Statistical Analysis
All the results were analyzed by SPSS software (Version 10). P values less than 0.05 were considered statistically significant. The data are expressed as mean ±SD. Pearson’s correlation coefficient was used to quantify the relationship between the variables under study. Chi square analysis was also performed. Receiver operating characteristics (ROC) curves to determine the diagnostic performance of aOxLDL as a predictor of acute myocardial infarction and a marker of unstable angina progression

3 Results
As presented in table 1 and figure 1, The lipid profile, cholesterol, triglycerides and LDL serum levels were higher in patients groups than in controls and this difference was statistically highly significant ( p=0.000, p=0.000, p=0.000 respectively ). Except for HDL serum level, it was slightly lower in acute myocardial patients than both unstable angina and control (47.80±9.26 mg/dl VS 51.13 ±9.86 mg/dl and 49.20±9.15 mg /dl respectively). This difference was not statistically significant between groups as ( p = 0.630). Also, the triglyceride serum level was higher in unstable angina patients than in AMI than controls (164.80 ± 70.4 mg/dl VS 152.80 ± 43.9 mg/dl and 95.0 ± 17.6 mg/dl, respectively).

This difference between groups didn’t reach the level of significance as (P = 0.006). Regarding to other biochemical parameters, CRP, CK, AND CK-MB serum levels. They were higher in acute myocardial patients than in unstable angina patients and this difference was statistically highly significant (15.88 ±6.73mg/Lvs 6.38±3.40 mg/l, p=0.000, 213.73 ±34.88IU /ml VS 127.47±27.90IU/ml, p=0.000 and 32.88±6.04 IU/ml VS 12.68 ±3.97 IU/ml, p=0.000, respectively).

Table 1: Statistical analysis of TG, cholesterol, HDL, LDL, CRP, CK, CK-MB, troponine I and anti-oxidized LDL by ANOVA among the three groups of patients.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>F</th>
<th>p.value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG mg/dl</td>
<td>5.890</td>
<td>.006</td>
</tr>
<tr>
<td>Cholesterol . mg/dl</td>
<td>14.269</td>
<td>.000</td>
</tr>
<tr>
<td>HDL mg/dl</td>
<td>.468</td>
<td>.630</td>
</tr>
<tr>
<td>LDL mg/dl</td>
<td>11.400</td>
<td>.000</td>
</tr>
<tr>
<td>CRP mg/l</td>
<td>27.519</td>
<td>.000</td>
</tr>
<tr>
<td>Ck IU/ml</td>
<td>49.811</td>
<td>.000</td>
</tr>
<tr>
<td>CK-MB IU/ml</td>
<td>98.228</td>
<td>.000</td>
</tr>
<tr>
<td>Tropl ng/ml</td>
<td>78.167</td>
<td>.000</td>
</tr>
<tr>
<td>aOxLDL mU/ml</td>
<td>10.606</td>
<td>.000</td>
</tr>
</tbody>
</table>

P is considered significant when <0.05.
As presented in Table 1, the mean concentrations of aOxLDL were significantly higher in acute myocardial infarction patients than in unstable angina patients or control group (418.40 ±202.05 mU/ml, 287.13 ± 101.75 mU/ml, 182.90 ± 51.026 u/ml, respectively p=0.000) as shown in figure 3. Regarding to the multiple comparisons of aOxLDL between every two studied groups as shown in Table 2, there was a statistical significant difference of aOxLDL concentrations between unstable angina patients and myocardial infarction patients (p=0.015) on the other hand there was a highly significant difference between myocardial infarction patients and controls (p=0.000) Persons correlation of serum titers of aOxLDL in myocardial infarction patients with the variables of lipid profile revealed no significant correlations were identified between serum titers of aOxLDL. The different parameters of lipid profile or the other clinic pathological parameters including CRP, CK, CK-MB and troponine I in the different three studied groups as shown in fig.2.

Table 2. Statistical multiple comparison of aOxLDL Serum titers between the unstable angina, AMI, and control groups

<table>
<thead>
<tr>
<th>Dependent variable Group</th>
<th>Group</th>
<th>P*-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>aOxLDL (mU/ml)</td>
<td>Unstable angina</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Myocardial infarction</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0.237</td>
</tr>
<tr>
<td></td>
<td>Myocardial infarction</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Unstable angina</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0.237</td>
</tr>
<tr>
<td></td>
<td>Unstable angina</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Myocardial infarction</td>
<td></td>
</tr>
</tbody>
</table>

P* = multiple comparisons between each two groups, P is significant when <0.05.
Fig. 2: Pearson’s correlation of serum titers of aOxLDL and different parameters of lipid profile. 

- **a)** LDL in unstable angina \( (r = -0.473, p = 0.075) \),
- **b)** TG in unstable angina \( (r = -0.265, p = 0.340) \),
- **c)** Cholesterol in AMI \( (r = -0.100, p = 0.722) \).

Correlation is significant at the 0.05 level (2-tailed).

Fig. 3: Serum titers of aOxLDL in different studied Groups expressed as mean ± SD.

### 3.1 Diagnostic performance of the aOxLDL

Receiver operating characteristic (ROC) curve analysis was performed to indicate the sensitivity and specificity of aOxLDL in acute myocardial infarction \( (n=15) \) and unstable angina \( (n=15) \) (figure 4). The area under the curve, the cut off value (C.O.V) and the youden index of serum aOxLDL levels were obtained from the % sensitivity and specificity\(^{(23)}\).

The Roc curve analysis presented a significant area under the curve of 0.751, 80% sensitivity, and 80% specificity and cut off point of aOxLDL level at 307.5 mU/ml.

Beyond which the occurrence of acute myocardial infarction is dramatically increased. However, as shown in Table 3 within the acute myocardial infarction group and unstable angina, those were >307.5 considered as positive (80 %, 33.3% respectively). While, those were <307.5 considered as negative (20 %, 66.6 % respectively). This is yielding a significantly elevated relative risk of 8 fold more increased risk of developing academic acute myocardial infarction \( (OR =8.00, 95 \% CI 1.5 -42.0, P=0.025) \).
Biochemical parameter | ROC Area under the curve | sig | Youden’s Index | Sensitivity | Specificity | Cut off value
--- | --- | --- | --- | --- | --- | ---
A Ox LDL | 0.751 | 0.019 | 0.47 | 80% | 80% | 307.5

Youden index=(specificity+ sensitivity )-1

Fig. 4: Diagnostic performance of a Ox LDL in AMI group (n=15) against unstable angina group (n=15).

Table 3: Frequency distribution of aOxLDL among AMI cases and unstable angina cases.

<table>
<thead>
<tr>
<th>Group</th>
<th>AOxLDL</th>
<th>Odds ratio</th>
<th>95 % CI</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMI (N=15)</td>
<td>&lt;307.5</td>
<td>&gt;307.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>( _ve)</td>
<td>(+ve)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20 %</td>
<td>80 %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UA (N=15)</td>
<td>66.6 %</td>
<td>33.3 %</td>
<td>8.00</td>
<td>1.522-42.04</td>
</tr>
</tbody>
</table>

P* : X² test Fisher exact: statistically significant (<0.05)

4 Discussion
Oxidative modification of LDL alters its structure, allowing LDL to be taken up by scavenger receptors on macrophages, endothelial and smooth muscle cells (24). Uptake of oxidatively modified LDL by macrophages seems to bypass a negative feedback mechanism. This unrestricted uptake allows increased influx of LDL in to macrophages, creating foam cell. The earliest recognized abnormality in atherosclerotic deposition (25). OLDL is able to induce both the humeral and cellular immune response (26).

Oxidation of LDL induces the formation of aOxLDL, which has been used by several investigators as a marker of Oxidative stress (27). Antibody to OLDL is detected in human sera, although its biological significance is not well established.

The main findings of this study are that, aOxLDL is significantly higher in acute myocardial infarction patients than in unstable angina patients than control subjects (p=0.000). Previous studies showed raised serum aOxLDL in those with advanced atherosclerosis (28). Several studies have shown the presence of auto antibodies to Ox LDL in patients with coronary artery disease compared to normal subjects (confirming partially the results of our study). That finding indicates that these titters may have a diagnostic and or prognostic value (29,31). However, there were two studies in 1994 and 1998 according to which no differences have been observed in the titters of the auto antibodies, between patients and healthy controls (32,33). The causes for this inconsistency in the results of various clinical studies are not known.

Confirming our results, Meuwissen et al. (34) found that the serum concentrations of aOxLDL was significantly higher in both groups of patients (myocardial infarction and unstable angina) than those in controls. More over they also found that the concentrations of aOxLDL were higher in myocardial infarctions than in angiina (p=0.001).

In a good agreement with our results Inoue et al., (35) when they measured the aOxLDL titer in 108 patients with (CAD) and another 31 patients had no significant (CAD) as control. They found that the titer was higher in acute myocardial infarction patients than unstable angina than old myocardial infarction patients. Our findings are also supported by Sfrijan et al., (36) as their
study was conducted to test the titers of a Ox LDL. The highest values belong to patients with acute myocardial infarction and unstable angina. It was detectable in stable angina patients and healthy subjects but in the lowest values. In contrast, studies in familial hypercholesterolemia (37) showed that those patients having myocardial infarction had a lower aOxLDL than those who had not had MI. (38) Roden- burg et al., (39) found that children with a family history of hypercholesterolemia had higher level of auto antibodies (isotype IgG and IgM) and the immune-complexes anti-apo B and anti-malondialdehyde (isotype – IgM). Therefore, there is consensus on the involvement of aOxLDL, although the impact of their increasing or decreasing on chronic disease such as obesity and atherosclerosis still demands further investigation. The results presented by Leticia et al., (40) confirmed the presence of aOxLDL in adolescents and established their relationship with anthropometric and biochemical parameters. They also suggested that it is probable that an increase in aOxLDL is an early marker of cardiovascular risk in obese adolescents.

In the present study the aOxLDL in the three groups is not correlated with serum total cholesterol, high density lipoprotein and low density lipoprotein and also triglycerides, except that in acute myocardial infarction group of patients, aOxLDL was positively correlated. It was reported that the aOxLDL and metabolic changes to the lipid profile vary in proportion with anthropometric parameters, which makes aOxLDL a potential biochemical indicator of risk of metabolic syndrome Erikkila et al., (30) A higher aOxLDL & a positive correlation between it and LDL was found in young healthy adults who had little atherosclerosis compared to middle aged individuals. Suggesting a protective role for a Ox LDL during young age only, and its effectiveness maybe diminished as the years pass.

Concluding that a Ox LDL is higher in patients with acute myocardial infarctions and is correlated with myocardial damage than with the severity of coronary atherosclerosis and lipid profiles.

In the present study, by examining diagnostic performance of the studied parameters aOx LDL is found to be significant prognostic marker for the progression of unstable angina to acute myocardial infarctions (sensitivity 80 % and specificity 80 %). Theses findings made aOxLDL a good marker for detecting highly progressive unstable angina cases. Data from our study showed that there was an 8 fold more increased risk of developing an acute myocardial infarction for those cases having UA and aOxLDL is exceeding the cut off value. These results are in agreement with that previously described by Puurnen et al, (43). Where they observed elevated aOxLDL after adjustment for age, smoking, blood pressure and high density lipoprotein. Cholesterol level, there was a 2-5 fold increased risk.

5 Conclusion

High concentration of aOxLDL suggests an increase in oxidative stress that would contribute to disease severity. The immune response to LDL may play role in the pathogenesis of atherosclerosis. The elevated levels of aOxLDL may be useful for identifying patients at higher risk for cardiac event and it may also be a marker of plaque instability to be considered in the therapeutic strategy in acute coronary syndrome.

References:


[38] Shoji T., Nishizawa Y., Fukumoto M., et al., Inverse relationship between circulating oxidized low density lipoprotein (oxLDL) and anti-oxLDL antibody levels in healthy subjects, Atherosclerosis, 148, 2000, 171–177.


