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ROLE OF OXIDIZED-LDL (OX-LDL) AND ANTI-OXIDIZED-LDL (ANTIOX-LDL) ANTIBODIES IN MYOCARDIAL INFARCTION

Biochemistry

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ABSTRACT

Introduction: Coronary Artery Disease (CAD) is looming large at the new epidemic affecting Indians earlier in life than in other ethnic groups. Oxidized low density lipoprotein (oxLDL) plays important roles in the formation of atherosclerotic lesions. Anti-Ox LDL Abs binds with Ox-LDL and produces immune complex which got deposit in tissues and then it can cause damages. Our objective was to evaluate the role of lipid oxidation markers, Ox-LDL and anti-Ox-LDL antibodies in Myocardial Infarction patients with plaque instability. **Material and methods:** 200 patients of acute MI were included and were further sub-grouped into first episode of MI and recurrent MI. Serum levels of Ox –LDL and antibody to ox-LDL were estimated by ELISA. **Results:** The Ox-LDL levels were lower in Group A(3.8385±6.0492) as compared to Group B(6.8538 ±236.0338) but was not statistically significant. Also, anti-ox-LDL abs levels were higher in Group A(1.8940±1.9435) as compared to Group B(1.3366 ±1.8303) and again the result was not statistically significant. **Conclusion:** Our study showed that there might be no direct role of Ox-LDL and anti-Ox-LDL antibodies in MI patients as we didn't get significant results. A detailed study in extended sample size with patients follow-up is compulsory to confirm the significance of oxidative stress biomarkers in Myocardial Infarction.

KEYWORDS

Myocardial infarction ,Atherosclerosis , Oxidative stress , Ox-LDL , Anti ox-LDL antibodies

Introduction

At the threshold of the new millennium, coronary artery disease (CAD) is looming large at the new epidemic affecting Indians earlier in life than in other ethnic groups, relatively higher in younger ages with more severity and extensiveness, to follow a malignant course[1], we are in the midst of a true cardiovascular pandemic. It has been apparent for the past decade that heart disease is the leading cause of death, disability, and healthcare expenses worldwide, according to world health organization (WHO) estimates, cardiovascular disease killed 14.7 million individuals in 1990 and 17 million in 1999. In 1990, there were 783000 deaths due to CAD in India and this is projected to double by the year 2015[2-4].

The formation of atherosclerosis is increasingly recognized as an inflammatory process in the arterial wall, including the accumulation of macrophages and activated T-lymphocytes[5]. Oxidized low density lipoprotein (oxLDL) plays important roles in the formation of atherosclerotic lesions. Examples for the proatherogenic role of oxLDL include taking up of extensively oxLDL by macrophages leading to foam cell formation, cytotoxicity and expression of adhesion molecules, and cellular proliferation induced by ox- LDL[6].

Oxidative modification of LDL results in the appearance of new epitopes that render LDL more antigenic[7], and thus anti-oxLDL antibodies are formed. Although the level of autoantibodies against oxLDL might be an indirect qualitative measure for the in vivo oxidation of LDL, it is not established yet whether the immune response to oxLDL is proatherogenic or antiatherogenic in vivo. Oxidative stress as an overproduction of reducing equivalents such as NAD(P)H may result in increased redox cycling of substances that can undergo repetitive rounds of oxidation/reduction, ultimately which leads to the increased generation of superoxide anion radical(O^{2-}) and secondary oxidants. This can be implicated in the formation of ROS by hypoxia like metabolic imbalances[8].

Studies over the years depicts that oxidation of low density lipoprotein might have role in origin of atherosclerosis[9-11]. As defined, Ox-LDL is ox datively modified LDL which contains protein components that were modified by aldehyde products and creates net negative charges that were important for its interaction and uptake by macrophages[11-12]. One of the important role of Ox-LDL has been proposed for lipoxygenase[13-14] as it is expressed in atherosclerotic plaque but not in normal blood vessel of humans[15-18]. There are the scavenger receptors which mediate the uptake of oxidized LDL by macrophages. This Ox-LDL is immunogenic molecules that stimulate the production of anti-ox-LDL abs[19]. Anti-ox-LDL antibodies are present in both healthy individuals and in CAD patients. Some studies show that they play dual role in individuals by being either protective or pathogenic. They can block the uptake of Ox-LDL by macrophages and play its protective role[20]. While some other studies show that there is negative association of Anti- Ox LDL and CAD[21-22]. Anti-Ox LDL Abs binds with Ox-LDL and produces immune complex which got deposit in tissues and then it can cause damages[19].The oxidative modification hypothesis of antioxidant protection of the LDL in the extracellular spaces needs to be focused. Through this perspective, the cellular deposition of Ox-LDL can be considered as hallmark for the atherosclerosis. There are so many hypotheses which describe the inverse relationship of antibody against Ox-LDL titres with atherosclerosis.

Now, we further wanted to see whether the levels of serum auto antibody (anti-Ox LDL) against oxidized low density lipoprotein in the patients are associated with plaque instability leading to an acute coronary event like MI, as our previous study shows the link & this suggests the study of these lipid oxidation markers in MI patients.

Objective:

To evaluate the role of lipid oxidation markers: Ox-LDL and anti-Ox-LDL antibodies in Myocardial Infarction patients with plaque instability.

Materials and Method:

Place of study:

The study was being conducted in the Department of Biochemistry, Lady Hardringe Medical College & associated SSKH in ollaboration with the Department of Cardiology, GB Pant Hospital New Delhi.

Study population:
We enrolled patients (N=200) of Myocardial Infarction presenting within 24hrs of the event to Cardiology Department / Emergency of GB Pant Hospital. The subjects were enrolled after confirmation of the diagnosis by clinical Evaluation, ECG and blood tests as per accepted criteria.

Group A: Patients of MI who do NOT have a family history (N=100), which are further sub grouped into:
Group A1: Patients who had MI for the first time (N=48)
Group A2: Patients who had recurrent MI (N=52)

Group B: Patients of MI who have a family history (N=100), which are further sub grouped into:
Group B1: Patients who had MI for the first time (N=33)
Group B2: Patients who had recurrent MI (N=67)

Inclusion criteria : Myocardial Infarction within 24 hours

Exclusion criteria: Patients of Diabetes Mellitus, Cancers, Patients of Chronic liver disease, Disease of inflammatory pathology like arthritis.

Collection and processing of blood samples:
Five milliliter (5ml) of peripheral blood was collected using all aseptic procedures, from all the participants within 24hrs of MI after confirmation of the diagnosis by clinical evaluation, ECG. Informed consent was taken before sampling. The serum was separated at 2000rpm for 10 minutes. Serum was stored at minus 20 degrees(in suitable aliquots) before the batch analyzed for various ELISA tests as detailed below.

A. Enzyme linked immunosorbent assays (ELISA):
The patient serum which were stored in aliquots at -20 degree centigrade were analyzed for
1. Human Oxidized- Low Density Lipoprotein
2. Human Anti-Oxidized-Low Density Lipoprotein

Methodology for ELISA :
Ox- LDL and anti-Ox-LDL abs. levels were estimated by using commercially available 'SincereBiotech' kits, according to the manufacturer's protocol.

Statistical analysis:
Categorical variables were presented in number and percentage (%) and continuous variables were presented as mean ± SD and median. Normality of data was tested by Kolmogorov-Smirnov test. If the normality was rejected then non parametric test was used.

Results:
The Ox-LDL levels were lower in Group A(3.8385±6.0492) as compared to Group B(6.8538 ±236.0338) but both are not significant in our results. However, anti-ox-LDL abs levels were higher in Group A(1.8940±1.9435) as compared to Group B(1.3366 ±1.8303) but again both are not significant according to our results.

Table-1(Oxidative stress biomarkers levels in study subjects)P

Parameters	Group A1 (N=16)	Group A 2 (N=17)	Group B1 (N=9)	Group B 2 (N=18)	*p-value between groups
Ox-LDL (µg/ml)	23.81±3.83	23.72±6.04	22.41±6.85	22.47±7.8	A1 Vs. A2 0.9602 B1 Vs. B2 0.4901
Anti-Ox-LDL abs (µg/ml)	14.67±1.89	14.02±1.92	14.50±1.33	15.02±1.83	A1 Vs. A2 0.3414 B1 Vs. B2 0.4526

Anti-oxidised low density lipoprotein antibody; Oxidised low density lipoprotein; µg/ml,

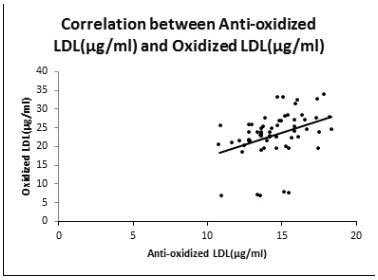

Figure 1:-Correlation between Anti-oxidized LDL(µg/ml) and Oxidized LDL(µg/ml)

Table 2:-Univarite logistic regression to find out effect of anti-oxidized LDL(µg/ml) and oxidized LDL(µg/ml) on recurrence of MI.

Recurrent MI	Beta coefficient	Standard Error	P value	Odds ratio	95% C.I.for Odds ratio	
					Lower	Upper
Anti-oxidized LDL(µg/ml)	-.021	.145	.883	.979	.736	1.302
Oxidized LDL(µg/ml)	-.009	.044	.845	.992	.910	1.080

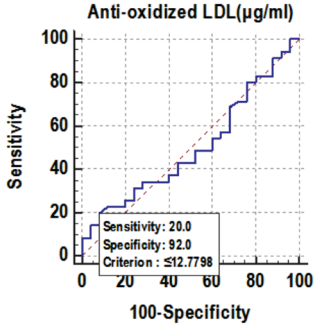

Figure 2:-Receiver operating characteristic curve of anti-oxidized LDL(µg/ml) to find out cut off point and area under curve to predict recurrent MI.

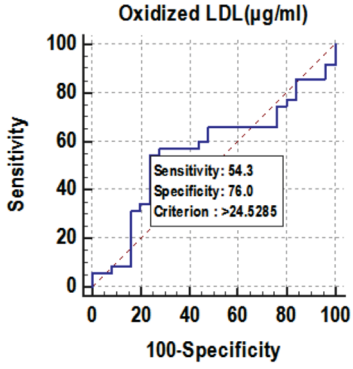

Figure 3:-Receiver operating characteristic curve of oxidized LDL(µg/ml) to find out cut off point and area under curve to predict recurrent MI.

DISCUSSION:
CAD is one of the most common types of heart disease. It leads to MI which is the major cause of death in men and women nowadays. And the most common cause of MI is atherosclerotic plaque. The levels of antioxidants play an important role in atherosclerosis. Their reduced activity promotes oxidative stress and ROS generation which further oxidizes the lipids. Oxidative modification of LDL alters its structure and allows the uptake of LDL by scavenger receptors on macrophages, endothelial and smooth muscle cells[22-24]. After its uptake by macrophages, it outflanks a negative feedback mechanism. This unrestricted uptake allows increased invasion of LDL into

macrophages while creating foam cells which is the primitive markers for atherosclerotic plaque deposition[25].

Our previous studies with angiographically proven atherosclerotic CAD patients have significantly higher levels of Ox-LDL[25].

These findings suggest that lipid oxidation and the consecutive induction of an immune response, in addition to the direct pro-inflammatory effects of Ox-LDL and anti-Ox LDL, are all mechanisms which might play role both in atherogenesis and in the pathogenesis of acute coronary syndromes.

Ox-LDL appears to be an immunogenic molecule which triggers the induction of anti-ox LDL antibodies. Thus, it is clear that there is an association between the presence of anti-ox LDL antibodies and atherosclerosis.

Our study shows that there might be no direct role of Ox-LDL and anti-Ox-LDL antibodies in MI patients as we didn't get significant results but few other studies suggests that these parameters plays an important role[22-24]. So, it might be possible that reduced activity of antioxidants promotes oxidative stress and ROS generation leading to the development of atherosclerosis and other CADs. While still there is no clear evidence that they play role in MI. It is quite possible that these insignificant results are due to smaller sample size we had studied. Therefore, a detailed study in extended sample size with patients follow-up is compulsory to confirm the significance of oxidative stress biomarkers in Myocardial Infarction.

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