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OxLDL/HDL ratio and its correlation to myeloperoxidase activity in ACS patients

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***Abstract*---**Background: Oxidative stress in ACS patients results in oxidation of lipoproteins. Resultant oxLDL (oxidized Low Density Lipoprotein) is a marker for lipid peroxidation. OxLDL/HDL ratio can be used as predictor of oxidative stress and inflammation in atherosclerotic cardiovascular diseases. Myeloperoxidase (MPO), oxidative stress related enzyme, increases in inflammatory conditions. Aim and Objectives: To evaluate oxLDL/HDL ratio in ACS patients. Correlation analysis of oxLDL/HDL ratio to oxidative stress markers, MPO and Malondialdehyde (MDA) were also attempted. Materials and Method: 60 ACS patients from Hospital's Cardiac Care Unit and 60 controls from Hospital's Health Checkup were selected. Results: oxLDL and oxLDL/HDL ratio were significantly higher in ACS patients. Significant increase was observed in Myeloperoxidase, MDA, Total cholesterol, Low Density Lipoprotein and Triglycerides in ACS patients. Significant positive correlation was observed between

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oxLDL/HDL and MPO and also between oxLDL/HDL and MDA. Conclusion: Increase in MPO, oxLDL and oxLDL/HDL ratio in ACS is indicative of the significance of MPO in oxidative damage to lipoproteins in cardiovascular disease (CVD) and in incidence of acute events in CVD. We suggest that oxLDL/HDL ratio and MPO can be used as predictive markers to monitor the precipitation of acute events in CVD.

***Keywords*---**acute coronary syndrome, myeloperoxidase, oxidized LDL, oxLDL/HDL ratio.

Introduction

Early stage of Acute Coronary Syndrome is linked to lipid peroxidation and production of reactive oxygen species (ROS) in arterial intima [1]. Atherosclerosis is a key contributor to Cardio Vascular Disease and is characterized by endothelial dysfunction and sub endothelial accumulation of lipids .In the presence of risk factors and genetic predisposition endothelial dysfunction occurs activating monocytes and attaching them to dysfunctional area. It activates and aggregates platelets propagating the pathological event. Ultimately monocytes turn into macrophages; Low Density Lipoprotein (LDL) undergoes oxidative damage, is phagocytosed by vessel wall macrophages and converted to oxLDL. Presence of this oxLDL triggers series of proinflammatory reactions spreading the stimulation of macrophages. They take up oxidized lipids and become foam cells [2]. It can be noted that foam cells are formed when endothelial cells are activated by inflammation and the resulting uncontrolled accumulation of oxLDL and cholesterol esters within the macrophages located in intima [3].

High-density lipoprotein (HDL) is proven to have antioxidant properties which prevents the occurrence and improves the course and outcome of cardiovascular diseases. This protective function may be due to the components of HDL, associated enzymes which provides protection to HDL by reducing synthesis of free radicals or reactive oxygen species .It acts as a lipoprotein that acquires harmful pro-atherogenic lipids and keeps them restricted to an atmosphere where they can be inactivated or transferred to liver for excretion [4].Clinical trials have been conducted with nutritional intervention which shows correlation between increased antioxidant property of HDL to decreased risk of atherosclerosis [5].

Myeloperoxidase (MPO) a peroxidase in nature containing heme, is present in neutrophils and monocytes. Chloride and hydrogen peroxide combines in the presence of MPO to generate hypochlorous acid .The powerful oxidant property of MPO is attributed to this reaction. Many inflammatory diseases are linked to this MPO generated oxidants. This oxidant role of MPO cannot be ignored while assessing the oxidative stress in Acute Coronary Syndrome patients [6]. In studies that deal with oxidative stress, Malondialdehyde (MDA) has been a preferred marker for detecting the extent of lipid peroxidation [7].

With oxidative markers MPO, MDA, oxLDL and antioxidant HDL in mind, this study was designed to assess oxLDL/HDL ratio in acute coronary syndrome

patients and to correlate oxLDL/HDL ratio with oxidant markers MPO and MDA. There are studies showing significance of oxLDL in Acute Coronary Syndrome, but, not many studies are available that shows the correlation of oxLDL/HDL ratio with oxidative stress markers.

Materials and Methods

Study Setting: Cross sectional study conducted in Biochemistry Department from January 2020 till January 2021.
Sample Size: With 90 % power and significance level of 5 %, assuming a difference of 100 U/ml of oxLDL and a standard deviation of 150, the sample size required is 49 per group.
Inclusion criteria: Clinically proven ACS patients were included in the study
Exclusion criteria:

1. Patients of age > 70 years were excluded.
2. Patients with renal, liver disease and cancer were excluded.

Informed Consent: Written consent to participate in the study was obtained from all participants before start of study.
Ethical approval: Ethical approval was obtained from the institute where research was carried out.
With 90 % power and significance level of 5 %, assuming a difference of 100 U/ml of oxLDL and a standard deviation of 150, the sample size required is 49 per group.

60 Acute Coronary Syndrome patients were selected from the critical care unit (CCU) of the hospital .60 subjects without any history of CAD ,within the same age group was selected as controls from Health checkup of our Hospital. At the time of admission to CCU, 4 ml peripheral blood was drawn from all subjects. Informed consent was obtained as per the criteria laid down by the institute scientific and ethics committee. Serum was separated from blood samples by subjecting it to centrifugal force of 3000 rpm for 10 minutes. Separated samples were stored at -80 ℃ till the time of analysis. Demographic and other information relevant for this study were recorded using Proforma. Following biochemical parameters were analyzed - Total Cholesterol (TC), High Density Lipoprotein Cholesterol (HDL-C), Low Density Lipoprotein Cholesterol (LDL-C), Triglyceride (TG), oxidized Low Density Lipoprotein (oxLDL), Myeloperoxidase (MPO) and Malondialdehyde (MDA). VLDL and oxLDL/HDL ratio was calculated from the available data.

Oxidized Low Density Lipoprotein was measured using commercially available IMTEC-ox-LDL antibody Ig (GM) ELISA kit. Spectrophotometric assay of Matheson et al.1981 was used to determine MPO. Yagi's & Satoh's (1984) method was used to estimate MDA. Plasma Lipid Profile was estimated in fully automated chemistry analyzers available in the hospital laboratory.

Statistical Analysis

Normally distributed data are represented as mean ± SD.t Test was used to find the difference in baseline characteristics between two groups. Correlation Analysis between oxLDL and MPO were tested by calculating Correlation Coefficient (Karl Pearson).Results were considered to be statistically significant if the P value is <0.05. Data was analyzed using SPSS software.

Result

The study was conducted to assess the level of oxLDL together with oxidant markers like MPO and MDA. An attempt was also done to correlate oxLDL /HDL ratio with Myeloperoxidase enzyme activity and level of MDA in ACS patients. Serum lipid profile was estimated in ACS patients. The values obtained were compared with control subjects of the same age group. The test population comprised of 60 subjects that included 42 (70%) males and 18 (30%) females. Control group consisted of 60 subjects that included 40 (68%) males and 19 (32%) females. The age of the test group included 36 to 69 years with mean age 52.5 years, whereas that of control subjects varied from 34 to 67 years with a mean age 50.5 years. Baseline characteristics of ACS patients and healthy controls are shown in Table 1.

Table 1: Baseline characteristics of ACS and controls

Baseline characteristics	ACS (n=60)		Control (n=60)	
Age	36-69 (52.5)		34-67(50.5)	
Gender	M	F	M	F
	42 (70%)	18 (30%)	41 (68%)	19 (32%)
Waist circumference	95.5 (69-122)		76.5 (56-103)	
BMI (kg/m^2)	29.32 ± 2.41		24.14 ± 2.07	

Distribution of test and control group according to the conventional risk factors of ACS is given in Table 2.

Table-2: Distribution of test and control according to the risk factors

RISK FACTORS	ACS (n=60)	Control (n=60)
DIABETES	(63%)	(40 %)
OBESITY	(56 %)	(21%)
HYPER TENSION	(67%)	(28 %)
CURRENT SMOKING	(72%)	(19 %)
HYPERLIPIDMIA	(68%)	(12 %)
ALCOHOL	(37 %)	(22 %)
FAMILY HISTORY OF CAD	(33 %)	(9 %)

Lipid profile in ACS patients and controls were compared. Mean value of TC, TG, HDL, LDL, VLDL, of these two groups are given in table 3.

Table 3. Lipid profile in ACS and control subjects

PARAMETERS mg/dl	ACS patients	Control	*P*-Value
TOTAL CHOLESTEROL (mg/dl)	231.48 ± 52.18	199.51 ± 31.86	.003
HIGH DENSITY LIPOROTEIN (mg/dl)	39.54 ± 8.17	52.14 ± 11.07	.000
LOW DENSITY LIPOPROTEIN (mg/dl)	159.53 ± 23.36	142.22 ± 36.90	.018
VERY LOW DENSITY LIPOPROTEIN (mg/dl)	34.41 ± 15.12	22.90 ± 7.78	.000
TRIGLYCERIDE (mg/dl)	172.09 ± 75.64	114.51 ± 38.91	.000

Results are expressed in mean ± SD
TC, TG, LDL and VLDL showed significant increase in ACS patients compared to that of controls .HDL value was significantly high in controls.

Oxidative stress related enzyme MPO, Lipid peroxidation product MDA, and oxidation product oxLDL was evaluated in ACS patients and controls. OxLDL/HDL ratio was calculated from the obtained data. Mean value of MPO, MDA, oxLDL and oxLDL/HDL ratio in ACS patients and controls were compared in table 4.

Table 4: MDA, MPO, oxLDL, oxLDL/HDL ratio in ACS and controls

Parameters	ACS patients	Control	*P*-Value
MDA ng/ml	3.02 ± 0.85	1.98 ± 0.44	.000
MPO U/ml	372.18 ± 156.80	162.16 ± 97	.000
oxLDL ng/ml	52.78 ± 57.91	16.67 ± 4.22	.001
OxLDL/HDL	1.39 ± 1.52	0.33 ± 0.107	.000

Results are expressed in mean ± SD
MPO, MDA, oxLDL and OXLDL/HDL ratio in ACS patients were significantly higher compared to controls.

Correlation analysis was done between oxidative stress indicator, oxLDL/HDL ratio and pro-oxidant enzyme, myeloperoxidase. Correlation analysis was also done between oxLDL/HDL ratio and malondialdehyde. Correlation Analysis is given in Table 5.

Table 5. Pearson correlation analysis of oxLDL/HDL ratio with MPO and MDA

	ACS	MPO	MDA
oxLDL/HDL ratio	Pearson Correlation	0.748**	0.489**
	Sig. (2-tailed)	<0.0001	0.005

**Correlation is significant at 0.01 level (2-tailed).

A statistically significant positive correlation was observed between oxLDL/HDL and MPO. Significant positive correlation was observed between oxLDL/HDL and MDA also.

Discussion

This study was conducted to evaluate the role of oxLDL /HDL ratio in predicting the acute events in Acute Coronary Syndrome. In the present study, it was observed that males (70%) in the age group of 36-69 were more affected with ACS rather than females. Waist circumference (95.5 cm) and BMI (29.32 ± 2.41kg/m^2) were also more in ACS group as compared to controls. Mirza et al [8] in their study in 2018 showed that the mean age of the ACS patients was 36 years and 85% of their study population was males. The mean BMI (29 kg/m2) and waist circumference (98 cm) of the patients were higher than the controls. In our study we found that 72 % of ACS patients were current smokers. Yagi et al [9] has indicated that cigarette smoking is the ultimate trigger for the onset of ACS. They had also suggested that by quitting smoking after CAD is diagnosed, patients can avoid subsequent cardiac events relatively early.

In our study population, Hypertension and hyperlipidemia were also found to be more in ACS patients. Study conducted by Ge J et al [10] showed that hypertension on its own, pose as a risk factor for multivessel coronary artery disease among ACS patients. Hyperlipidemia is another proven risk factor for acute coronary syndrome. A tendency of obesity with high prevalence of smoking and hyperlipidemia was seen in young adults with ACS [11]. Lipid profile was assayed in the patients of ACS group. Total cholesterol, LDL, Triglycerides and VLDL were significantly increased in patients compared to that of controls. HDL was found to be low in patients when compared to controls. Similar findings were observed by Bounafaa et al [12] whose study also showed significant increase in TG and LDL and also significant lowering of HDL.

The level of oxidized LDL was assessed in patients and control subjects. A significant increase in oxLDL in ACS group was observed in our study. Study conducted by Trpkovic et al [13] showed that oxLDL was involved in every step of development of atherosclerosis. OxLDL was also increased in comorbidities like diabetes mellitus, obesity and metabolic syndrome. After comparing many cohort studies, Gao et al [14], has shown that increased ox-LDL was associated with increased risk of Atherosclerotic Cardiovascular events.

Level of oxidative stress marker, MDA was assayed in this study. MDA level significantly increased in ACS patients. Study by Emam et al [15] shows that MDA increased significantly in ACS group compared to controls. Study by Maly et al [16] also showed significant increase in MDA levels in ACS group than in

controls. Level of prooxidant Myeloperoxidase activity was assessed in study population to know the extent of oxidative stress. MPO was found to be increased with significance in ACS patients than in controls. Study conducted by Govindarajan et al [17] to analyze the prognostic value of plasma myeloperoxidase in patients with suspected acute coronary syndrome showed that Plasma MPO in MI patients were significantly high when compared to patients with chest pain in both physical activity or rest.

Sawicki et al [18] had conducted a study in ACS patients with chest pain which aimed at finding the diagnostic importance of MPO and/or when it was analyzed together with cardiac troponin I. They suggested MPO as a dependable marker at the early stages of ACS especially in Troponin negative patients. If MPO is assessed in early stages, disease diagnosis can be accelerated. The diagnostic efficacy of MPO can be further enhanced by combining it with TnI. Another study conducted in 2016 concluded that serum MPO can be used to predict acute coronary syndrome [19].

Studies show a strong interrelationship between MPO and CVD. Elevated levels of MPO indicates bad prognosis and there is also risk of increase in death due to CVD.MPO is therefore considered a biomarker for patients with acute coronary syndrome with high risk. Due to this reason MPO is hinted as a versatile target for cardiovascular protection [20]. In our study an attempt was made to calculate oxLDL/HDL ratio from the obtained values. The ratio of oxLDL/HDL was significantly high in ACS patients than in controls. Study conducted in Navajo population by Harmon et al [21] showed that the ratios of oxLDL/HDL and oxLDL/LDL may be more informative than oxLDL alone. They have also reported a matching study in Type 2 Diabetes Mellitus patients, ratio of oxLDL to both HDL and LDL were correlated with atherosclerosis.

Correlation of oxLDL/HDL ratio with oxidative markers, Myeloperoxidase and MDA was also attempted. Not many studies are available that shows correlation of oxLDL/HDL with MPO and MDA. It was seen in our study that oxLDL/HDL showed a statistically significant positive correlation with MPO and MDA. There are not many studies that show correlation between oxLDL/HDL ratio and oxidative stress markers. One study conducted in Navajo population [21] reported that correlational analysis showed that oxLDL alone was not associated with HbA1c, but oxLDL/HDL, were significantly associated with HbA1c, CRP and glucose.

Conclusion

oxLDL, MPO, MDA and oxLDL/HDL ratio were significantly high in Acute Coronary Syndrome patients. This shows that there is strong oxidative damage leading to increase in oxidative markers. Findings of this study also points to the role of MPO in oxidative damage to lipoproteins in cardiovascular disease and in incidence of acute events in CVD. Therefore we propose that oxLDL/HDL ratio and MPO can be used as a predictive marker to know the precipitation of acute events in CVD patients.

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Authors' contributions: This work was carried out in collaboration among all authors.

1. SV: Acquisition of Data, Conception and design.
2. RV: Methodology, Supervision, Final Approval
3. JR: Software, Validation.
4. MMP: Visualization, Investigation
5. SSA: Analysis and interpretation of data, Reviewing and Editing.

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