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Biochemistry Section

Oxidised LDL Cholesterol (Ox-LDL-C) and Ox-LDL-C/HDL Cholesterol (HDL-C) Ratio in Acute Coronary Syndrome Patients versus Chronic Coronary Artery Disease Patients on Statin Treatment

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ABSTRACT

Introduction: Oxidised Low Density Lipoprotein Cholesterol (Ox-LDL-C) and High Density Lipoprotein Cholesterol (HDL-C) have antagonistic action in the development of atherosclerotic cardiovascular disease. Use of statins reduces cardiovascular risk by reducing LDL-C levels and also by increasing HDL-C. No systematic studies were carried out to study the role of HDL-C as an antioxidant and its effect in decreasing Ox-LDL-C.

Aim: To compare the values of Ox-LDL-C/HDL-C in patients with chronic Coronary Artery Disease (CAD) and patients with Acute Coronary Syndrome (ACS) and to evaluate the levels of Ox-LDL-C and Ox LDL-C/HDL-C ratio in patients treated with the two different statins.

Materials and Methods: In this cross-sectional study 30 patients with ACS and 30 patients with chronic CAD on rosuvastatin or atorvastatin were included in the study. Apparently normal 27 age and sex-matched controls without CAD was included in the study. Lipid profile was estimated using fully auto analyser and Ox-LDL-C was estimated using ELISA kits. Statistical analysis was done using SPSS version 16 software. A p-value of <0.05 was considered as statistically significant.

Results: Hypertension and diabetes were found to be significantly associated with CAD (p-value 0.03). There was significant correlation between total cholesterol, triglycerides, with CAD. The levels of triglycerides, Ox-LDL-C and Ox-LDL-C/HDL-C were significantly higher (p<0.05) in ACS patients compared to chronic CAD and normal. Total cholesterol and LDL-C were lower in chronic CAD patients on atorvastatin treatment compared to patients on rosuvastatin treatment.

Conclusion: Ox-LDL-C/HDL-C ratio is a better predictor of acute coronary events. In addition to lipid lowering action, statins have pleiotropic benefits including prevention of LDL oxidation.

Keywords: Diabetes, Hypertension, Oxidsied low density lipoprotein cholesterol

INTRODUCTION

Ox-LDL-C plays a potential role in the development of atherosclerosis by stimulating infiltration of monocyte. It also induces migration of smooth muscle cell and its proliferation. HDL-C has antagonistic action to that of Ox-LDL-C because of its antioxidant and anti-inflammatory properties [1]. Atheroprotective activities of HDL-C also include its maintenance of endothelial cell functions and mediating reverse cholesterol transport. HDL-C levels are inversely related to the level of Ox-LDL-C and risk of CAD. The antioxidant action of HDL-C contributes to the inverse relationship between HDL-C and CAD. The enzymes like paraoxonase and Lecithin Cholesterol Acyl Transferase (LCAT) are associated with HDL-C and prevent the oxidation of LDL-C which is an important step in the pathogenesis of atherosclerosis [2]. Ox-LDL-C inhibits the action of these enzymes. Hence, Ox-LDL-C and HDL-C are antagonists in their action for the development of atherosclerotic cardiovascular disease.

The use of statins has major role in the management of individuals with cardiovascular risk factors. There are several effects of statins that help in reducing the cardiovascular risk. The most important benefit of statins is reduction of LDL-C which is supported by various studies [3-6]. However, there are also several evidences that suggest that use of statins also increases the levels of HDL-C which may contribute to its beneficial effects [7]. Apart from its lipid lowering effect, statins also have anti-inflammatory and antioxidant effects.

Several studies have been done to evaluate the lipid lowering effects of different types of statins [8-10]. No systematic studies were carried out to study the role of HDL-C as an antioxidant and its effect in decreasing Ox-LDL-C and no systematic studies have been done to evaluate and compare the pleiotropic benefits of different types of statins. Therefore, the present study was under taken to compare the values of Ox-LDL-C/HDL-C in patients with chronic CAD and patients with ACS and also compare the levels of Ox-LDL-C and Ox-LDL-C/HDL-C ratio in patients treated with the two different statins thereby assessing the pleiotropic statin benefit.

MATERIALS AND METHODS

A cross-sectional study was conducted in which 30 patients with ACS referred from coronary care unit and 30 clinically proved CAD patients on statin treatment (atorvastatin/rosuvastatin treatment at least for two year) referred from Out Patient Department of tertiary care hospital in South Kerala during the period of December 2017 to July 2018 were included. Twenty seven healthy, age and sex-matched subjects without CAD were selected from the staff of the hospital formed the control group.

Group 1: ACS patients

Group 2: CAD patients on statin treatment (Atorvastatin/Rosuvastatin)

Group 3: Normal controls

Patients with history of exposure to ionic radiations, chemotherapy and other mutagenic agents were excluded. Subjects with any form of malignancy and other chronic disorders are excluded. Pregnant ladies are also excluded from the present study.

The study was approved by Institutional Ethics Committee (No. PIMS & RC/E1/388A/2017) at Pushpagiri Institute of Medical Sciences and Research Centre, Thiruvalla, Kerala, India. Once included, detailed clinical and other relevant data were obtained from the subjects using proforma. After taking written informed consent, five mL of venous blood was collected after 12 hours of fasting from all the subjects. Total cholesterol by CHOD-PAP method [11], Triglycerides by Enzymatic GPO method [12], HDL-C by homogeneous enzymatic colorimetric assay [13] and LDL-C by homogeneous enzymatic colorimetric assay [14] were estimated in automated clinical chemistry analyser Beckman Coulter AU680 using company provided reagents on the same day of collection. Ox-LDL-C was estimated using commercially available ELISA kit (Human IMTEC ELISA KIT Catalog No: ITC59500). The test was based on the simultaneous incubation of serum samples with both Ox-LDL-C (immobilised on microtiter strips) and the native LDL-C (immobilised on the pins of the TSP plate). Subsequent binding of anti Ox-LDL-C antibodies from patient's serum to the microtiter plate was detected with peroxidase labeled secondary antibody that is directed against human IgG and IgM. Substrate was added. Intensity of the colour was directly proportional to the concentration of the detected antibodies. Following the addition of stop solution, the colour changed from blue to yellow. Absorbance was read at 450 nm [15]. The ratio of Ox-LDL-C and HDL-C was calculated and tabulated.

STATISTICAL ANALYSIS

The statistical analysis was done with SPSS version 16 software. Association between risk factors among the CAD patients and normal subjects without CAD was done by chi-square test. Logistic regression analysis was done to find out the independent variables for CAD. Comparison of lipid profile parameters, Ox-LDL-C and Ox-LDL-C/HDL-C of the three groups were done by Post-Hoc Analysis. A p-value of <0.05 was considered as statistically significant.

RESULTS

Hypertension and diabetes were found to be significantly associated with CAD. Subjects with hypertension have 3.05 times more risk for developing CAD than those without hypertension (OR=3.05) Subjects with diabetes have 3 times higher risk than subjects without diabetes for developing CAD [Table/Fig-1].

Life style/Risk factors	OR	CI	χ^2	p-value
Smoking	0.96	0.324-2.896	0.003	0.95
Alcoholism	0.53	0.186-1.529	1.392	0.23
Hypertension	3.05	1.125-8.291	5.015	0.03*
Diabetes	3.50	1.451-9.281	5.25	0.04*
Family history of CAD	2.20	0.443-10.982	0.972	0.49

[Table/Fig-1]: Odds's ratio (OR), 95% Confidence interval (CI) and significance for test and control group according to lifestyle/risk factors for CAD (Chi-square test).
**: significant*

The variables which are found to be statistically significant in the logistic regression analysis were total cholesterol and triglycerides [Table/Fig-2].

	OR	95.0% CI		p-value
Total cholesterol (mg/dL)	1.07	1.003	1.148	0.04*
Trigylcerides (mg/dL)	0.97	0.958	0.991	0.01*
HDL-C (mg/dL)	0.95	0.860	1.060	0.38
LDL-C (mg/dL)	0.95	0.893	1.024	0.19
Ox-LDL-C (U/mL)	0.80	0.636	1.023	0.07
Ox-LDL-C/HDL-C ratio	2.36	0.491	1.14	0.07

[Table/Fig-2]: Logistic regression analysis showing correlation between Lipid profile, Ox-LDL-C and Ox-LDL-C/HDL-C ratio value with Coronary artery diseases (CAD).
**: significant*

Association between risk factors among the CAD patients and normal control with Ox-LDL-C was done by Chi-square test. Smoking was found to be significantly associated with high Ox-LDL-C (>15.5 U/dL). Subjects with smoking have 4.4 times more risk for causing high Ox-LDL-C than those without smoking (OR=4.468, CI=1.191-16.765). Association between risk factors among the CAD patients and normal control with Ox-LDL-C/HDL-C was done. Hypertriglyceridemia (Triglyceride >150 mg/dL) was found to be significantly associated with high Ox-LDL-C/HDL-C ratio (>0.38). Subjects with hypertriglyceridemia have 3.09 times more risk for having high Ox-LDL-C than those with normal triglyceride level (OR=3.09, CI=1.244-7.679).

The levels of triglycerides, Ox-LDL-C and Ox-LDL-C/HDL-C were significantly higher (p<0.05) in ACS patients compared to chronic CAD and normal. No such difference was observed between chronic CAD and normal subjects [Table/Fig-3].

Parameter	ACS n=30	Chronic CAD n=30	Normal n=27	F	p-value
Total cholesterol (mg/dL)	201.48±48.76	183.83±62.34	213.22±39.75	2.36	0.10
Triglycerides (mg/dL)	168.76±77.20	118.93±51.9	125.26±69.58	4.86	0.01*
HDL cholesterol (mg/dL)	44.88±15.39	49.37±12.75	49.56±12.72	1.096	0.33
LDL cholesterol (mg/dL)	136.53±35.145	117.33±52.48	142.22±36.9	2.74	0.07
Oxidised LDL cholesterol (U/mL)	45.1167±61.21	22.1±25.31	20.75±19.05	3.38	0.03*
Oxidised LDL cholesterol/HDL cholesterol ratio	1.0819±1.50	0.4554±0.45	0.4676±0.55	3.91	0.02*

[Table/Fig-3]: Levels of Total cholesterol, Triglycerides, HDL-C, LDL-C, Ox-LDL-C, Ox-LDL-C/HDL-C ratio (Post-Hoc Analysis).
**: significant*

Lipid profile parameters, Ox-LDL-C and Ox-LDL-C/HDL-C of atorvastatin (Group 1) versus rosuvastatin (Group 2) treated chronic CAD patients were compared using ANNOVA in [Table/Fig-4]. Total cholesterol and LDL-C were lower in chronic CAD patients on atorvastatin treatment compared with chronic CAD patients on rosuvastatin treatment.

Parameters	Groups	Mean±Std. Dev	Z	p-value
Total cholesterol (mg/dL)	1	176.31±69.888	1.102	0.27
	2	192.43±53.702		
Triglycerides (mg/dL)	1	127.88±55.178	-0.977	0.33
	2	108.71±47.811		
HDL cholesterol (mg/dL)	1	46±12.237	-1.601	0.11
	2	53.21±12.656		
LDL cholesterol (mg/dL)	1	113.31±56.807	-0.956	0.35
	2	121.93±48.772		
Oxidised LDL cholesterol (U/mL)	1	27.8438±4.42659	-1.485	0.15
	2	15.5357±33.8658		
Oxidised LDL cholesterol/ HDL cholesterol ratio	1	0.5842±0.58832	-2.703	<0.01*
	2	0.3081±0.13016		

[Table/Fig-4]: Comparison of lipid profile parameters, Ox-LDL-C and Ox-LDL-C/HDL-C of atorvastatin versus rosuvastatin treated chronic CAD patients (ANNOVA).
**: significant*

Group 1- Chronic CAD patients treated with atorvastatin

Group 2- Chronic CAD patients treated with rosuvastatin

DISCUSSION

Various risk factors such as diabetes, hypertension, smoking and excess alcohol contribute to the development of CAD. Type 2 Diabetes has an independent and causal effect on the risk of major cardiovascular events. According to Baguet JP et al., among the

numerous risk factors associated with coronary artery disease, hypertension plays a major role due to its high frequency and physiopathogenesis [16]. This is supported by present study as we found a significant association for CAD with hypertension and diabetes mellitus.

A poor diagnostic quality for total cholesterol and LDL cholesterol was observed by Johnston N et al., for identifying CAD patients in his studies [17]. According to Manurung D, the combination of low HDL cholesterol level and high triglycerides is the most significant risk factor in ACS patients [18]. In the present study, no significant difference was observed in the levels of total cholesterol, LDL-C and HDL-C among the three groups. High levels of triglycerides levels was observed in ACS patients compared to chronic CAD patients with statin treatment and normal subjects without CAD which is the similar to the findings of the previous study.

As per the studies by Johnston N et al., the assessment of Ox-LDL-C to HDL-C ratio is an important blood lipid test for identifying risk among possibly healthy men and women [17]. Ghosh J et al., had same observation [19]. Since HDL-C decrease the oxidation of LDL-C, decrease in HDL-C increases the Ox-LDL-C to HDL-C ratio. A significant correlation between Ox-LDL-C to HDL-C ratio with CAD was observed in present study.

Atorvastatin, which is a competitive inhibitor of HMG-CoA reductase is found to have role in lowering LDL-C, but has a very little influence on HDL-C [20]. Rosuvastatin which is a synthetic statin have larger number of binding interaction with HMG-COA reductase and hence have higher affinity towards the enzyme. For lowering LDL-C rosuvastatin is the most effective among the available statins. Apart from that, it increases HDL-C and reduces triglyceride-rich lipoprotein particles [21]. According to Jones PH et al., rosuvastatin has HDL-C raising ability than atorvastatin [22]. Present study findings are in agreement with the above studies as we also found that CAD subjects with rosuvastatin treatment have high HDL-C and significantly low triglyceride levels. Ox-LDL-C/HDL-C ratios were found to be significantly increased in ACS patients compared to CAD subjects on statin treatment and normal subjects without CAD in present study. Also, a significant decrease of Ox-LDL-C/HDL-C ratio is observed in CAD subjects with rosuvastatin treatment. Present study findings are supported by the study by Vasankari T et al., [23].

LIMITATION

Subjects were not categorised based on age or lifestyle factors which may also have significant effect on the parameters used in the study. A large study including more number of subjects with various lifestyle factors is required for clarifying the results of this study.

CONCLUSION

There is significant association of CAD with hypertension and diabetes mellitus. Subjects with smoking have 4.4 times more risk for causing high oxidised LDL-C than those without smoking. There is significant correlation between oxidised LDL-C to HDL-C ratio with CAD. The levels of triglycerides, Ox-LDL-C and Ox-LDL-C/HDL-C ratio were significantly higher ($p<0.05$) in ACS patients compared to chronic CAD and normal subjects. Hence Ox-LDL-C/HDL-C ratio is a better predictor of acute coronary events. In addition to lipid lowering action statins have pleiotropic benefits including prevention of LDL oxidation. According to this study Ox-LDL-C/HDL-C ratio lowering ability of rosuvastatin, was superior to atorvastatin.

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