

Comparison of Serum Oxidized Low Density Lipoproteins in Healthy Individuals and Patients with Ischemic Heart Disease

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ABSTRACT

Background At present, approximately 300 variables have been included in the list of risk factors for coronary artery disease (CAD). However, atherosclerosis and its resultant complications may develop even if there is no apparent risk factor. Cases without identifiable risk factor(s) highlight the importance of further research regarding the etiology, pathogenesis, and interplay of various factors in the process of atherogenesis and it is anticipated that more risk factor(s) might be identified in future. It has been reported that modified low density lipoproteins (LDL) uptake and degradation by macrophages is an important event in the early stages of atherosclerosis and oxidized LDL (OxLDL) possess many potentially proatherogenic properties.

Aim: To compare serum OxLDL levels of healthy individuals and patients with ischemic heart disease (IHD).

Methods: A total of 137 subjects were included in the study, 90 patients of IHD as cases and 47 healthy subjects with no history of IHD as controls. We compared serum OxLDL levels of healthy individuals and patients with IHD.

Results: No significant difference was observed between serum OxLDL concentration of cases and controls.

Conclusion: There is no difference in serum OxLDL levels of healthy individuals and ischemic heart disease patients.

Key words: Acute myocardial infarction, Coronary artery disease, Ischemic heart disease, oxidized LDL

INTRODUCTION

A number of prospective studies, most notably the Framingham study and the multiple risk factor intervention trial have helped to identify the risk factors that predispose to atherosclerosis.¹ At present, approximately 300 variables have been included in the list of risk factors for CAD.² Recently, elevated levels of fibrinogen, plasminogen activator inhibitor-1 and homocysteine have been included in this list.³ Moreover, the role of inflammatory processes in atherogenesis is also under consideration.^{4,5} However, atherosclerosis and its resultant complications may develop even if there is no apparent risk factor.¹ Cases without identifiable risk factor(s) highlight the importance of further research regarding the etiology, pathogenesis and interplay of various factors in the process of atherogenesis and it is anticipated that more risk factor(s) might be identified in future.

In 1979, Goldstein et al. reported that modified LDL uptake and degradation by macrophages is an

important event in the early stages of atherosclerosis⁶. It has been reported that oxidized LDL possess many potentially proatherogenic properties⁷.

The OxLDL generated in the vessel wall may diffuse into the circulation or alternatively; LDL in the circulation may in part be oxidatively modified. Although either is possible, evidence favors the former as the mechanism by which circulating OxLDL is generated⁸⁻¹⁰. It has been hypothesized that the OxLDL present within unstable plaques may be released into the blood stream in patients with severe endothelial injuries, such as plaque erosion or rupture¹¹. At present, plasma levels of OxLDL are being measured by Enzyme linked immunosorbent assay (ELISA)¹².

Ehara et al. were first to report that OxLDL levels relate directly to the severity of coronary syndromes. They reported that the levels of OxLDL were \approx 4 times higher in patients with AMI¹¹. Holvoet et al. has reported that circulating OxLDL is a sensitive marker of CAD and suggested that addition of OxLDL to the established risk factors may improve cardiovascular risk prediction¹³.

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MATERIALS AND METHODS

This analytical cross-sectional study was conducted at University of Health Sciences (UHS) and Lahore Medical and Dental College (LM&DC), Lahore in collaboration with Punjab Institute of Cardiology (PIC), Lahore. The study included 90 patients of IHD (40-60 years, both sexes) as cases and 47 healthy subjects (40-60 years, both sexes) with no history of IHD as controls.

Inclusion Criteria: Diagnosed cases of acute myocardial infarction (AMI) (within first 48 hours of AMI) admitted in different wards of PIC were selected as cases. Diagnosis of AMI was based on the typical history, suggestive ECG changes, and serum cardiac biomarkers.

Controls: Forty seven healthy subjects with no history of IHD were selected as controls.

Exclusion Criteria: Subjects suffering from acute and chronic inflammatory conditions and smokers were excluded. It has been reported that serum OxLDL/LDL ratio is higher in smokers, so we excluded smokers¹⁴.

Selected subjects were informed about the study and consent was obtained on the consent forms. Relevant history was recorded in proformas. Venous blood samples (10 ml) were collected after overnight fast (i.e., 12-14 hours fast) between 8:00 am to 9:00 am. As plasma triglyceride levels rise and both HDL and LDL cholesterol levels fall modestly after a fat-containing meal, plasma lipids are preferably measured after a 12-hour fast¹⁵.

Five ml of blood was transferred to labeled collection tubes and was analyzed for fasting lipid profile assay (cholesterol and triglycerides were determined by enzymatic colorimetric method on Hitachi 912 modular analyzer, HDL was determined by Homogenous enzymatic colorimetric test on Hitachi 912 modular analyzer). We calculated serum LDL cholesterol concentrations using the Friedewald equation ($\text{LDL cholesterol} = \text{Total cholesterol} - (\text{HDL} + \text{Triglycerides}/5)$).

The rest of 5 ml was transferred to labeled BD vacutainers (SST II Advance 5 ml) and placed in an ice box. Samples were transported within an hour of collection to Biochemistry laboratory, UHS, Lahore for further processing. Blood was allowed to clot and serum was separated by centrifugation. Approximately 500-700 μL of serum was transferred to each of two labeled aliquots (one for serum OxLDL and an extra one). Aliquots were stored at -80°C . Storage temperature was checked regularly twice a day i.e., at 9:00 am and 3:00 pm. Mercodia OxLDL ELISA kit was used for the quantitative measurement of OxLDL in human serum.

The data was entered and analyzed using SPSS (16.0). Independent sample t test was applied

to observe group mean differences. A p -value of <0.05 was considered as statistically significant.

RESULTS

Among cases, minimum and maximum LDL concentration was 41 mg/dl and 211mg/dl respectively. Among controls, minimum and maximum LDL concentration was 25 mg/dl and 178 mg/dl respectively. No significant difference was observed between LDL concentration of cases and controls (104.83 ± 26.895 vs. 110.21 ± 26.569 mg/dl, $p = 0.267$) (Table 1 and Figure 1). One participant had triglycerides >400 mg/dl and we could not calculate his LDL by Friedewald equation. According to Well's et al. and National Cholesterol Education Program III (NCEP) criterion, only two subjects in our study had high LDL (i.e., >160 mg/dl)¹⁶. Among cases, one male subject had LDL of 211mg/dl. Moreover, one male subject among controls had LDL of 178mg/dl. Among cases, minimum and maximum serum OxLDL concentration was 31.33U/L and 131.22U/L respectively. Among controls, minimum and maximum serum OxLDL concentration was 34.55 U/L and 131.29 U/L respectively. No significant difference was observed between serum OxLDL concentration of cases and controls (80.074 ± 27.490 vs. 86.558 ± 28.014 U/L, $p=0.195$) (Table 2 and Fig. 2).

Table 1: Differences in LDL of cases and controls

Parameter	Cases (Mean \pm SD)	Controls (Mean \pm SD)
LDL (mg/dl)	104.83 \pm 26.895	110.21 \pm 26.569

P Value: 0.267

Table 2: Differences in serum OxLDL of cases and controls

Parameter	Cases (Mean \pm SD)	Controls (Mean \pm SD)
Serum OxLDL(U/L)	80.074 \pm 27.490	86.558 \pm 28.014

P value: 0.195

Fig. 1: Differences in LDL of cases and controls

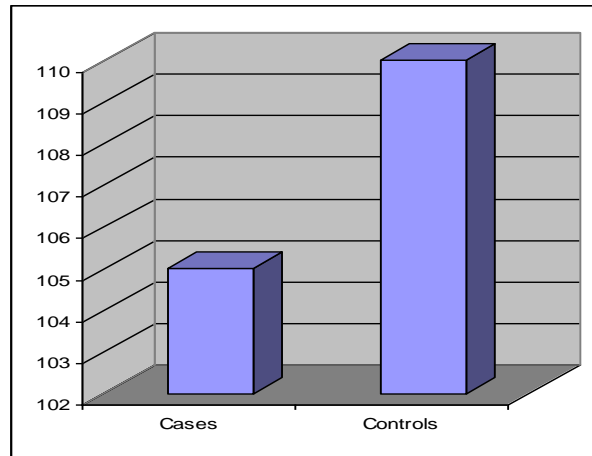
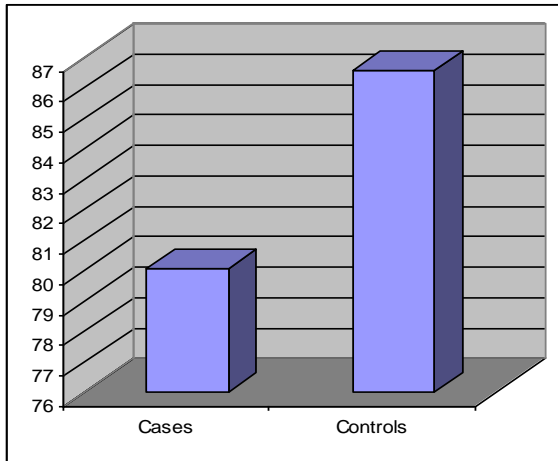


Fig. 2: Differences in serum OxLDL of cases and controls



DISCUSSION

Among cases, minimum and maximum LDL concentration was 41 mg/dl and 211 mg/dl respectively. Among controls, minimum and maximum LDL concentration was 25 mg/dl and 178 mg/dl. No significant difference was observed between LDL concentration of cases and controls (104.83±26.895 vs. 110.21±26.569mg/dl, p=0.267). Comparatively lower mean LDL concentration among cases (i.e., 104.83 mg/dl) than controls (i.e., 110.21mg/dl) might have resulted from the widespread prescription of HMG-CoA reductase inhibitors in IHD patients. These hypolipidemic drugs inhibit the rate-limiting step in hepatic cholesterol biosynthesis (the conversion of HMG-CoA to mevalonate), resulting in an increase in LDL receptors levels in hepatocytes and enhanced receptor mediated clearance of LDL cholesterol from the circulation. These drugs decrease total cholesterol by 20-30% and LDL cholesterol by 25-40%¹⁵.

We carried out the present study to compare serum OxLDL in the study population assuming that OxLDL may have a role in atherogenesis consequently leading to IHD. Among cases, minimum and maximum OxLDL concentration was 31.33 U/L and 131.22 U/L respectively. Among controls, minimum and maximum OxLDL concentration was 34.55 U/L and 131.29 U/L respectively. No significant difference was observed between serum OxLDL concentration of cases and controls (80.074±27.490 vs. 86.558±28.014 U/L, p=0.195).

Our results are generally in agreement with the ones reported by Johnston et al. They have reported mean ±SD of OxLDL (U/L) as 76.21±19.48 and 53.82±14.34 among cases and controls respectively¹². Although our values of mean OxLDL are comparatively higher in both groups, the

difference seems to be more obvious among controls. Moreover, another finding that is contrary to the other studies is the higher values of OxLDL among controls but statistical analysis revealed this difference to be insignificant. A notable factor that might have contributed is the frequent use of HMG-CoA reductase inhibitors in patients with IHD lowering LDL and consequently OxLDL fraction among cases. At this point we would like to suggest that OxLDL/LDL ratio (proportion of LDL that is oxidized in individual subjects) might prove more meaningful in assessing the oxidative stress than OxLDL alone. Sevanian et al. have also reported that the absolute amounts of LDL⁻ (oxidatively modified LDL) are related to plasma LDL cholesterol levels and can be much greater in hypercholesterolemic subjects¹⁷.

Comparatively higher values in both groups might have resulted from storing the serum samples at -80°C until analysis (as recommended by the manufacturer of the kit). Toshima et al. have reported that OxLDL levels fluctuated when the samples were frozen. Although the reason for the increase in OxLDL levels when the blood is frozen is not clear at present, one likely explanation is the higher activity of reactive oxygen species when blood is frozen or freeze-thawed⁸. This explanation seems to be relevant, because Johnston et al. employed the methodology, similar to ours for estimation of OxLDL with the exception of storage temperature (they stored samples at -70°C until analysis) and their values are comparatively lower than ours, in both groups¹².

We have not found any significant difference between OxLDL of cases and controls. A number of factors might have contributed. The one that deserves consideration is the fact that the oxidation of LDL is an extremely complex process. All the components of LDL particle including proteins, lipids and even the antioxidants can be oxidatively modified⁷. Moreover, oxidative susceptibility of LDL is influenced by both intrinsic and extrinsic factors. Intrinsic factors include antioxidant content and fatty acid composition of the LDL particle, presence of preexisting fatty acid peroxides in LDL, size of the particle and inter-particle reactions. Elevated levels of LDL⁻ in total LDL appear to contribute to oxidative susceptibility^{17,18}. Extrinsic factors include the pH of the microenvironment, local antioxidant concentrations, transition metal availability and presence of specific enzyme systems¹⁸. Moreover, consumption of diet rich in linoleic acid increases oxidative susceptibility of LDL and oleic acid and vitamin E rich diets confer significant protection to these particles¹⁷. However, contribution of smoking and diseases like diabetes mellitus (DM) is not yet

established. At present, little is known about the clearance of OxLDL from plasma⁸.

As OxLDL is a heterogeneous mixture of particles containing molecules that have been modified to different degrees and may even differ qualitatively, the exact quantification by any of the available methods is not possible leading to variation in the results of different studies. Johnston et al. have also reported that results vary significantly when different methods were employed for measurement of OxLDL (sandwich ELISA and competitive ELISA).¹² Ehara et al. reported that the mean plasma OxLDL for AMI patients corresponded to 0.04% of the total LDL. They also compared their values with the ones quoted by Holvoet et al. and concluded that the proportion of LDL that is reported to be oxidized varies in different studies. Their values for the oxidized proportion of LDL ($\approx 0.04\%$) were lower than the one quoted by Holvoet et al. ($\approx 5\%$) signifying the difference emerging from the employment of different assay methodologies. Ehara et al. used sandwich ELISA whereas Holvoet et al. utilized competitive ELISA method.¹¹ Moreover, we have not taken into consideration the various intrinsic and extrinsic factors affecting oxidative susceptibility of LDL.

Another note worthy factor that might have contributed is the time of collecting blood samples. Ehara et al. measured OxLDL in plasma samples collected within 24 hours after the onset of chest pain, whereas we assayed OxLDL in serum samples collected within first 48 hours of AMI, during which time much of the released OxLDL might have been cleared from plasma leading to the levels comparable to the control group.

CONCLUSION We conclude that there is no difference in serum OxLDL levels of healthy individuals and ischemic heart disease patients.

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