Experimental and Validation of Significance and Accuracy of Oxidized Low-Density Lipoproteins and Myeloperoxidase in the Screening of Cardio-Vascular Disease

Deepti Mandorsorwale, Bindu Sharma

The aim. To access the superiority of myeloperoxidase & oxidized low-density lipoproteins over each other acts as a better predictive marker gaining information regarding the severity of cardiovascular disease.

Materials and methods. 215 subjects are taken into consideration of which 54 are healthy controls, 52 are from stable angina pectoris, 53 are taken from unstable angina pectoris and 36 subjects are from acute myocardial infarction. Lipid profile parameters, oxidative stress markers, plasma myeloperoxidase and plasma oxidized low density lipoproteins were estimated by kit methods, thiorbarbituric acid reactive substances method, and colorimetric assay, sandwich and competitive enzyme linked immunosorbent assay techniques, respectively. Results were present as mean±SD, p<0.05 as significant, and Student’s unpaired “t” test. Comparative analysis by box and whiskers plot to check skewness and deviations within the values. Data analysis was performed by software package SPSS version 17.0.

Results. The oxidized low density lipoproteins levels found significantly elevated in all three cases subgroup contrary to insignificant levels of myeloperoxidase in stable angina pectoris compared to control. Box and whisker plot of myeloperoxidase levels showed no skewness in stable angina pectoris (non-significant), whereas unstable angina pectoris and acute myocardial infarction showed right skewness (highly significant), whereas plots of oxidized low-density lipoproteins show extensive interquartile range in the stable angina pectoris subgroup, suggesting scattered deviation in the mean values compared to unstable angina pectoris and acute myocardial infarction subgroup.

Conclusions. The study concluded that significantly elevated level of oxidized low-density lipoproteins in stable angina pectoris, unstable angina pectoris, and acute myocardial infarction subgroups with a scattered deviation of oxidized low density lipoproteins levels in the stable angina pectoris subgroup reflects its low prognostic reliability compared to plasma myeloperoxidase with marginal deviation and in insignificant elevation in stable angina pectoris. Thus, plasma myeloperoxidase and oxidized low density lipoproteins levels serve as independent predictors of cardiovascular disease, but plasma myeloperoxidase levels predict an increased risk over oxidized low density lipoproteins for subsequent cardiovascular events in stable and unstable angina and extend the prognostic information gained from traditional biochemical markers.

Keywords: cardiovascular disease, myeloperoxidase, oxidized low density lipoprotein, oxidative stress marker

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1. Introduction
Cardiovascular disease (CVD) includes group of diseases that affect the heart and circulation, referred clinically as coronary heart disease and stroke became the leading cause of mortality in India due to increased risk factors such as sedentary lifestyle, smoking, hypertension, diabetes, stress, and unhealthy eating habits. In 2016, the estimated prevalence of CVDs in India was estimated to be 54.5 million. One out of 4 deaths in India are now because of CVDs responsible for >80 % of this burden affecting patients in the most productive years of their lives [1].

Atherosclerosis is an inflammatory disease involving a crosstalk between vascular cells, monocytes, proinflammatory cytokines, chemokines, and growth factors, detected in the initiation and progression of the disease. As per several studies, accumulation of LDL in macrophages occurs only in modified form and modification particularly oxidation of low-density lipoproteins (LDLs) bears prime interest [2]. LDL is oxidized within the sub-endothelial space by various reactive species including superoxide, myeloperoxidase (MPO), 15-Lipoxygenase, and peroxynitrite [3].

Ox-LDL is widely described as a critical component of atherogenesis and triggers the inflammatory processes of the disease. It induces several potentially proatherogenic activities such as the production of proinflammatory cytokines and chemokines by endothelial cells, monocytes, and smooth muscle cells [3]. LDL receptors are utilized by monocytes that cannot bind, due to high levels of Ox-LDL and transform into macrophages that continues to engulf the modified lipopro-
teins leading to the formation of foam cells having an accumulation of many intracellular lipids, an essential feature of atherosclerosis [4].

Growing evidence demonstrates the action of MPO as a critical participant shunting between inflammation and oxidative stress in cardiovascular disease. The myeloperoxidase, along with hydrogen peroxide, forms free radicals and diffusible oxidative substances with antimicrobial activity. It also promotes oxidative damage of host tissue by exercising pleiotropic effects in the vascular system with potential impact in development, endothelial dysfunction, plaque, and response in ventricular remodelling after ischemic injury [5]. Moreover, MPO-derivated oxidants like hypochlorous acid, hydroxyl radical, nitrogen dioxide, and peroxynitrite could interact with both LDL and HDL to induce oxidation. Thus, MPO itself is involved in the oxidative modification of LDL to generate ox-LDL [6]. Earlier studies have documented Ox-LDL and MPO are cardiovascular risk factors [7]. However, the controversy regarding the superiority of MPO and Ox-LDL over each other as predictors of cardiovascular events has not been settled yet.

The aim of the research. The present study was to compare both plasma ox-LDL and plasma MPO to find out a better prognostic marker with increasing severity of the disease in three cases subgroup i.e., SAP, UAP, and AMI.

2. Materials and methods

The present observational case-control study consists of total 215 subjects divided into two groups: 54 control and 161 cases. Cases group is further divided into three sub-groups – SAP with 52 cases, UAP with 53 cases and 56 cases are in AMI subgroup, respectively. Sample size calculation for unmatched case-control study was done by power analysis method by using Open Epi, version 3, open-source calculator - Power CC, at 95 % confidence interval. All recruited subjects were selected from series of consecutive indoor and outdoor patient department attending the coronary clinic under the Department of General Medicine, Varun Arjun Medical College and Rohilkhand Hospital, Shahjahanpur from March 2018 to January 2020.

4 ml of overnight fasting blood samples were collected in the morning from the cubital vein in vials containing EDTA anticoagulant and transported immediately to the Clinical Biochemistry Laboratory under the Department of Biochemistry, VAMC& RH, Shahjahanpur, for further analysis.

Lipid profile parameters namely, total cholesterol (TC), triglycerides (TG), HDL-C, LDL-C, and VLDL-C were estimated in all the study subjects by commercially available kit methods. Oxidative stress markers (Plasma MDA and Catalase) were estimated by the TBARS method and colorimetric assay respectively. Plasma levels of MPO were determined by kit method (AbFrontier) based on sandwich ELISA technique whereas plasma Ox-LDL estimation was performed by competitive ELISA kit method (Mercodia). Established CVD cases with deranged lipid profiles were selected for the study. Evaluation of the cardiovascular disease was performed by experienced investigators blinded for study aim.

Study approval was taken by an institutional ethical committee (VAMC/IEC/2018/XV and dated 18/3/2018), and informed consent from every individual subject was obtained according to the principles of the Helsinki declaration. The patient’s demographic profile, socioeconomic status, behavioural risk factors (sedentary lifestyle, dietary habits), and disease risk factor histories were recorded. Patients with diabetes mellitus, thyroid disorders, renal diseases, respiratory diseases, acute infection, or any other systemic illness and regular smokers and alcoholics were excluded from the study. Furthermore, patients on lipid-lowering drugs for the past three months were also excluded out from the study. None of the control subjects had clinical or laboratory evidence of any disease that might have affected the parameters to be measured.

Statistical analysis. Results are shown as Mean ± SD. Continuous variables of demographic and baseline characteristics were assessed with the use of the Student’s unpaired ‘t’ test to compare the levels of both the ratios between the test and control group. Comparative analysis between plasma MPO and ox-LDL was also performed by box and whiskers plot to check the values’ skewness and deviations. All p<0.05 were considered significant. Data analysis was performed by software package SPSS version 17.0.

3. Research results

To analyze the risk factor and plasma levels of MPO in the current study, 215 subjects are taken into consideration. The details regarding control and target subgroups in reference to SAP, UAP and AMI respectively are given in Table 1. The demographic and baseline characteristics of control and cases subgroups were compared using unpaired Student’s t-test comprised of sex, age, BMI, systolic and diastolic blood pressure (expressed as Mean ± SD) taken in the study. Highly significant (p<0.001) results were obtained on comparison between control and all the three case subgroups, namely stable angina pectoris SAP, unstable angina pectoris UAP, and acute myocardial infarction AMI except for age in the SAP subgroup where it is moderately significant (p<0.01). Statistically non-significant (p>0.05) results were obtained for sex in all the three cases subgroup, namely SAP, UAP, and AMI against control as shown in Table 1.

Table 1 show the clinical and laboratory characteristics of all the three case sub-groups i.e., SAP, UAP & AMI, compared with age and sex-matched control subjects. In Table 2, total cholesterol, LDL-C, VLDL-C, and triglycerides were found highly elevated (p<0.001) in the patient subgroups than in healthy controls. On the contrary, HDL-C was higher (p<0.001) in control with respect to patient subgroups. A comparison of oxidative stress markers in control with the cases sub-group indicates highly significant (p<0.001) results with respect to the levels of lipid peroxidation-(MDA) and catalase.
Table 1
Baseline characteristic of control and cases subgroup, mean ± SD

<table>
<thead>
<tr>
<th>Baseline characters</th>
<th>Control (n=54)</th>
<th>SAP (n=52)</th>
<th>UAP (n=53)</th>
<th>AMI (n=56)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, male (person)</td>
<td>p=0.56±0.49</td>
<td>0.5±0.50</td>
<td>0.62±0.48</td>
<td>0.64±0.48</td>
</tr>
<tr>
<td>Sex, female (person)</td>
<td>p=0.42±0.49</td>
<td>0.46±0.50</td>
<td>0.37±0.48</td>
<td>0.40±0.49</td>
</tr>
<tr>
<td>Age (years)</td>
<td>p=41.37±8.08</td>
<td>46.90±8.92</td>
<td>50.55±8.05*</td>
<td>49.92±7.34*</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>p=23.55±1.28</td>
<td>25.64±1.71</td>
<td>26.06±1.44*</td>
<td>25.06±1.41*</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>p=112.70±9.04</td>
<td>139.88±10.59*</td>
<td>135.13±7.60*</td>
<td>129.32±10.36*</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>p=79.96±6.57</td>
<td>89.5±6.77</td>
<td>86.90±6.57</td>
<td>85.56±7.29*</td>
</tr>
</tbody>
</table>

Note: –*p<0.001 found on comparison between control and cases subgroups

Table 2
Level of lipid profile and oxidative stress markers in control and cases subgroup, mean±SD

<table>
<thead>
<tr>
<th>Biochemical parameters</th>
<th>Control (n=54)</th>
<th>SAP (n=52)</th>
<th>UAP (n=53)</th>
<th>AMI (n=56)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. TC (mg/dl)</td>
<td>185.20±10.28</td>
<td>215.69±16.04*</td>
<td>212.03±12.85*</td>
<td>213.69±15.5*</td>
</tr>
<tr>
<td>2. TG (mg/dl)</td>
<td>150.72±14.10</td>
<td>218.74±14.79*</td>
<td>218.07±15.34*</td>
<td>218.56±14.93*</td>
</tr>
<tr>
<td>3. HDL-C (mg/dl)</td>
<td>42.47±4.68</td>
<td>35.42±3.52*</td>
<td>39.17±3.38*</td>
<td>37.56±3.53*</td>
</tr>
<tr>
<td>4. VLDL-C (mg/dl)</td>
<td>30.14±2.82</td>
<td>37.34±4.33*</td>
<td>36.17±3.16*</td>
<td>36.51±2.98*</td>
</tr>
<tr>
<td>5. LDL-C (mg/dl)</td>
<td>112.58±8.61</td>
<td>142.92±14.94*</td>
<td>136.68±4.10*</td>
<td>139.61±5.38*</td>
</tr>
<tr>
<td>6. MDA (µ mol/L)</td>
<td>2.15±0.51</td>
<td>6.35±0.762*</td>
<td>4.50±0.722*</td>
<td>4.87±0.772*</td>
</tr>
<tr>
<td>7. CAT (µmol/min/gmHb.)</td>
<td>129.59±6.01</td>
<td>67.26±7.33*</td>
<td>61.97±8.60*</td>
<td>64.36±8.62*</td>
</tr>
</tbody>
</table>

Note: –*p<0.001 found on comparison between control and cases subgroups

Table 3
Level of biochemical parameters shown in control and cases subgroup, mean ± SD

<table>
<thead>
<tr>
<th>Biochemical Parameters</th>
<th>Control (n=54)</th>
<th>SAP (n=52)</th>
<th>UAP (n=53)</th>
<th>AMI (n=56)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Oxidized-LDL (U/L)</td>
<td>95.27±3.70</td>
<td>104.5±4.37*</td>
<td>103.64±2.43*</td>
<td>105.92±2.96*</td>
</tr>
<tr>
<td>2. Plasma MPO (ng/ml)</td>
<td>60.17±7.69</td>
<td>62.96±8.21</td>
<td>85.24±12.04*</td>
<td>91.01±11.74*</td>
</tr>
</tbody>
</table>

Note: –*p<0.001 found on comparison between control and cases subgroups

Analysis of the data showed significant elevation in the levels of Ox-LDL from control to case subgroups. The differences were found highly significant (p<0.001) between control and all the three cases sub-group (SAP, UAP & AMI). An insignificant result (p>0.05) was found on a comparison between SAP and UAP. As shown in Table 3, there is a significant result between the two groups SAP and AMI (p<0.05) and seen a highly significant result between UAP and AMI subgroup (p<0.001).

According to the box and whiskers plot, the interquartile range in the SAP subgroup was found to be very large, suggesting scattered deviation in the mean values as compared to the UAP and AMI subgroup. Also, a longer lower whisker suggests the existence of most of the individual mean value towards the lower levels in the SAP subgroup. Box plots of UAP and AMI subgroup showed right skewness, suggesting the existence of mean values towards the higher side, as depicted in Fig. 1.

As compared with controls, plasma MPO levels were found highly significant in UAP (p<0.001) and AMI (p<0.001). There is no significant difference in plasma MPO levels in the SAP subgroup (p>0.05) regarding control. Furthermore, plasma MPO levels were significantly higher in AMI and UAP as compared with the SAP subgroup (p<0.001), but there is no significant correlation observed between UAP and AMI subgroup (p>0.05) (Table 3). Box and whiskers plot of MPO levels showed no skewness in the SAP subgroup, suggesting a non-significant variation in the mean values regarding control, whereas UAP and AMI subgroup showed right skewness, suggesting a highly significant difference in the mean values in comparison to the control subgroup (Fig. 2).
4. Discussion of research results

Cardiovascular disorder (CVD), consisting mainly of coronary artery disease (CAD) and stroke, remains a major cause of death and disability in the world today. A sedentary lifestyle, changing food habits, rapid urbanization, and an abnormal lipid profile are thought to contribute to this rising epidemic [8]. Several inflammatory molecules are playing a considerable role to use as biomarkers for plaque vulnerability. Recent studies have drawn attention towards two such biomarkers i.e., MPO, one of the enzymes of the innate immune system, and ox-LDL, a modified form of lipoprotein as potential markers of CVD and potential targets for treatment. Therefore, there is a constant requirement to search for new and more reliable biomarkers that will predict the occurrence of cardiovascular complications in the future. Thus, the present study aims to access the superiority of MPO & ox-LDL over each other as a better predictive marker gaining information regarding the severity of CVD.

In present findings, the Mean ± SD levels of both oxidized LDL and plasma MPO were found elevated in cases subgroup (SAP, UAP, and AMI) in comparison to controls are depicted in Tab. 3. Oxidized LDL (Ox-LDL) plays a crucial role in the pathogenesis of atherosclerosis. Several studies have demonstrated that higher circulating Ox-LDL concentrations are associated with subclinical atherosclerosis and clinical coronary heart disease [3]. According to Linton et. al., 2019 the main sources of accumulation of intracellular cholesterol that are generally accepted are the multiple forms of modified LDL [9]. Also, as per the study conducted by Orekhov, 2018 atherogenic multiple-modified LDL circulates in the blood of atherosclerosis patients and triggers the intracellular lipid accumulation in the areas that are most prone to atherosclerosis [10].

The present study highlights a highly significant results (p<0.001) for Ox-LDL in all the three cases subgroup – SAP, UAP, and AMI subgroups respectively in comparison to control. Furthermore, a comparison of UAP with AMI showed a highly significant (p<0.001) association whereas comparison of SAP with AMI indicates nearly significant (p<0.05) result and that of SAP vs UAP showed non-significant association (p>0.05) as shown in Table 3. Moreover, according to the box and whiskers plot Fig. 1 observations of the mean±SD levels of ox-LDL in all the three patient subgroups indicate larger deviation in the SAP subgroup in comparison to the UAP & AMI subgroup, suggestive of increased future risk of myocardial infarction in both SAP and UAP subgroup. However, at the same time, a non-significant correlation between SAP and UAP subgroup and large scattered deviation in the mean values of the SAP subgroup implies that the elevated levels of ox-LDL cannot be used to distinguish the severity level of the disease.

According to one of the study comparisons between groups of plasma oxidized LDL levels and malondialdehyde (MDA)-modified LDL with acute coronary syndromes and stable CAD found that plasma levels of oxidized LDL were independent of LDL-C but correlated inversely with HDL-C levels. Thus, they concluded that elevated plasma levels of oxidized LDL are associated with CAD. Elevated plasma levels of MDA modified LDL suggests plaque instability and may be useful for identifying patients with acute coronary syndromes [11, 12].

Several pieces of evidence implicate the role of myeloperoxidase (MPO) in the pathogenesis of atherosclerosis. MPO serves as an enzymatic source of eicosanoids and bioactive lipids generate atherogenic forms of both LDL and HDL. These factors are likely to contribute to clinical studies demonstrating that increased systemic levels of MPO and its oxidation products predict increased cardiovascular risk. As a result, interest has focused on the potential to target MPO for the development of new risk markers, imaging, and therapies to prevent cardiovascular events [13].

According to the present study, plasma MPO levels were significantly elevated in patients with UAP (p<0.001) and AMI subgroup (p<0.001) compared with control. There is no significant difference in plasma MPO levels in patients with SAP (p>0.05) and controls as per Table 3 and Fig. 2. Similar results were obtained in the studies conducted by Govindarajan S, 2016 suggesting that elevated plasma MPO levels...
were able to differentially diagnose chest pain between AMI and that due to stable or unstable angina [14]. Thus, the present study confirms MPO to be a useful predictive marker of CVD.

Similarly, a case-control study conducted by LIAihua et.al, 2010 on 219 coronary heart diseases (CHD) patients and 70 controls explored the relationship between myeloperoxidase (MPO) and CHD to predict the risk of CHD and concluded that MPO is a marker of the instability of plaque in the coronary artery, correlated with coronary heart disease [15]. Likewise, Kubala et.al., 2008 quantified plasma levels of MPO and traditional CAD risk factors in African American and Caucasian patients (n=557) undergoing elective coronary angiography. Their results suggest that the plasma level of MPO did not identify patients with stable CAD and was also independent of gender and ethnicity [16].

As per the study performed by Tang et. al, 2011, an assessment of plasma MPO concentrations of 1895 patients with known atherosclerotic burden undergoing elective coronary angiography observed an increased MPO level found significantly associated with the incidence of major adverse events over a 3-year follow-up study [17]. This suggests that MPO could serve as a better prognostic marker for adverse events in stable patients with coronary artery disease (CAD).

Hence in the present study, relatively non-significant but elevated levels of MPO in the SAP subgroup were found. Also, levels of MPO were found significantly elevated in UAP and AMI subgroups. Therefore, it can be said that MPO levels are increasing with the increasing severity of the disease. Therefore, it can be used as a predictive marker to assess the severity of CVD.

**Study limitations.** ROS production via MPO catalyzed pathway imparts a substantial impact on inflammatory events contribute to determine the immune responses. MPO also enhances the tissue damages resulting due to modification of lipoprotein particles. Therefore, both MPO and Ox-LDL seems to be an attractive target for the development of prognostic biomarkers and therapeutic interventions in the prevention of CVD. Our study deals with very small sample size, and population study is much needed to establish their roles as biomarkers.

**Prospect for further research.** There is considerable evidence that ox-LDL and MPO not only correlate with the presence of CVD, but also with disease severity. Future research will clarify if these techniques will find practical clinical use.

5. Conclusion

Levels of ox-LDL were found significantly elevated in SAP, UAP, and AMI subgroups indicate the diagnostic importance of ox-LDL in CVD as compared to control. However, scattered deviation in box whisker plot of ox-LDL in SAP subgroup suggests its poor prognostic reliability in assessing the severity of the disease. On the contrary, significantly elevated plasma MPO levels in UAP and AMI subgroups signify the role of MPO as a marker of oxidative stress leading to inflammation associated with the severity of CVD. Furthermore, plasma MPO levels in the SAP subgroup found slightly higher as compared to control in the present study suggests its prognostic value, of adverse events in healthy control and patients with SAP. Thus, plasma MPO and Ox-LDL levels serve as independent predictors of CVD, but plasma MPO levels predict an increased risk over ox-LDL for subsequent cardiovascular events in stable and unstable angina and extend the prognostic information gained from traditional biochemical markers.

**Conflict of interests**

The authors declare that they have no conflicts of interest.

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