Research Article **Biochemistry**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

MYELOPEROXIDASE - A PROGNOSTIC MARKER IN CARDIO VASCULAR DISEASE

DEEPTI MANDSORWALE[1], SURYAKANT NAGTILAK*[1], AARTI LALCHANDANI[2] AND AJAI KUMAR SRIVASTAVA[3].

[1]Department of Biochemistry Subharti Medical College Meerut (UP)-250005
[2]Department of Medicine GSVM Medical College Kanpur (UP)-208024
[3]Department of Biochemistry GSVM Medical College Kanpur (UP)-208024

ABSTRACT

Myeloperoxidase (MPO), a leukocyte derived enzyme linked to both inflammation and oxidative stress. Present study comprising 215 subjects [control (n=54), three patient subgroups (SAP=52, UAP=53 and AMI=56 respectively)] selected to evaluate systemic release of MPO, a characteristic feature in various referred sub-groups suffering from CVD correlated with levels of oxidative stress markers and traditional coronary disease risk factors in study subjects. Lipid profile, MDA, catalase and plasma MPO were analyzed by established techniques. Data suggests that CVD cases had significantly elevated levels of MDA, catalase and Lipid profile except HDL-C. Plasma MPO was found significantly elevated ($p < 0.001$) in UAP and AMI subgroups compared with control and SAP subgroup counterparts. Study concludes that plasma MPO levels play an important role in diagnosis of UAP & AMI subgroups and may be used as prognostic marker in evaluation of risk in patients with SAP.

KEY-WORDS: Cardiovascular disease, Myeloperoxidase, Oxidative stress, Angina pectoris, Acute myocardial infarction (AMI)

SURYAKANT NAGTILAK
Department of Biochemistry Subharti Medical College Meerut (UP)-250005

***Corresponding author**

INTRODUCTION

India has one of the highest burdens of cardiovascular diseases (CVD). The prevalence of cardiovascular disease (CVD) in rural 7.4%, and in urban India is 11%. The incidence of ischemic heart disease (IHD) and coronary heart disease (CHD) is steadily increasing in the Indian subcontinent[1]. According to World Health Report 2002, CVD will be the largest cause of death in India by 2020. It is predicted that 2.6 million Indians will die due to CHD. This number will represent 54.1% of all CVD deaths in the age group, 30–69 years [2]. The mortality and morbidity of CVD are promoted by major risk factors, such as hyperlipidemia, hypertension, and smoking. The sequence of events leading to CVD includes endothelial dysfunction, atherosclerotic plaque formation, and rupture. Inflammation has been implicated in all these stages in the evolution of atherosclerotic plaques [3]. Moreover, oxidative stress is currently considered a major step in development of CVD [4]. Growing evidence demonstrate the action of Myeloperoxidase (MPO) as central participant linked to both inflammation and oxidative stress in CVD. MPO is a hemoprotein that is stored in azurophillic granules of polymorphonuclear neutrophills (PMN) and macrophages, which is released in a state of inflammation, catalyzes the formation of several reactive species, including hypochlorous acid and thus has a role in host defense against micro-organisms [5]. MPO and its products display a diversity of pro-inflammatory and pro-atherogenic properties including catalytic consumption of endothelium-derived nitric oxide, LDL oxidation, modulation of metalloproteinase activities, and activation of PMN in a cytokine-like manner independent of the catalytic activity[6,7]. The significance of MPO in the development of coronary artery disease (CAD) has been demonstrated in studies showing association of systemic MPO level and expression of MPO with the prevalence of CAD or with chronic heart failure [8]. Interestingly, MPO serum and plasma levels are markedly elevated in patients with acute coronary disease, forming a firm mechanistic link between PMN activation, MPO release, and compromised vascular reactivity[9,10]. The diversity of clinical presentations in patients with CVD leads to evaluate whether systemic release of MPO is a characteristic feature in the various sub-groups suffering from CVD. The aim of the present study was to assess levels of plasma MPO in various sub-groups of patients with different cardiovascular diseases i.e., UAP, SAP and AMI and their relationship with oxidative stress markers, Malondialdehyde (MDA), Catalase and traditional coronary disease risk factors.

MATERIALS AND METHODS

The study cohort consists of 215 subjects, divided into four groups i.e., healthy control (n=54), established patients with stable angina pectoris (SAP, n=52), unstable angina pectoris (UAP, n=53), and patients with acute myocardial infarction (AMI, n=56). All recruited subjects were selected from series of consecutive outdoor patient department attending coronary clinic and indoor patient department of Laajpat Singhania Institute of Cardiology, GSVM, Medical College, Kanpur. Overnight fasting 4.0 ml blood samples were collected in the morning from the cubital vein in vials containing EDTA anticoagulant and transported immediately to the department of Biochemistry, GSVM, Medical College, Kanpur for further analysis. Lipid profile, parameters namely, Total Cholesterol (TC), Triglycerides (TG), HDL-C, LDL-C, and VLDL-C was estimated in all the study subjects by commercially available kit methods. Plasma MDA was estimated by TBARS method[11], Catalase was estimated by colorimetric assay[12] and plasma levels of MPO were determined by kit method (AbFrontier) based on ELISA technique. Established CVD patients with deranged lipid profile were selected for the study. Evaluation of the cardiovascular disease was performed by experienced investigators blinded for study aim. Patients with diabetes mellitus, renal diseases, respiratory diseases, thyroid disorders, acute infection or any other systemic illness and on lipid lowering drugs for the past three months were excluded from the study. The study was approved by an

institutional ethical committee, and informed consent was obtained from each subject in accordance with principles of the declaration of Helsinki. Family history of CAD, diabetes, hypertension and other major illness in the past, personnel history of smoking, alcohol, diet and drug history was recorded for each subject on computerized working proforma. None of the control subjects had clinical or laboratory evidence of any disease that might have affected the parameters to be measured.

Statistical Analysis

Data analysis was performed by software package SPSS version 17.0. Continuous variables of demographical and baseline characteristics were compared with the use of Students t-test for two groups or analysis of variance by using one way ANOVA for multiple comparisons. P value < 0.05 was considered significant.

RESULTS

A total of 215 subjects recruited in the study for analyzing the risk factors and plasma levels of MPO, comprising 54 healthy control (31 male, 23 female), patients with stable angina pectoris (SAP) 52 (28 male, 24 female), patients with unstable angina pectoris (UAP) 53 (33 male, 20 female) and patients with acute myocardial infarction (AMI), 56 (34 male, 22 female) respectively (Table 1A).

Table 1A
Demographical characteristics of control and patient subgroups.

S. No.	DEMOGRAPHICAL CHARACTERS	CONTROL (n=54)	PATIENT SUBGROUPS		
			SAP (n=52)	UAP (n=53)	AMI (n=56)
1.	Sex, male, n (%)	31 (57.4%)	28 (53.84%)	33 (62.26%)	34 (60.7%)
3.	Diet, (Non-veg) n (%)	28 (51.8%)	29 (55.76%)	31 (58.49%)	30 (53.57%)
4.	Smoking, n (%)	18 (33.3%)	21 (40.38%)	20 (37.73%)	24 (42.85%)
5.	Alcohol n (%)	3 (5.55%)	6 (11.53%)	5 (9.43%)	8 (14.28%)
6.	Family history n (%)	21 (38.8%)	25 (48.07%)	32 (60.37%)	30 (53.57%)

The baseline characteristics in control and patient's sub-groups such as age, BMI, Systolic and diastolic blood pressure (expressed as Mean ± SD). Results highlight that in subgroups namely SAP, UAP and AMI patients baseline characteristics were highly significant ($p < 0.001$) except for age in SAP subgroup where it is moderately significant($p < 0.01$) (Table 1B).

Table 1B
Baseline characteristics of control and patient subgroups.

S. No.	BASELINE CHARACTERS	CONTROL (n=54)	PATIENT SUBGROUPS		
			SAP (n=52)	UAP (n=53)	AMI (n=56)
1.	Age (yrs)	41.31 ± 8.13	46.88 ± 8.96*	49.81 ± 8.06**	50.01 ± 7.25**
2.	BMI (kg/m²)	23.54 ± 1.29	25.63 ± 1.70**	26.07 ± 1.45**	25.02 ± 1.40**
4.	SBP (mmHg)	112.66 ± 9.06	139.78 ± 10.67**	135.05 ± 7.67**	129.28 ± 10.42**
5.	DBP (mmHg)	79.88 ± 6.58	89.5 ± 5.80**	89.52 ± 5.68**	85.69 ± 7.36**

Values are expressed as mean ± SD *p value: <0.01 moderately significant, **p value : <0.001 highly significant.

The clinical and laboratory characteristics such as Total Cholesterol, LDL-C, VLDL-C, and Triglycerides found highly elevated ($p < 0.001$) in patient subgroups than in healthy controls. On the contrary HDL-C was higher ($p < 0.001$) in control with respect to patient sub-groups. Comparison of oxidative stress markers in control with patient's subgroup indicates highly significant ($p < 0.001$) correlation with respect to the levels of MDA and Catalase (Table 2). Plasma MPO levels were significantly higher in patients with UAP ($p < 0.001$) and AMI ($p < 0.001$) compared with controls. There is no significant difference in plasma MPO levels in patients with SAP ($p > 0.05$) and controls. Furthermore, plasma MPO levels were significantly higher in AMI and

UAP compared with SAP ($p < 0.001$), but there is no significant difference between AMI and UAP ($p > 0.05$) (Table 2).

Table 2
Biochemical parameters of control and patient subgroups.

S. No	BIOCHEMICAL PARAMETERS	CONTROL (n=54)	PATIENT SUBGROUPS		
			SAP (n=52)	UAP (n=53)	AMI (n=56)
1.	Total Cholesterol (TC) (mg/dl)	185.20 ± 10.28	215.69 ± 16.04***	212.03 ± 12.85***	213.69 ± 15.5***
2.	HDL-C (mg/dl)	42.47 ± 4.68	35.42 ± 3.52***	39.17 ± 3.38***	37.56 ± 3.53***
3.	VLDL-C (mg/dl)	30.14 ± 2.82	37.34 ± 4.33***	36.17 ± 3.16***	36.51 ± 2.98***
4.	LDL-C (mg/dl)	112.58 ± 8.61	142.92 ± 14.94***	136.68 ± 14.10***	139.61 ± 15.38***
5.	Triglycerides (TG) (mg/dl)	150.72 ± 14.10	186.74 ± 21.69***	180.87 ± 15.8***	182.56 ± 14.93***
6.	Lipid peroxidation (MDA) (µ mol /L)	2.15 ± 0.51	6.35 ± 0.76***	4.50 ± 0.722***	4.87 ± 0.77***
7.	Catalase (CAT) (µ mol/min/gmHb.)	129.59 ±6.01	67.26 ± 7.33***	61.97 ± 8.60***	64.36 ± 8.62***
8.	Plasma MPO (ng/ml)	60.17 ± 7.69	62.96 ± 8.21*	85.24 ± 12.04***	91.01 ± 11.74***

Values are expressed as mean ± SD *p value: >0.05 non significant, **p value: <0.01 moderate significant, ***p value: <0.001 highly significant.

The ROC curve analysis in CVD cases reveals elevated specificity (98.14%) and comparatively low sensitivity (64.5%) at cut-off points > 74.76 ng/ml (AUC = 0.856; CI 95 % = 0.808 – 0.904) (Fig. 1).

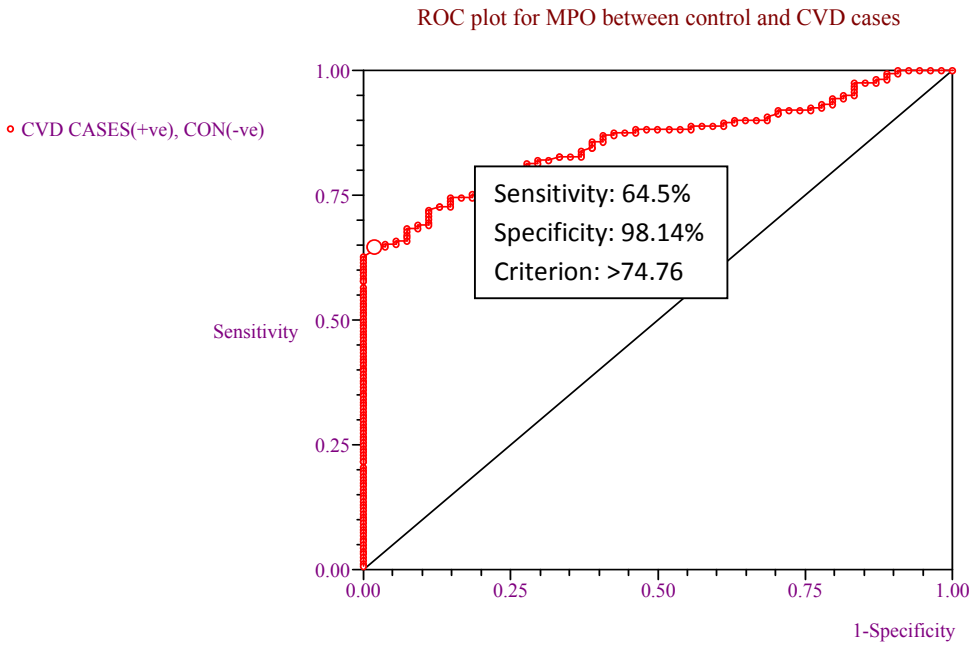

Figure 1
ROC curve for MPO between Control & CVD cases

DISCUSSION

The CVD is a heterogeneous disease with a wide range of clinical presentations and outcomes. The current study aimed to determine the role of plasma MPO levels in assessment of the patients with various grades of cardiovascular diseases i.e SAP, UAP and AMI respectively. The systemic markers of inflammation have been

investigated and linked to identify patients at risk of CAD and predict future cardiovascular events [13]. The first epidemiological report assessing the association between MPO and CVD was a case-control study, reported by *Zhang et al*[14] that MPO activity was higher in patients with CAD than angiographically verified normal controls. Thus the increased activity was significantly associated with presence of CAD. The results of this study correlates with our observations, we also found a significant increase in plasma MPO levels in patients with UAP and AMI, compared with controls. Multivariate analysis performed by *Dominguez et al* [15], MPO was the strongest independent predictor of CVD outcome. Furthermore, this study reports that a group of 38 patients with ST-segment myocardial infarction presenting with cardiogenic shock, treated with percutaneous coronary interventions, baseline MPO was an independent predictor of Indoor Patient Department mortality. *Mocatta et al,*[16] measured plasma MPO in AMI patients and found significant association of MPO with follow-up events. Study concludes that MPO has an additional prognostic value on the top of ejection fraction and Brain Natriuretic Peptide (BNP), a finding observed by *Khan et al,*[17] in similar population with ST- Elevated Myocardial infarction (STEMI). A non-significant association between control and SAP sub-group of present study resembles with the study conducted by *Kubala et al,* [18]. Recently, the importance of PMN degranulation of MPO in the coronary circulation has been illustrated by the fact that systemic plasma and serum MPO are markedly elevated and emerged as powerful predictors of adverse outcome in patients with acute CAD. *Buffon et al*,[19] showed that patients with stable CAD have significantly lower or no evidence of PMN recruitment and activation, as evidenced by unchanged CD11b expression and MPO content in PMN. Moreover, in patients with resolving unstable angina the decreased MPO content in PMN returned to levels similar to that in patients with chronic stable angina or in non-CVD subjects[20]. The comparison between levels of SAP with UAP and AMI respectively, showed highly significant correlation while nearly significant correlation was found between UAP vs AMI, similar to the study reported by *Lobbes et al,* [21]. The ROC curve analysis in present study indicates, the best MPO cut-off point for CVD cases was identified as > 74.76 ng/ml (AUC = 0.856; CI 95 % = 0.808 – 0.904) revealed elevated specificity (98.14%) with relatively low sensitivity (64.5%) corresponding to the studies conducted by *Esporcatte et al* [22] *and Brevetti et al* [23]. An oxidative stress plays a decisive role in the pathophysiology of CVD. The MDA and catalase belongs to the group of oxidative stress markers. The MDA on one hand is a product of lipid peroxidation involved in the generation of oxidative stress while catalase on the other hand is an antioxidative enzyme providing protection against the toxic effect of oxidants. In the present study, the MDA mean ± SD levels were found elevated in patient sub-groups whereas levels of catalase were decreased. Our findings correlates with the similar studies conducted by *Karajibani* et al [24] and *Gupta & Kumar* [25]. An elevated level of MPO is indicative of CVD risk which confirms in the present study. The levels of MPO are directly proportional to the severity of CVD i.e *SAP < UAP < AMI*. The MPO may be used as a diagnostic and prognostic marker for assessment of cardiovascular disease. Study suggests that MPO is mechanism based marker of inflammation and oxidative stress that has been consistently elevated in patient subgroups and might be involved in the progression and severity of cardiovascular disease.

CONCLUSION

The present study highlights that plasma MPO is an independent diagnostic predictor of CVD. There is significant elevation of MDA and decrease level of Catalase in patient subgroups with respect to control indicates strong association with these oxidative stress biomarkers with CVD. The Plasma MPO levels were not elevated in SAP subgroup, suggested that systemic release of MPO is not a characteristic feature of SAP sub-group of CVD. Significantly elevated levels of plasma MPO in UAP and AMI subjects justify MPO as a marker of inflammation and oxidative stress involved in the progression and severity of the disease.

REFERENCES

1. Huffman MD, Prabhakaran D, Osmond C et al., "Incidence of cardiovascular risk factors in an Indian urban cohort: results from the New Delhi Birth Cohort," *Journal of the American College of Cardiology*, vol. 57; no. 17: 1765–1774 (2011).
2. Jha Sudha, Ahmad Naved, Nagtilak Suryakant and Chawla Maheshwar. Coronary Heart Disease, A gift of modern civilization. Int. J. Sci. Res.,2(11): 378-380: (2013).
3. Ross R. Atherosclerosis—an inflammatory disease. N Engl J Med; 340: 115–26 (1999).
4. Stocker R, Keaney JF Jr. Role of oxidative modifications in atherosclerosis. Physiol Rev; 84: 1381–478 (2004).
5. Klebanoff SJ. Myeloperoxidase: friend and foe. J Leukoc Biol; 77: 598–625 (2005).
6. Eiserich JP, Baldus S, Brennan ML, et al. Myeloperoxidase, a leukocyte-derived vascular NO oxidase. Science; 296: 2391–4 (2002). [PubMed: 12089442]
7. Zhang R, Brennan ML, Fu X, et al. Association between myeloperoxidase levels and risk of coronary artery disease. Jama; 286: 2136–42 (2001). [PubMed: 11694155]
8. Tang WH, Brennan ML, Philip K, et al. Plasma myeloperoxidase levels in patients with chronic heart failure. Am J Cardiol; 98: 796–9 (2006). [PubMed: 16950188]
9. Cavusoglu E, Ruwende C, Eng C, et al. Usefulness of baseline plasma myeloperoxidase levels as an independent predictor of myocardial infarction at two years in patients presenting with acute coronary syndrome. Am J Cardiol; 99: 1364–8 (2007). [PubMed: 17493461]
10. Tang WH, Tong W, Troughton RW, et al. Prognostic value and echocardiographic determinants of plasma myeloperoxidase levels in chronic heart failure. J Am Coll Cardiol; 49: 2364–70 (2007). [PubMed: 17572253].
11. Satho K. Serum lipid peroxidation in cerebrovascular disorders determined by a new colorimetric method. Clin. Chem., Acta; 90: 37-43 (1978).
12. Sinha A K,. Colorimetric assay of Catalase. Analytical Biochemistry; 47: 389-394 (1972).
13. Fichtlscherer S, Heeschen C, Zeiher AM. Inflammatory markers and coronary artery disease. Curr Opin Pharmacol; 4: 124–31 (2004). [PubMed: 15063355]
14. Zhang C, Patel R, Eiserich JP, Zhou F, Kelpke S, Ma W, Parks DA, Darley-Usmar V, White CR. Endothelial dysfunction is induced by proinflammatory oxidant hypochlorous acid. *Am J Physiol Heart Circ Physiol*; 281: H1469–H1475 (2001).
15. Dominguez-Rodriguez A, Samimi-Fard S, Abreu- Gonzalez P, Garcia-Gonzalez MJ, Kaski JC. Prognostic value of admission myeloperoxidase levels in patients with ST-segment elevation myocardial infarction and cardiogenic shock. Am J Cardiol; 101: 1537– 40 (2008).
16. Mocatta T. J, Pilbrow A.P, Cameron V. A, et al. "Plasma concentrations of myeloperoxidase predict mortality after myocardial infarction," *Journal of the American College of Cardiology*; vol. 49, no. 20: pp. 1993–2000 (2007).
17. Khan SQ, Keely D, Quinn P, Davies JE, Ng LL. Myeloperoxidase aids prognostication together with N-terminal pro-B-Type natriuretic peptide in high-risk patients with acute ST elevation myocardial infarction. Heart; 93: 826-31 (2007).
18. Kubala L, Lu G, Baldus S, Berglund L, Eiserich JP. Plasma levels of myeloperoxidase are not elevated in patients with stable coronary artery disease. Clin Chim Acta; 394: 59–62 (2008).
19. Buffon A, Biasucci LM, Liuzzo G, et al. Widespread coronary inflammation in unstable angina. N Engl J Med; 347: 5–12 (2002). [PubMed: 12097534]
20. Biasucci LM, D'Onofrio G, Liuzzo G, et al. Intracellular neutrophil myeloperoxidase is reduced in unstable angina and acute myocardial infarction, but its reduction is not related to ischemia. J Am Coll Cardiol; 27: 611–6 (1996). [PubMed: 8606272]

21. Lobbes M. B. I, Kooi M. E, Lutgens E, Ruiters A.W, Lima Passos V, Braat S. H. J. G, Rousch M, Ten Cate H, van Engelshoven J. M. A, Daemen M. J. A. P., and Heeneman S,. Leukocyte Counts. Myeloperoxidase, and Pregnancy-Associated Plasma Protein A as Biomarkers for Cardiovascular Disease: Towards a Multi-Biomarker Approach. International Journal of Vascular Medicine;10: 1-9 (2010).
22. Esporcatte R, Rey H.C.V, Rangel F.O.D, Rocha R.M, Hugo Tannus Furtado de Mendonça Filho, Hans Fernando Rocha Dohmann, Francisco Manes Albanesi Filho. Predictive value of myeloperoxidase to identify high risk patients admitted to the hospital with acute chest pain. Arq Bras Cardiol; 89(6):341-347 (2007).
23. Brevetti G, Schiano V, Laurenzano E, Giugliano G, Petretta M, Scopacasa F, and Chiariello M,. Myeloperoxidase, but not C-reactive protein, predicts cardiovascular risk in peripheral arterial disease. European Heart Journal; 29: 224–230 (2008).
24. Karajibani M, Hashemi M, Montazerifar F, Bolouri A and Dikshit M.The Status of Glutathione Peroxidase, Superoxide Dismutase, Vitamins A, C, E and Malondialdehyde in Patients with Cardiovascular Disease in Zahedan, Southeast Iran. *J Nutr Sci Vitaminol*; 55: 309–316 (2009).
25. Gupta Vani and Kumar Sandeep. Alpha tocopherol, oxidative stress and diabetic vascular complications. Int J Pharm Bio Sci . July; 4(3): (B) 1065 – 1074 (2013).