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Thejaswini Muppala., Sukanya Shetty., Ashalatha V Rao and Subramanyam K

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RESEARCH ARTICLE

CORRELATION BETWEEN MYELOPEROXIDASE AND NITRIC OXIDE LEVELS IN ACUTE ISCHEMIC HEART DISEASE

Thejaswini Muppala[1]*., Sukanya Shetty[1]., Ashalatha V Rao[1] and Subramanyam K[2]

[1]Department of Biochemistry, K.S.Hegde Medical Academy, Mangalore, Karnataka
[2]Department of Cardiology, K.S.Hegde Medical Academy, Mangalore, Karnataka

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ABSTRACT

Ischemic heart disease (IHD) occurs due to imbalance between myocardial oxygen supply and demand. The aim of this study is to estimate, compare and correlate serum myeloperoxidase (MPO) and nitric oxide (NO) levels in newly diagnosed IHD subjects and healthy subjects. 30 acute IHD subjects and 30 age and sex matched controls were included in this study. Myeloperoxidase and nitric oxide were estimated. SPSS 16 was used to analyze the statistical data. High serum myeloperoxidase levels and low serum nitric oxide levels were observed in acute IHD subjects in comparison to controls. There was a negative and moderately significant correlation between serum MPO and NO in acute IHD subjects. Determination of serum MPO and NO in IHD may be useful in taking proper measures to prevent IHD.

INTRODUCTION

Ischemic heart disease (IHD) is a condition in which there is an inadequate supply of blood and oxygen to a portion of the myocardium and it typically occurs when there is an imbalance between myocardial oxygen supply and demand. It is the leading cause of cardiovascular death worldwide and majority of deaths occur in countries like India (Rajeev G *et al*, 2012).

Myeloperoxidase (MPO) is a hemoprotein, with molecular weight of 144 kD and consists of two identical dimers linked by a disulfide bridge and each dimer is composed of one heavy subunit and one light subunit (Hansson M *et al*, 2006). It is stored in the granules of neutrophils and monocytes, and is released in response to leukocyte activation (Loria V *et al*, 2008). MPO can amplify oxidative potential of hydrogen peroxide by producing a variety of reactive oxidants, including chlorinating and nitrating species (Delporte C *et al*, 2013). Thus MPO catalyses the production of hypochlorous acid from hydrogen peroxide and chloride ions (Loria V *et al*, 2008). These reactant oxidant species play an important role in thrombosis, plaque instability, and vasoconstriction from nitric oxide depletion (Loria V *et al*, 2008, Singh TP *et al*, 2011).

Nitric oxide (NO) produced by endothelial nitric oxide synthase is a powerful vasodilator and plays a critical role in the regulation of vascular tone. It relaxes coronary arteries and also prevents activation and adhesion of platelets (Richard OC III *et al*, 1998). NO suppresses binding of circulating cells to the endothelium and inhibits proliferation of smooth muscle cell in the vascular wall. Insufficient production and/or increased scavenging of NO may impair vascular function and accelerate atherosclerosis. (Loria V *et al*, 2008, Hansson M *et al*, 2006).

Myeloperoxidase activity diminishes NO bioavailability and leads to endothelial dysfunction (Meuwese MC *et al*, 2007). It plays an important role in the development of cardiovascular disorder especially IHD by promoting atherogenesis (Loria V *et al*, 2008). This study has been taken up to estimate, compare and correlate myeloperoxidase and nitric oxide levels in subjects with newly diagnosed ischemic heart disease.

MATERIALS AND METHODS

This case control study was conducted in the Department of Biochemistry, in collaboration with the Department of Cardiology, K.S.Hegde Charitable Hospital, Mangalore.

Corresponding author:* **Thejaswini Muppala
Department of Biochemistry, K.S.Hegde Medical Academy, Mangalore, Karnataka

Ethical clearance was obtained from the Institutional Ethical Committee before the start of the study. 30 IHD subjects for the study were recruited from those who were admitted to the cardiology ward, K S Hegde Charitable Hospital. The subjects were selected based on clinical history and clinical examination. The diagnosis of IHD was based on the findings in ECG or echocardiogram (presence of regional motional valve abnormality) or elevation of cardiac markers. The number of individuals with diagnosis of STEMI (ST-segment elevated Myocardial Infarction), NSTEMI (Non-ST-segment elevation Myocardial Infarction) and UA (Unstable Angina) were 24, 3 and 3 respectively. 30 healthy age and sex matched subjects were selected from the general population as controls. IHD subjects had hemoglobin $\geq$ 12 gm/dL and were within the age group of 30 – 60 yrs. Patients with history of diabetes and hypertension who were on regular treatment were also included in this study. Among acute IHD subjects, 1 subject had diabetes (DM), 9 had hypertension (HTN) and 3 had both diabetes and hypertension. 6 subjects were alcoholics, 9 were smokers and 2 had history of both alcoholism and smoking in the IHD group. Control group subjects did not have any risk factors or addictions. Patients with a history of chronic kidney disease (with positive microalbuminuria), chronic liver disease, blood donation in the past 3 months, on iron or antioxidant therapy, infections, polycythemia, malignancy, anemia (with haemoglobin <12 gm/dl) and pregnant women were excluded from the study.

The informed consent was obtained from all the subjects before the start of the study. 5ml of blood samples were collected in plain red-topped vaccutainer tubes containing clot activator. Blood samples were centrifuged and sera were separated. Sera were stored at -20^{0}c till analysis for the estimation of nitric oxide and myeloperoxidase. Serum myeloperoxidase was estimated by Matheson *et al* method and nitric oxide was estimated by Griess reagent method using spectrophotometer.

Statistical Analysis

Data were analysed using Statistical Package for Social Sciences (SPSS) version 16. Results were expressed as mean $\pm$ SD. Categorical variables were expressed as frequencies. The groups were compared using student t test. Correlation between different parameters was determined using Karl Pearson's correlation coefficient. A p value of less than 0.05 was considered statistically significant.

RESULTS

In both IHD and control groups, 24 were males and 6 were females. The mean age and body mass index (BMI) of IHD and control groups have been shown in Table 1. There was no significant difference between the two groups.

Table 1 Characteristics of the study population

	Acute IHD subjects (30)	Controls (30)
Male/Female	24/6	24/6
Age in years (Mean $\pm$ S.D)	54.3 $\pm$ 6.6	55.8 $\pm$ 4.5
BMI in Kg/m^2 (Mean $\pm$ S.D)	22.8 $\pm$ 2.2	23.5 $\pm$ 1.6

Table 2 Serum myeloperoxidase and nitric oxide levels in acute IHD subjects and controls.

Study Groups	Serum levels(Mean $\pm$ S.D)	
	MPO (µmoles/L)	NO (µmoles/L)
Acute IHD subjects (30)	351.6 $\pm$ 89.9	11.0 $\pm$ 1.3
Controls(30)	78.6 $\pm$ 20.8 a*	18.1 $\pm$ 3.2 b*

a. comparison of MPO levels of acute IHD subjects with controls
b. comparison of NO levels of acute IHD subjects with controls

*- p value of <0.001

Serum myeloperoxidase levels were significantly increased in acute IHD subjects(351.6 $\pm$ 89.9 µmoles/L) in comparison to controls(78.6 $\pm$ 20.8 µmoles/L) and serum nitric oxide levels were significantly decreased in IHD subjects (11.0 $\pm$ 1.3 µmoles/L) compared to controls (18.1 $\pm$ 3.2 µmoles/L).(Table 2, Figure 1and 2)

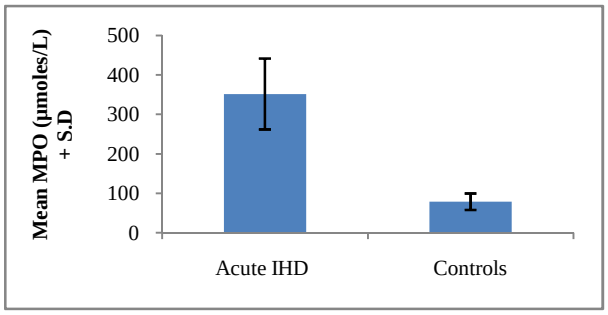

Figure 1 Serum myeloperoxidase levels in acute IHD subjects and controls.

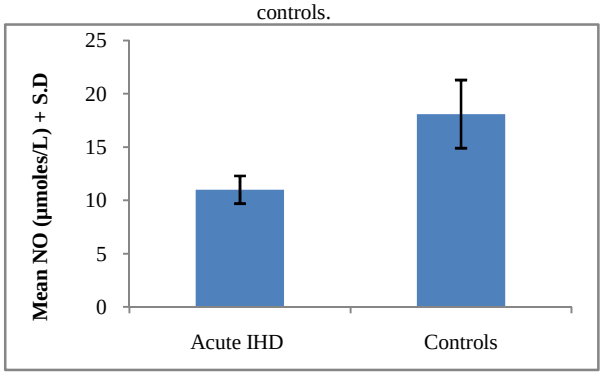

Figure 2 Serum nitric oxide levels in acute IHD subjects and controls.

There was a negative and moderately significant correlation between myeloperoxidase and nitric oxide (r =-0.723, p $\leq$ 0.001). (Figure 3)

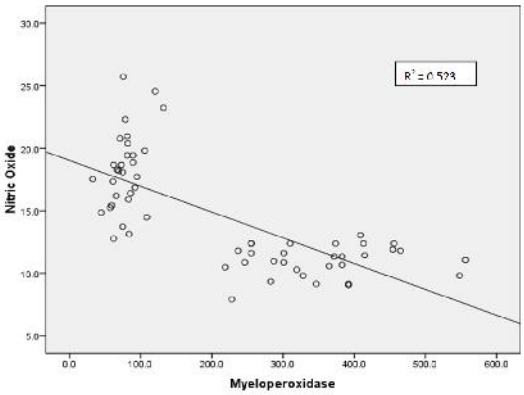

Figure 3 Correlation between serum myeloperoxidase and nitric oxide levels

DISCUSSION

IHD contributes to 12.2% of total deaths worldwide and is expected to be one of the four major causes of death by 2030 (Akheel MM *et al*, 2012). Major cause of Ischemic heart disease is atherosclerosis (Antman EM *et al*, 2008).

MPO generates a number of reactive species, including hypochlorous acid, chloramines, tyrosyl radicals, and nitrogen dioxide, that oxidize the protein, lipid, and antioxidant constituents of low density lipoprotein (LDL). Modified LDL (Mox-LDL) can contribute to atherogenesis by promoting cholesterol deposition and transformation of macrophages into foam cells**.** Mox-LDL also inhibits fibrinolysis process via endothelial cell interaction (Delporte C *et al*, 2013, Podrez EA *et al*, 1999). High density lipoprotein (HDL) is a selective in vivo target for MPO-catalyzed oxidation by a non specific molecular mechanism. MPO converts cardioprotective lipoprotein into a dysfunctional form. Dysfunctional HDL particles lack atheroprotective properties and promote pro- inflammatory effects(Loria V *et al*, 2008).

MPO generates free radicals, induces inflammation, decreases NO levels and is involved in all stages of atherogenesis from endothelial dysfunction to plaque rupture. This leads to acute coronary syndromes (Roger KS *et al*, 2009, Loria V *et al*, 2008, Meuwese MC *et al*, 2007). In the present study, serum MPO levels were significantly increased in new IHD cases when compared to controls indicating its role in inflammation. These results are in accordance with those observed by (Hameed RM *et al*, 2007, Ndrepepa G *et al*, 2008) who have also reported high serum MPO levels in acute coronary syndromes when compared to controls. (Zhang R *et al*, 2001) observed that blood and leukocyte MPO activity were higher in patients with coronary artery disease (CAD) than angiographically verified normal controls. Individuals who possess MPO levels in the fourth quartile were 15-20 fold more likely to demonstrate abnormal coronary angiograms compared to subjects in the lowest quartile.

MPO levels have been observed to differ significantly with difference in age and BMI (Scharnagl H *et al*, 2014, Shetty S *et al*, 2012). Hence in the present study controls were matched age and sex wise in order to remove the false variation created by these factors. Also there was no significant difference in the mean BMI between all the three groups.

(Kaya MG *et al*, 2012) observed higher plasma MPO levels in patients with acute STEMI when compared to controls and also found that there was a higher risk of major adverse cardiovascular events (MACE) in patients with higher MPO levels among the cases after a follow up period of 2 years. (Meuwese MC *et al*, 2007) in EPIC (European Prospective Investigation into Cancer and nutrition) – Norfolk prospective population study have evaluated the association of MPO levels with risk of future coronary artery disease (CAD) in healthy individuals.

MPO levels were significantly higher in case of subjects who later developed CAD during 8 years of follow up. MPO levels correlated with C-reactive protein (CRP) and white blood cell count. Patients with higher MPO levels and a significant coronary artery calcium score were at an increased risk of cardiovascular disease events after a follow up of 3.8 years in a study done by (Wong ND *et al*, 2009).

Low levels of NO causes vasoconstriction and endothelial dysfunction. This leads to decreased tissue perfusion, increased vascular resistance and formation of thrombus. This acts as a predisposing factor for formation of IHD (Sozmen B *et al*, 1998). Diminished nitric oxide bioactivity causes constriction of coronary arteries and contributes to myocardial ischemia (MI) in patients with CAD. It also facilitates vascular inflammation that could lead to oxidation of lipoproteins and formation of atherosclerotic plaque (Richard OC III *et al*, 1998). This supports the findings of low serum nitric oxide levels in IHD cases in this study. (Rizk A *et al*, 2004, Bhardwaj S *et al*, 2012) have also observed low nitric oxide levels in IHD cases when compared to controls.

In the present study, there was a negative and moderately significant correlation between serum MPO and NO. MPO consumes nitric oxide and impairs nitric oxide dependent vasodilation. This impairs vascular function and promotes atherosclerosis (Loria V *et al*, 2008). These findings are in accordance with Ziaaldin *et al* who reported high MPO and low NO levels in CAD patients (Samsamshariat SZ *et al*, 2011). (Baldus S *et al*, 2004) also reported increased MPO levels in patients with acute MI and is of the opinion that MPO enhances consumption of NO. (Vita JA *et al*, 2004) observed a strong and independent relation between serum MPO level and endothelial dysfunction based on brachial artery flow mediated dilation studies.

As scavenging of NO by MPO- derived reactive substances may reduce the bioavailability of NO, MPO might play a role in altering guanylate cyclase activation as well as other NO-dependent signaling events during development of vascular disease (Abu-Soud HM *et al*, 2000). Hypochlorous acid can react with nitrogen atoms of the endothelial nitric oxide synthase (eNOS) substrate arginine to produce chlorinated arginine species that are inhibitors of all isoforms of NOS and promotes vascular dysfunction under inflammatory conditions (Yang J *et al*, 2006).

Serum MPO and NO levels couldn't be compared between subgroups of IHD due to small sample size. The risk factors like diabetes and smoking can increase myeloperoxidase levels (Scharnagl H *et al*, 2014). Study done by Leonard *et al* observed association of MPO with blood pressure in patients with diabetes and obesity (Van der Zwan LP *et al*, 2010). (Nahon P *et al*, 2009) observed that increased alcohol consumption leads to reactive oxygen species formation and is associated with high MPO levels. In this study, the effect of single risk factor could not be studied because of small sample size and most of the study subjects had multiple risk factors. However, it was observed that serum MPO levels were higher in subjects with risk factors like hypertension, smoking or both. Sample size was small and serial estimations were not done during the acute period. Follow up studies were not done.

CONCLUSION

High MPO levels and low NO levels were observed in acute IHD subjects. There was a negative and moderately significant correlation between serum MPO and NO in acute IHD subjects. This is a matter of concern as they are associated with risk of adverse cardiovascular events. Hence determination of serum MPO and NO in general population may be useful in taking proper measures to prevent IHD.

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