Myeloperoxidase and Paraoxonase Activity in Acute Coronary Syndrome Patients

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Background: Myeloperoxidase (MPO), an oxidative stress related enzyme is elevated in Coronary Artery Disease (CAD) and is involved in development of atherosclerotic plaque. Paraoxonase (PON) an enzyme protein associated with HDL serves as an antioxidant and plays an important role in preventing the formation of Oxidized LDL (OxLDL). This suggests a conflicting role of MPO and PON in development of cardiovascular disease and atherosclerosis.
Aim: Present study was done to evaluate and compare MPO/PON ratio in Acute Coronary Syndrome (ACS) patients with controls. The study evaluates and compares the pro oxidant and pro inflammatory enzyme, MPO and anti-oxidant and anti-inflammatory enzyme, PON in ACS patients with controls. Oxidative marker, Malondialdehyde (MDA) and anti-oxidant marker, Reduced Glutathione (GSH) was assessed in ACS patients and compared with controls. An attempt was also made to correlate MPO/PON ratio to markers of oxidative stress (MDA and GSH).
Methods: Cross-sectional study carried out in Dr. Somervell Memorial CSI Medical College, Trivandrum, Kerala, India.50 ACS patients from Cardiac Care Unit and 50 age and sex matched controls without CAD from Medical College Health Checkup was selected.

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Results: MPO and MPO/PON ratio were significantly high and PON was significantly lower in ACS patients compared to controls. Total cholesterol, Triglyceride, LDL, MDA were significantly high in ACS patients. Statistically significant positive correlation was observed between MPO/PON and MDA. Significant negative correlation was observed between MPO/PON and GSH.

Conclusion: Myeloperoxidase and MPO/PON ratio was significantly high in ACS patients than controls. This is suggestive of the role of MPO in oxidative damage to lipoproteins in CAD patients. Prooxidant, Paraoxonase ,and antioxidant, GSH is lowered in ACS patients as a result of the increased oxidative stress. This study suggests that MPO/PON1 ratio can be used as a predictive marker of ACS.

1. INTRODUCTION

Coronary artery disease (CAD) isone of the principal reasons behind death due to Non communicable diseases in developed countries. World Health Organizationhas stated that close to seven million people die of CAD every year all around the world[1]. Among these sudden deaths, 80% are triggered by atherosclerotic CAD [2]. Atherosclerosis occurs as a result of development of fatty lesions,plaque formation andresulting inflammation. This sequence of events may occur as a consequence of continuous oxidative stress building up inside the cell [3].Acute coronary syndrome (ACS) is acomplex form of coronary heart disease (CHD). It includes unstable angina (UA), non-ST segment elevation myocardial infarction (NSTEMI), and ST segment elevation myocardial infarction (STEMI). Rupture of atherosclerotic plaque and the thrombosis resulting from this are the leading cause of ACS [4,5]. One established reason for atherosclerotic plaque formation is the low-density lipoproteins (LDL) undergoing oxidative modifications and formation of oxLDL inside the arterial wall.

Among the enzymes that bring about oxidation of LDL, most important one is Myeloperoxidase (MPO).It is a lysosomal heme containing protein capable of breaking up different types of peroxides. Profusely present in the neutrophil granulocytes, MPO displays potent pro-oxidative and proinflammatory properties. MPO is involved in the reaction of hydrogen peroxide and chloride ion to form hypochlorous acid. This is responsible for the microbicidal action of phagocytes. It is found to be present in large amounts in ruptured plaque [6]. MPO is also involved in the oxidation of lipids associated with LDL cholesterol and for the oxidative modification of lipoproteins in the artery wall [7]. MPO is apparently elevated in ACS patients and is therefore an established marker of inflammation and resulting oxidative stress.

Low Density Lipoprotein has direct correlation with development of CAD [8]. On the contrary, HDL and its major Apo lipoprotein, Apo AI, are inversely related to risk of CAD. HDL becomes an easy target for oxidation by MPO, which converts this atheroprotective lipoprotein to a dysfunctional one. Because of this alteration in vivo, HDL is considered as an objective for therapeutically preventing many vascular diseases [9].Through its antioxidative and antiatherogenic processes, this lipoprotein safeguards against development of atherosclerosis [10,11]. This therapeutic capacity of HDL is attributed to effect of Paraoxonase1 (PON1), an anti atherogenic enzyme associated with this lipoprotein [12,13].

Paraoxonaseis an enzyme protein made up of 354 amino acids. It has a molecular weight of 43 kDa [14,15].Action of PON is to bind reversibly to its substrate like organophosphorouscompounds and it is hydrolyzed.PON has materialized as an essential part of HDL that helps it to metabolize lipid peroxides and prevents them from accumulating on LDL particles. Lipid peroxidation of LDL particles was prevented by purified PON to a great extent [16,17]. PON1 was extensively effective in protecting LDL against oxidation more than Lecithin Cholesterol Acyl Transferase, LCAT or apoA-I.It has been proven that antioxidant effect of HDL is also due to associated Paraoxonase enzyme [18].

Based on these conclusions, PON can be further studied as a causative factor for development of Coronary Artery Disease. Even though many studies have been conducted to test the ability of PON to prevent atheroma, further information is required about the pharmacological and therapeutic effects of this HDL associated enzyme. More light can be thrown into the protective effect of Paraoxonase by studying in detail its interrelationship with other atherogenic inflammatory enzymes like myeloperoxidase.

The specific objective of the present study was to assess and compare the Myeloperoxidase/ Paraoxonase (MPO/PON) ratio in patients with Acute Coronary Syndrome and normal subjects. An attempt was also made to correlate MPO/PON ratio to markers of oxidative stress (MDA and GSH).

2. MATERIALS AND METHODS

This study was carried out in Department of Biochemistry, Dr. Somervell Memorial CSI Medical College, Trivandrum, Kerala, India.

2.1 Sample size

100 (~50 in each group) Estimation of Minimum sample size: with the power of 90 % and significance level of 5 %, assuming a difference of 100 U/ml of PON (assume standard deviation 150) ,the required sample size is 49 per group.

50 ACS patients were selected from critical care unit (CCU) and Medical Intensive Care Unit (MICU) of our institute.50 age and sex matched subjects without CAD wasselected as controls from Health checkup of Somervell Memorial Medical college Hospital.

Blood samples were collected from all the patients after getting written informed consent as per the criteria. Sample from each subject was collected by venipuncture as and when admitted to CCU. Serum samples were separated by centrifugation at 3000 rpm for 10 minutes. Samples were frozen at -80℃ until further analysis.

Detailed demographic and other relevant information were recorded using Proforma. 4 ml of peripheral blood was collected from each patient and the following parameters were analyzed - Total Cholesterol (TC),Triglyceride(TG), High Density Lipoprotein Cholesterol(HDL-C), Low Density Lipoprotein Cholesterol (LDL-C), Malondialdehyde (MDA), Reduced Glutathione (GSH), Paraoxonase -1 (PON-1) and Myeloperoxidase

(MPO).VLDL,MPO/PON ratio was calculated from the available data.

MPO & PON1 were done spectrophotometrically, using the method of Matheson et al.1981 and the method of Beltowski et al. 1985, respectively. MDA was estimated by Yagi's & Satoh's (1984) method. GSH was estimated using commercial assay kits (Alcaraz et al 2004). Plasma Lipid Profile was estimated in fully automated chemistry analyzers using commercial assay kits.

2.2 Statistical Analysis

In case of normally distributed variables, data are presented as mean ± SD. Difference in baseline characteristic between two groups were tested using t test.Correlation Analysis between MPO/PON and MDA and GSH were tested by calculating Karl Pearson Correlation Coefficient. *P* value of <0.05 was considered to be statistically significant. The statistical analysis was performed using SPSS software.

3. RESULTS

The study was conducted to assess the contradictory effects of prooxidant enzyme Myeloperoxidase, and antioxidant enzyme Paraoxonase in ACS patients. Pro inflammatory MPO, anti atherogenic PON-1, lipidperoxidation product MDA, an endogenous antioxidant GSH and serum lipid profile were estimated in ACS patients. These values were compared with that of age and sex matched controls.

The test population comprised of 50 subjects that included 34 (68%) males and 16 (32%) females. Control group consisted of 50 subjects that included 33 (65%) males and 17 (35%) females. The age of the test group varied from 35 to 70 years with mean age 52.5 years, whereas that of control subjects varied from 36 to 70 years with a mean age 53 years. Baseline characteristics of ACS patients and controls are shown in Table 1a. Distribution of test and control group according to the conventional risk factors of CAD is given in Table 1b.

Table 1a. Baseline characteristics of Acs patients and control group

Baseline characteristics		ACS	CONTROL
Age		52.5(35-70)	53(36-70)
Gender	Male	34	33
	Female	16	17
BMI (kg/m^2)		27.48 ± 4.25	24.16 ± 3.04

Table 1b. Distribution of test and control according to the risk factors

Risk factors	Acs patients		Control	
	Yes	No	Yes	No
Diabetes	57%	43%	42%	58%
Hypertension	61%	39%	29%	71%
Smoking	22%	72%	0	0
Alcohol	36%	64%	26%	74%

3.1 Biochemical Characteristics

3.1.1 Oxidative stress and Antioxidant status

Oxidative stress related enzyme MPO and antioxidant enzyme PON1 are evaluated and compared in ACS patients and controls. The mean value of MPO, PON1 and MPO/PON1 of ACS patients and control subject were compared and given in table 2. MPO and MPO/PON1 of ACS patients were significantly high compared to control subjects. PON1 was significantly lowered in ACS patients compared to that of controls.

Lipid peroxidation product MDA, endogenous antioxidant GSH levels was evaluated in ACS patients and controls. Mean value of MDA and GSH in ACS patients and controls were compared in table 3. MDA level of ACS patients were significantly higher compared to controls. Level of GSH was found to be significantly lowered in controls as compared to ACS patients.

Correlation analysis was done between MPO/PON ratio and markers of oxidative stress (MDA and GSH) and is given in Table 4. A statistically significant positive correlation was observed with MPO/PON and MDA. Significant negative correlation was observed in case of MPO/PON and GSH.

Lipid profile in ACS patients and controls were compared. Mean value of TC, TG, HDL, LDL , VLDL, Non-HDL and LDL/HDL ratio of these two groups are given in table 5.TC, TG, LDL, VLDL, Non-HDL and LDL/HDL showed significant increase in ACS patients compared to that of controls .HDL value was high in controls but there was no statistical significance.

4. DISCUSSION

Many conventional risk factors like diabetes, hypertension, smoking and family history have already been established in the progress of CAD. Hypertension is a principal risk creating factor for

Table 2. MPO, PON1 and MPO/PON1 ratio in ACS and control subjects

Parameters(U/ml)	ACS patients	Control	*P*-Value
MPO	180 ± 50.73	139.85 ± 50.11	.003
PON1	190.72 ± 43.96	256.85 ± 54.75	.000
MPO/PON1	1.01 ± 0.45	0.562 ± 0.20	.000

Results expressed as mean ± SD

Table 3. Levels of MDA, GSH in ACS and control subjects

Parameters	ACS patients	Control	*P*-Value
MDA ng/ml	2.56 ± 0.9	1.9 ± 0.3	.000
GSH mg/ml	7.93 ± 1.2	9.52 ± 2.4	.001

Results are expressed in mean ± SD

Table 4. Pearson correlation analysis of MPO/PON with oxidative stress markers, MDA and GSH

		MDA	GSH
ACS	Pearson Correlation	0.341**	-0.32*
	Sig. (2-tailed)	0.007	0.01
CONTROL	Pearson Correlation	-0.173	0.103
	Sig. (2-tailed)	0.18	0.300

**Correlation is significant at the 0.01 level (2-tailed); *Correlation is significant at the 0.05 level (2-tailed)

Table 5. Lipid profile in ACS and control subjects

Parameters	ACS patients	Control	*P*-value
Total Cholesterol (mg/dl)	224.7 ± 49.07	196.6 ± 24.05	.009
Triglycerides (mg/d)l	155.15 ± 61.83	117.33 ± 52.41	.014
High Density Lipoprotein (mg/d)l	45.97 ± 9.58	50.77 ± 12.73	.110
Low Density Lipoprotein (mg/d)l	159.07 ± 32.42	138.48 ± 20.61	.006
Very Low Density Lipoprotein (mg/d)l	31.03 ± 12.37	23.46 ± 10.48	.006
Non-HDL (mg/d)l	567.83 ± 110.61	477.22 ± 74.98	.000
LDL/HDL	3.61 ± 1.06	2.85 ± 0.65	.000

Results are expressed in mean ± SD

cardiovascular disease [19]. Diabetes is also exceedingly prevailing in patients with ACS. Previous studies have demonstrated considerable excess risk in mortality rate and many adverse cardiovascular events were seen [20]. The present study also agrees with the fact that hypertension and Diabetes Mellitus forms a major risk factor for development of Coronary Artery Disease.

Our study clearly indicates an increase in oxidative stress enzyme myeloperoxidase in ACS patients when compared to controls. Study conducted in 2019 [21] suggests that increased Myeloperoxidase is associated with increased risk of mortality. They also suggest that MPO can be used in risk analysis models that can be used to guide therapy in ACS patients.

Also in our study, PON was lowered in ACS patients. It is seen in previous studies that reduced PON1 arylesterase activity can be used as predictive elements for mortality rate after Myocardial infarction [22]. MPO/PON ratio calculated in our study subjects were elevated in patients with ACS compared to controls. In an earlier study by Bacchetti et al, it is clearly mentioned that high MPO/PON1 ratio characterizes patients with atherosclerotic cardiovascular disease ASCVD and can be used as a potential marker of dysfunctional HDL [23]. Another study by Vasylchenko et al shows that myeloperoxidase/ paraoxonase-1 ratio was elevated many folds in patients with cardiovascular complications [24].

In order to assess the oxidant and antioxidant status in the study subjects, Lipid peroxidation product MDA, and endogenous antioxidant GSH levels were estimated in both ACS patients and controls. MDA levels in ACS patients included in this study were significantly higher compared to control subjects. Level of GSH was significantly lowered in control subjects when compared to

ACS patients. This finding is well in accordance with study conducted by Nguyen et al. [25] where MDA levels in the ACS group were significantly higher than that present in controls.

Another objective of this study was to correlate MPO/PON ratio with oxidative stress markers, like MDA and GSH in ACS patients. We observed a positive correlation between MPO/PON and MDA, which was statistically significant. Also a negative correlation with statistical significance was observed between MPO/PON and GSH.

5. CONCLUSION

Oxidative stress inducing MPO, antioxidant and antiatherogenic PON-1 plays a significant conflicting role in the initiation of ACS. The present study shows that Myeloperoxidase and MPO/PON ratio was significantly high in ACS patients than controls. This is suggestive of the role of MPO in oxidative damage to lipoproteins in CAD patients. Paraoxonase, a prooxidant enzyme as well as GSH an antioxidant is lowered in ACS patients as a result of this increased oxidative stress. Conclusions derived from this study is suggesting that MPO/PON1 ratio can be used as a predictive marker of ACS.

6. LIMITATIONS

Even though we have made much progression in understanding the pathogenesis of atherosclerosis, care should be taken before using these markers in clinical practice. Well-designed studies with large sample sizes are required to confirm the interaction between these inflammatory markers and to provide a scientific explanation behind this correlation. Genetic studies of well-defined groups would help us to learn more about the precise role of the above markers in development and management of atherosclerosis.

CONSENT

All authors declare that written consent to participate in the study was obtained from the participants before the start of the study.

ETHICAL APPROVAL

Ethical approval was obtained from the institute where research was carried out, SMCSIMCH/EC(PHARM)02/02/12.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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