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Original Article

Myeloperoxidase, protein carbonyls and oxidative stress in coronary artery disease

G. Tejaswi[a], M.M. Suchitra[a,*], D. Rajasekhar[b], V.S. Kiranmayi[a], P.V.L.N. Srinivasa Rao[a]

[a] Department of Biochemistry, Sri Venkateswara Institute of Medical Sciences, Tirupati 517507, Andhra Pradesh, India
[b] Department of Cardiology, Sri Venkateswara Institute of Medical Sciences, Tirupati 517507, Andhra Pradesh, India

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ABSTRACT

Objective: Oxidative stress and inflammation play important roles in the pathogenesis of coronary artery disease (CAD). Myeloperoxidase (MPO) produced by circulating neutrophils plays an important role in both processes of inflammation and oxidative stress. MPO generates reactive oxygen species which cause oxidative damage to cellular lipids and proteins. Generation and accumulation of oxidative damage plays an proatherogenic role initiating a cascade of damaging cellular changes. This study was designed to evaluate the association of MPO with oxidative stress and to assess MPO as a predictor of CAD.
Methods and results: Thirty patients diagnosed with CAD following angiography along with thirty age matched healthy controls were taken into the study. CAD cases had significantly higher triglyceride ($p = 0.012$), lower HDL cholesterol, higher oxidative stress markers malondialdehyde (MDA), protein carbonyl content (PCO), and MPO levels and lower antioxidant ferric reducing ability of plasma (FRAP) levels in CAD patients when compared to controls ($p < 0.001$). The oxidant markers were found to have good sensitivity and specificity at cut off values of MPO 106.25 IU/L, PCO 0.250 nmol/mg of protein and MDA 1.8 μmol/L. MPO was found to be a significant predictor of CAD.
Conclusion: Our study shows that an increase in MPO levels is associated with oxidative stress and MPO is also observed to be a significant predictor of CAD.

1. Introduction

Globally cardiovascular diseases (CVDs) are ranked as the number one cause of death. Similarly CVD is also proving to be the leading cause of disability and death in India also.[1] CVD includes coronary artery disease (CAD) which is an athero-inflammatory disease, the prevalence of which is reported to be approximately 11% in urban population and 7% in rural population across India.[2] CAD mortality and morbidity are promoted by major risk factors, such as hyperlipidemia, hypertension, and smoking. Oxidative stress and inflammation play important roles in the pathogenesis of CAD are considered as novel risk factors for CAD. In fact, inflammation was found to be involved in all the stages of atherosclerotic process, right from the evolution of atherosclerotic plaque till the stage of complications. Myeloperoxidase (MPO), a member of heme peroxidase family found within circulating neutrophils, produces hypochlorous acid (HOCl) during the neutrophil's respiratory burst, which is cytotoxic and is used by neutrophils to kill pathogens and in the same manner it may cause oxidative damage to host tissue structures. Hence MPO is linked to both inflammation and oxidative stress, the events that participate in the initiation and progression of plaque formation and also rendering plaques unstable.[3–5]

Stable intermediates and products formed during interaction of free radicals with bio molecules are commonly measured as indicators of oxidative status. Some of these include malondialdehyde (MDA), which is a by-product of lipid peroxidation and protein carbonyls (PCO), formed as a result of free radical attack on proteins.[6] Studies have evaluated the usefulness of MDA as a biomarker in coronary artery disease.[7] Protein carbonyls have become widely accepted biomarker of oxidative stress.[8] Ferric reducing ability of plasma (FRAP) is an estimate of total antioxidant status.[9] An increase in oxidative modifications in lipid, protein and DNA with a decrease in FRAP values has been observed in patients with CAD when compared to controls.[10] MPO levels in leukocytes and in the blood of patients with CAD were reported to be significantly increased when compared to controls.[11] Similarly, in a large prospective study, elevated MPO levels were found to predict the future risk of CAD in apparently healthy individuals.[12] Elevated MPO levels in CAD were found to progressively increase with

* Corresponding author.
E-mail address: suchitra.n@rediffmail.com (M.M. Suchitra).

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progression of CAD from stable CAD to non-ST-segment elevation acute coronary syndromes and to acute myocardial infarction.[13]

Identification of factors that are involved in this complex cascade not only helps to predict the risk of cardiovascular disease, but also helps in designing targeted effective therapeutic interventions. This prospective case control study was hence taken up to evaluate the association of MPO with oxidative stress and the predictive ability of MPO in CAD patients.

2. Material and methods

2.1. Subjects

Patients attending the cardiology department of Sri Venkateswara Institute of Medical Sciences, Tirupati and diagnosed with coronary artery disease following angiography along with age matched controls with a normal angiogram were taken into the study. Subjects with acute coronary syndrome, those who had coronary artery bypass graft and angioplasty were excluded from the study. Thirty CAD patients (28 males), and thirty controls (15 males) were included into the study after obtaining a written informed consent. The study was approved by the institutional ethics committee. The study was performed as per the ethical principles for medical research involving human subjects of the World Medical Association declaration of Helsinki.

2.2. Sample collection

6 ml of venous blood was collected into additive free tubes from all the subjects following an overnight fast of 8–12 h. The samples were allowed to stand for 30 min and the serum separated by centrifugation at 2000 rpm for 10 min. The separated serum was stored at −80 °C until further analysis.

2.3. Biochemical Analysis

Total Cholesterol, Triglycerides, HDL cholesterol were estimated by cholesterol oxidase-peroxidase method, glycerol phosphate oxidase method and Beckman system pack respectively on Beckmann Coulter Unicel DxC 600 autoanalyser. VLDL was calculated by using formula Triglyceride/5; LDL was calculated by using Friedwald's formula.[14] MDA,[15] protein carbonyls,[16] FRAP[8] and MPO[17] was measured by spectrophotometric method using Perkin Elmer UV/VIS spectrophotometer.

2.4. Statistical Analysis

Data was expressed as Mean ± Standard deviation. The difference between the parameters in the two study groups was assessed using *t*-test or Mann Whitney *U* test as appropriate. Correlation between the oxidative stress markers and antioxidant status was assessed by Pearson or Spearman correlation analysis as appropriate. Receiver operating characteristic curve (ROC) was constructed for the oxidant markers to study their diagnostic sensitivity and specificity in CAD. Logistic regression analysis was performed to assess MPO as a predictor of CAD. Statistical analysis was performed using Microsoft Excel Spread Sheets and SPSS for windows version 11.5. A 'p' value < 0.05 was considered as statistically significant.

3. Results

Table 1 shows the traditional cardiovascular risk factors in the two study groups. Patients with CAD had significantly higher triglyceride levels ($p = 0.012$) and lower HDL cholesterol levels ($p < 0.001$) when compared to the control group. The oxidant and antioxidant markers studied in study group and controls are shown in Table 2. MDA, protein carbonyl content, and MPO levels were found to be significantly higher and FRAP levels were significant lower in CAD patients when compared to the control group ($p < 0.001$). Fig. 1. shows the ROC curve analysis with significant areas under the curve for the oxidant markers ($p < 0.001$). Table 2. shows significant positive correlations between oxidant markers and significant negative correlations between oxidant and antioxidant markers ($p < 0.001$). Table 3. shows logistic regression analysis which found MPO as a predictor of CAD.

4. Discussion

Obesity, insulin resistance, dyslipidemia, type-2 diabetes mellitus act as powerful risk factors of CAD. It has been reported that total cholesterol, triglyceride and LDL cholesterol levels were significantly higher and HDL cholesterol to be significantly lower in CAD patients when compared to control group.[18] In the present study, we similarly found CAD patients had significantly higher levels of Triglycerides and significantly lower levels of HDL when

Table 2
Correlation between oxidant and antioxidant markers.

	MPO		PCO		MDA		FRAP	
	r value	p value	r value	p value*	r value	p value*	r value	p value*
MPO	–	–	0.673	<0.001	0.617	<0.001	−0.566	<0.001
PCO	–	–	–	–	0.676	<0.001	−0.736	<0.001
MDA	–	–	–	–	–	–	−0.621	<0.001

MPO- Myeloperoxidase, PCO-protein carbonyl, MDA- malondialdehyde, FRAP-ferric reducing ability of plasma.
* Statistically significant.

Table 1
Demographic data and biochemical parameters in the study groups.

Parameter	Controls (n = 30)	Cases (n = 30)	p value
Age (years)	48.14 ± 6.94	52.43 ± 10.12	0.069
Total Cholesterol (mg/dL)	171.17 ± 32.12	162.87 ± 40.71	0.384
Triacylglycerol (mg/dL)	123.70 ± 51.99	171.43 ± 85.75	0.012*
HDL-C (mg/dL)	36.93 ± 5.97	31.33 ± 5.20	<0.001*
LDL-C (mg/dL)	109.49 ± 27.48	97.23 ± 39.62	0.169
VLDL-C (mg/dL)	24.74 ± 10.40	34.30 ± 17.20	0.012*
MDA (μmol/L)	0.96 ± 0.48	4.41 ± 2.52	<0.001*
FRAP (mmol/L)	0.40 ± 0.06	0.25 ± 0.07	<0.001*
PCO (nmol/mg of protein)	0.13 ± 0.05	0.87 ± 0.35	<0.001*
MPO (IU/L)	70.80(50.90–88.50)	283.25(194.70–553.12)	<0.001*

TGL- Triglycerides; HDL- High density lipoprotein cholesterol; LDL-Low density lipoprotein cholesterol; VLDL- Very low density lipoprotein cholesterol, MDA-Malondialdehyde; FRAP- Ferric reducing ability of plasma; PCO-Protein carbonyl content, MPO- Myeloperoxidase.
* Statistically significant, Data expressed as Mean ± SD, median (inter quartile range).

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