Original article:

Study of Myeloperoxidase level in Coronary Artery Disease patients from tertiary care Hospital

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ABSTRACT

Background:

Coronary artery disease (CAD) is a major cause of morbidity and a leading contribution to mortality worldwide. Timely diagnosis allows clinicians to risk stratify their patients and select appropriate treatment. Levels of creatine kinase isoenzymes and cardiac troponins, which are diagnostic biochemical parameters of myocardial necrosis, are used either alone or in conjunction with levels of C-reactive protein as prognostic indicators of CAD. Many patients with chest pain have normal levels of creatine kinase isoenzymes or troponins at presentation but subsequently have a myocardial infarction, require revascularization, or die within six months. Additional biochemical measures, ideally based on the pathophysiology of plaque vulnerability are needed. Inflammation plays a critical role in acute myocardial infarction. One such inflammatory biochemical parameter is myeloperoxidase (MPO). Their role as a diagnostic biochemical parameter in coronary artery disease is unclear. We sought to investigate this.

Material & methods

We studied 50 control subjects & 150 patients of CAD which includes 50 cases each of acute myocardial infarction, unstable angina, and stable angina. Plasma levels of MPO and Serum total CPK, CK-MB, LDH, CRP, uric acid and lipid profile levels along with Troponin T were measured in all these patients.

Results

Plasma Myeloperoxidase (MPO) level was significantly increased in AMI [707.00 ± 67.42ng/ml], unstable angina [340.94 ± 45.28ng/ml] and stable angina [283.26 ± 30.68ng/ml] as compared to control mean value [112.12 ± 29.07ng/ml]. We observed 100% sensitivity and specificity for MPO in CAD, which is more than the other biomarkers. Inflammatory marker like CRP were significantly raised in Stable angina [3.73 ± 0.72], in unstable angina [18.22 ± 5.75], and in AMI [19.12 ± 8.64]. While serum uric acid were also significantly increased in AMI [8.18 ± 1.07], unstable angina [5.58 ± 0.76], and stable angina [3.55 ± 0.62], When compared with control mean value of CRP [1.39 ± .643mg/l] and uric acid [2.52 ± 0.51mg/dl]. Lipid profile and other cardiac biochemical

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parameter are also significantly increased; While Troponin T was positive in AMI and negative in unstable angina, stable angina and in control.

Conclusion:

Myeloperoxidase level is increased in all the three groups of coronary artery disease (CAD) and this increase is statistically significant when compared to the control group. Also this increase was in accordance with increase in other inflammatory biochemical parameter like serum CRP and serum uric acid. Hence myeloperoxidase serves as an early marker of the inflammation and vulnerability of plaque and one that can be used to identify patients at imminent risk for major adverse cardiac events. MPO can also be used as a diagnostic biochemical parameter in CAD.

Keywords: Coronary artery diseases, Myeloperoxidase, C- reactive protein, inflammation

Introduction:

Cardiovascular Disease (CVD) is a major cause of morbidity and a leading contribution to mortality worldwide. Although CVD mortality has declined in economically developed countries, the emergence of CVD during recent decades in developing countries like China, India, is increasing. The risk of CAD in Indians is 3-4 times higher than Americans, 6-times higher than Chinese, and 20-times higher than Japanese. Indians are prone as a community to CAD at a much younger age.[1, 2]CAD occurs when the arteries that supply blood to the heart muscle become hardened and narrowed hence, reduced or cutoff blood flow and oxygen supply to the heart muscle which can result in angina. Russel Ross (1986)[3] described the characteristics of plaques by explaining formation of fibrous cap and beneath the cap, lesion containing smooth muscle cells, macrophages laden with lipid droplets, surrounded by connective tissue. Beneath this there is area of necrotic tissue debris, cholesterol crystals and calcification. Several prospective epidemiologic studies have demonstrated that elevated levels of LDL-C and low levels of HDL-C are independent risk factors for CAD.The C-reactive protein (CRP), is an exquisitely sensitive systemic marker of disease with broad clinical utility for monitoring and differential diagnosis. Inflammation, the key regulator of CRP synthesis, plays a vital role in atherothrombotic cardiovascular disease. In consideration of the important role that inflammatory processes play in determining plaque stability, recent work has focused on whether plasma markers of inflammation like myeloperoxidase, CRP, and uric acid may help improve risk stratification. Of these markers, CRP has been the most widely studied[4].

Material and Methods:-

Study design and subjects:

The objective was to study Myeloperoxidase level in patients with CAD and healthy controls. The present study comprises of 150 clinically diagnosed cases of CAD like stable angina (SA), unstable angina and acute myocardial Infarction (AMI). Age and sex matched 50 normal individuals were studied as controls. Diagnosis was based on ECG/2DECHO evidence and enzyme profiles with the help of expert physicians of cardiac unit. The synopsis of the study protocol was submitted to the institutional ethics committee and approval was obtained. Department where the study was conducted has supported the work no other funding source was approached.

Informed consent was obtained from all subjects. Patients and controls with known active inflammatory diseases (other than atherosclerosis), and Patients with anemia, valvuar heart disease, pericardial heart disease, patients with clinical hypothyroidism were excluded. While patients with Chest pain or chest discomfort, shortness of breath. Age≥ 35, of either sex, family history of early heart disease. Swelling of feet and abdomen, fatigue. ECG/2DECHO evidence of IHD or both were included in the study.

About 10 ml of blood was obtained by venipuncture and collected in plain bulb and EDTA bulb. The blood in EDTA bulb used for Myeloperoxidase and Troponin T estimation. The serum was used for measurement of lipid profile, SGOT, LDH, Total CPK, CPK-MB, CRP, and U.A.

Statistical analysis

Statistical calculations were expressed as mean ± standard deviation (SD), A P value <0.05 was considered statistically significant. Receiver operating characteristic (ROC) analysis is used to calculate cutoff value and sensitivity and specificity of variables.

Observation & Results:-

Table 1:- Clinical data of controls and patients with Coronary artery disease

Parameters	N	Controls	AMI		Unstable angina		SA	
		Mean ± SD	Mean ± SD	P Value	Mean ± SD	P Value	Mean ± SD	P Value
Age (Yrs)	50	45.24 ±9.58	45.48 ±9.41	P>0.05	44.96 ±7.76	P>0.05	45.76 ±9.41	P>0.05
SBP (mmHg)	50	123.20 ± 6.61	125.94 ± 11.49	P>0.05	126.20± 10.38	P>0.05	128.76± 7.60	P<0.05
DBP (mmHg)	50	68.54 ± 4.68	73.64 ± 7.08	P<0.0001	76.42± 6.46	P<0.0001	74.90± 5.21	P<0.0001
Hb gm/dl	50	13.11 ± .93	9.68 ± 2.69	P<0.0001	13.02 ± 1.24	P>0.05	11.94 ± 1.57	P<0.005
WBC 10^3/ µL	50	6.85 ±1.05	10.38 ±2.39	P<0.0001	9.42 ±1.56	P<0.0001	7.54 ±1.35	P>0.05
Pulse/min	50	74.90 ± 3.75	102.24 ± 10.03		88.84 ± 14.35		78.20 ± 7.74	

There is a no statistically significant difference in age between all groups (p>0.05). The youngest patient was of 35 years while the oldest was of 74 years of age. Chest pain was present in all CAD patients [n=150(75%)], which was highly significant (p<0.0001) and absent in controls [n=50(25%)].

Table 2:- Lipid profile levels of controls and patients with coronary artery disease

Parameters	N	Controls	AMI		Unstable angina		SA	
		Mean ± SD	Mean ± SD	P Value	Mean ± SD	P Value	Mean ± SD	P Value
T. Cholesterol mg/dl	50	156.82 ±27.78	265.08±52.29	P<0.0001	256.26 ±61.16	P<0.0001	200.76± 79.25	P<0.001
TG mg/dl	50	103.06 ± 24.80	263.50±61.04	P<0.0001	244.16 ±61.82	P<0.0001	184.62± 65.77	P<0.0001
HDL mg/dl	50	53.00 ± 7.22	35.39 ±3.37	P<0.0001	38.24 ±2.64	P<0.0001	40.48 ±9.07	P<0.0001
LDL mg/dl	50	83.20 ± 23.74	176.99 ±42.23	P<0.0001	169.18 ±59.42	P<0.0001	123.35 ± 74.82	P<0.001
VLDL mg/dl	50	20.61 ± 4.96	52.70 ±12.20	P<0.0001	48.83 ±12.36	P<0.0001	36.92 ±13.15	P<0.0001
TC/HDL	50	2.97 ± .48	7.53 ±1.81	P<0.0001	6.76 ±1.84	P<0.0001	5.54 ±3.23	P<0.0001
LDL/HDL	50	1.57 ± .45	5.07 ±1.44	P<0.0001	4.47 ± 1.71	P<0.0001	3.52 ± 2.72	P<0.001

Lipid Profile levels in all three groups of CAD showed high significant increase as compared to h controls (p<0.0001).

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Table 3:- Data showing Cardiac enzymes level of controls and patients with coronary artery disease

Parameters	N	Controls	AMI		Unstable angina		SA	
		Mean ± SD	Mean ± SD	P Value	Mean ± SD	P Value	Mean ± SD	P Value
T. CPK IU/L	50	101.71 ±37.92	276.85 ±27.11	P<0.0001	256.92 ±20.02	P<0.0001	127.67 ±16.20	P<0.0001
CK-MB IU/L	50	10.52 ±5.58	99.37 ±36.33	P<0.0001	36.43 ±6.17	P<0.0001	23.38±3.71	P<0.005
SGOT IU/L	50	20.11 ±9.06	144.03 ±28.47	P<0.0001	33.82±9.69	P<0.0001	25.29±5.41	P>0.05
LDH IU/L	50	141.34 ± 25.48	315.03 ± 29.47	P<0.0001	207.17 ± 38.85	P<0.0001	159.85 ± 27.89	P<0.05

In table 3 all three groups of CAD show significant increase of Total CPK, CK-MB, LDH, and SGOT as compared to normal control mean value except SGOT in stable angina. Troponin T was positive in AMI and negative in unstable angina, stable angina and control subjects.

Table 4:- The mean ± SD values of myeloperoxidase, C-reactive protein, and uric acid, in controls and patients with coronary artery disease.

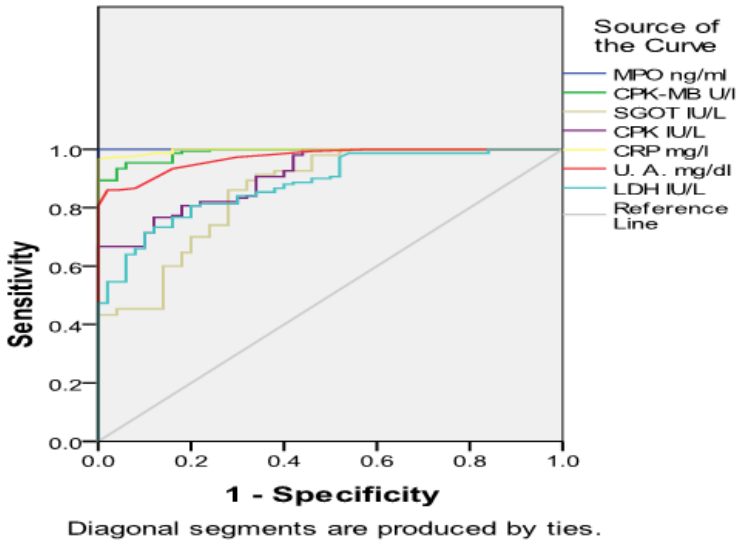
Parameters	N	Controls	AMI		Unstable angina		SA	
		Mean ± SD	Mean ± SD	P Value	Mean ± SD	P Value	Mean ± SD	P Value
MPO ng/ml	50	112.12 ± 29.07	707.00 ± 67.42	P<0.0001	340.94 ± 45.28	P<0.0001	283.26 ± 30.68	P<0.0001
CRP mg/L	50	1.39 ± .643	19.12 ±8.64	P<0.0001	18.22 ±5.75	P<0.0001	3.73 ± .724	P<0.0001
U.A. mg/dl	50	2.52 ± .51	8.18 ±1.07	P<0.0001	5.58 ± .76	P<0.0001	3.55 ± .62	P<0.0001

As shown in table 2, in the present study, plasma myeloperoxidase levels showed highly significant increase (p<0.0001) as [283.26] in stable angina, [340.94] in unstable angina and [707.00 ng/ml] in AMI as compared to healthy controls [112.12]. Which was highly significant (p<0.0001). The diagnostic efficacy of myeloperoxidase was determined by Receiver operating characteristic (ROC) analysis. When we analyses these results, we observed 100% sensitivity and specificity for MPO in CAD, which is more than the other biochemical parameters. So we can say that MPO is better biochemical parameter in comparison with other biochemical parameter in CAD.Inflammatory marker like CRP was significantly increased in AMI [19.12], unstable angina [18.22], and SA [3.73] (p<0.0001). When compared with controls of CRP [1.39mg/l]. While serum uric acid was also significantly increased in AMI [8.18], unstable angina [5.58], and SA [3.55] (p<0.0001) when compare with healthy controls [2.52mg/dl]. This suggests that there is inflammatory reaction in the foreground of CAD.

Table 5:- ROC analysis of MPO and other biochemical parameters in CAD.

Sr. No.	Variable(s)	Cut off	Sensitivity %	Specificity %	Area Under curve (AUC)	SE	Significance	95% Confidence Interval	
								Lower Bound	Upper Bound
1	MPO ng/ml	>180.5 ng/ml	100	100	1.00	0	<0.0001	0	1
2	SGOT IU/L	>21.80 IU/L	86	72	0.85	0.03	<0.0001	0.79	0.91
3	CPK IU/L	>133.17 IU/L	82	78	0.91	0.02	<0.0001	0.87	0.95
4	CK-MB U/l	>18 U/L	98	84	0.98	0.00	<0.0001	0.97	0.99
5	CRP mg/l	>2.75 mg/l	97	96	0.99	0.00	<0.0001	0	1
6	U. A mg/dl	>3.1 mg/dl	93	84	0.97	0.01	<0.0001	0.95	0.98
7	LDH IU/L	>159.6 IU/L	81	78	0.88	0.02	<0.0001	0.83	0.92

Graph 1:- ROC curve of MPO and other biochemical parameter in CAD



Graph 1 show the diagnostic efficacy of myeloperoxidase and other cardiac biochemical parameters was determined by ROC analysis in CAD. The cut-off values of MPO and other cardiac biochemical parameters were calculated in CAD.

Table 5 shows the cut-off level of MPO in 150 patients of CAD was determined as >180.5 ng/ml (AUC= 1; SE= 0; 95% CI= 0 – 1) using ROC curve analysis. We achieved a sensitivity of 100% and specificity of 100%. The cut-off level of CK-MB in patients of CAD was determined as >18 U/L (AUC= 0.98; SE=0.005; 95% CI= 0.97 – 0.99) using ROC curve analysis. We achieved a sensitivity of 98% and specificity of 84%. The cut-off level of AST in patients of CAD was determined as >21.80 IU/L (AUC= 0.85; SE=0.031; 95% CI= 0.79– 0.91). We achieved a sensitivity of 86% and specificity of 72%. The cut-off level of CPK in patients of CAl

determined as >133.17 IU/L (AUC= 0.91; SE=0.021; 95% CI= 0.87– 0.95). We achieved a sensitivity of 82% and specificity of 78%. The cut-off level of CRP in patients of CAD was determined as >2.75 mg/l (AUC= 0.99; SE=0.002; 95% CI= 0–1). We achieved a sensitivity of 97.33% and specificity of 96%. The cut-off level of LDH in patients of CAD was determined as >159 IU/L (AUC= 0.88; SE=0.025; 95% CI= 0.83–0.92) with sensitivity of 81.33% and specificity of 78%.

Discussion:

In this study we are trying to find out the diagnostic value of MPO with other cardiac biochemical parameters. MPO has been considered as an important biochemical parameter for inflammatory and auto immune diseases and cancer. Due to its role in the pathology of atherogenesis, MPO is also thought to be an important cardiac biochemical parameter.

MPO produces hypochlorous acid and other reactive molecules from hydrogen peroxide, a process that leads to lipid peroxidation and scavenging of nitric oxide. Because increase in these activities is found within atherosclerotic lesions, MPO is a marker of inflammation and plaque vulnerability. Recent investigations of atherosclerosis have focused on inflammation, providing new insight into mechanisms of disease. The concept of the involvement of inflammation in atherosclerosis has spurred the discovery and adoption of inflammatory biochemical parameters for cardiovascular risk prediction. CRP is currently the best validated inflammatory biochemical parameter; in addition, myeloperoxidase may provide additional information for cardiovascular risk stratification and prediction.

In short we estimated all routine biochemical parameters and MPO in CAD i.e. in stable angina, in unstable angina and in AMI. We observed a significant rise in all these biochemical parameters. So to differentiate we did ROC curve analysis and it was observed that MPO is the only parameter which showed 100% sensitivity and 100% specificity. Hence it can be concluded that it is a better biochemical parameter as compared to others. These findings are consistent with following studies.Nambi V[5]. (2005) had shows that MPO is believed to be one of the most promising cardiac markers. Recently it was demonstrated that an increased MPO level in patient's blood serves as a risk marker for atherosclerosis and CAD. Baldus S. et al[6]. (2003) and Brennan ML et al[7]. (2003) observed that MPO predicts the early risk of myocardial infarction, as well as the risk of other major adverse cardiac events in patients with chest pain in the ensuing 30-day and 6-month periods. The value of MPO as a marker is in that MPO predicts these outcomes independently of other known laboratory tested risk factors, including troponins, CK-MB, CRP and lipid profile. Moreover, unlike troponins I and T, CK-MB, and CRP, MPO makes it possible to identify patients at risk for cardiac events in the absence of myocardial necrosis. All these factors make MPO measurements in patients an indispensable procedure to reveal patients with chest pain that are at increased risk of cardiovascular complications. Zhang R, Brennan ML[8], (2001) suggests MPO levels may be elevated among individuals with CAD. Myeloperoxidase is an enzyme secreted by a variety of inflammatory cells, including activated neutrophils, monocytes, and certain tissue macrophages, such as those found in atherosclerotic plaque. The enzyme is not released until leukocyte activation and degranulation. Myeloperoxidase may convert LDL into a high-uptake form for

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macrophages, leading to foam cell formation, and may also deplete nitric oxide, contributing to endothelial dysfunction. In a recent case-control study, increasing levels of leukocyte-MPO and blood-MPO were significant predictors of the risk for CAD, such that after adjustment for white blood cell count and Framingham risk score, individuals in the highest quartile of blood-MPO had a 20-fold higher risk of CAD than individuals in the lowest quartile. Salonen I, Huttunen K, et al[9] (2012) followed Fifty-three patients with ischemic heart disease and observed that MPO was statistically associated with blood leukocyte, neutrophil, and lymphocyte concentrations of the patients (P=0.001-0.003). MPO was also statistically associated with high-sensitivity C-reactive protein (P=0.02) and was not associated with markers of lipid and glucose metabolism, of atherosclerosis, or of other chronic diseases. The results support previously reported view that MPO is a marker of inflammation in patients of ischemic heart disease.Roman, Rauel Melchior, Camargo PV, et al[10]. (2010) compared MPO levels among the patients with stable and unstable ischemic heart disease and evaluated their independent prognostic value for cardiovascular events. MPO and CRP levels were significantly higher among patients with ACS [MPO 93 (54-127) vs. 9.9 pmol/l (5-21) and CRP 11 (3-27) vs. 2.6 mg/l (1-5)]. Which suggest that this may participate in plaque vulnerability and instability process, whereas these findings suggest distinct role of the inflammatory markers studied in the pathophysiology of CAD.

Conclusion:

From the study it is clear that myeloperoxidase level is increased in all the three groups of CAD i.e. myocardial infarction (AMI), unstable angina and stable angina (SA), and this increase is statistically high significant ($p<0.0001$) when compared to the healthy control group. We achieved 100% sensitivity and specificity for MPO which is more than the other biochemical parameters. Also this increase was in accordance with increase in other cardiac biochemical parameter.

It could be concluded from these results that myeloperoxidase enzyme is precise in detecting CAD. Myeloperoxidase levels were increased in patients with not only myocardial infarction but also in patients with unstable and stable angina. Myeloperoxidase serves as biochemical parameter of the inflammation and vulnerability of plaque and one that can be used to identify patients at imminent risk for major adverse cardiac events. MPO can also be used as a diagnostic biochemical parameter in CAD.

Limitations:

Although the study has reached its aims, there are some unavoidable limitations & should be kept in mind.

1) This is a case control study comparing CAD patients with controls and the effect of previous treatment was not taken into account.
2) Serial measurements of these enzymes were not performed. Values not compared for different anatomical infarcts or with amplitude of ST elevation.
3) As this study was undertaken in tertiary care hospital coronary angiography was not possible in most of the patients for diagnosis of coronary artery disease.

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