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Lipid Peroxidation and Glutathione Peroxidase in Acute Myocardial Infarction

David Gangte[1*], Davina Hijam[1], Asitava Roy[1], Sonam Tashi Dolma[1], Lalnunpuii[1] and Th. Ibetombi Devi[1]

[1]Department of Biochemistry, Regional Institute of Medical Sciences, Imphal, Manipur, India.

Authors' contributions

This work was carried out in collaboration between all authors. Author TID designed the study, wrote the protocol. Authors DG, AR, STD and L applied the questionnaire and collected the blood samples. Authors DG and AR did the MDA, Gpx and lipid assay. Authors DG and DH wrote the manuscript and managed the analyses of the study. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: To evaluate the level of lipid peroxidation (by measuring malondialdehyde) and endogenous antioxidant enzyme (glutathione peroxidase) in acute myocardial infarction.
Study Design: Cross sectional study.
Place and Duration of Study: Department of Biochemistry in collaboration with Department of Medicine, Regional Institute of Medical Sciences (RIMS), Imphal, Manipur, India between November 2010 to April 2012.
Methodology: Fifty patients (32 male, 18 female) between the age group of 18-90 years suffering from acute myocardial infarction either attending emergency department, out patients department or admitted in intensive care coronary unit (ICCU) RIMS, within 6 hours from the complaint of chest pain were included in the study. Another thirty age and sex matched healthy individuals (18 male and 12 female) were taken as controls. Glutathione peroxidase (GPx) and malondialdehyde (MDA) levels were estimated by Beckman DU 640 spectrophotometer using commercially available kit. The data was analysed using SPSS version 16.
Results: The plasma malondialdehyde mean level (4.02±0.72 µmol/L in males and 3.77±0.58 µmol/L in females) was significantly high in the AMI patients compared to the control group (1.34±0.22 µmol/L in males and 1.30±0.26 µmol/L in females) however the

**Corresponding author: Email: dr.davidgangte@yahoo.in;*

glutathione peroxidase mean level (23.3±4.2 u/gHb in males and 23.0±3.6 u/gHb in females) was significantly reduced in AMI patients than the controls group (29.68±1.4 u/gHb in males and 29.57±1.0 u/gHb in females).

Conclusion: In conclusion AMI patients have increased oxidative stress and a compromised antioxidant defense system. Therefore, it is recommended that the management strategy for the patients of AMI should include specific antioxidant supplementation along with lowering of lipid peroxidation.

Keywords: Acute myocardial infarction; malondialdehyde; glutathione peroxidase.

1. INTRODUCTION

Acute myocardial infarction (AMI) is one of the major causes of morbidity and mortality in the world [1]. World Health Organisation (WHO) has predicted that from the years 2000 to 2020 disability adjusted life years (DALYs) lost from coronary heart disease(CHD) in India shall double in both men and women from the current 7.7 and 5.5 million respectively [2]. AMI occurs when there is an abrupt decrease in coronary blood flow following a thrombotic occlusion of a common artery previously narrowed by atherosclerosis. In most cases, infarction occurs when an atherosclerotic plaque fissures, rupture or ulcerates and a mural thrombus forms at the site of rupture and leads to coronary artery occlusion. The risk factors and predisposing factor for developing acute myocardial infarction are age, sex, family history, genetic factor which are not modifiable and other modifiable factors include cigarette smoking, hypertension, dyslipidaemia, diabetes, obesity and sedentary habits [3]. However, these factors explain only part of attributable cardiovascular disease. Evidence suggests that reactive oxygen species (ROS) may play an important role in the pathogenesis of myocardial infarction [4]. Following ischaemia, ROS are produced during reperfusion phase [5-6]. ROS are capable of reacting with unsaturated lipids and of initiating the self-perpetuating chain reactions of lipid peroxidation in the membranes [7-8]. Free radicals can also cause oxidation of sulfhydryl groups in proteins and strand scission of nucleic acids [9].

Myocardial antioxidants inhibit or delay the oxidative damage to subcellular proteins, carbo-hydrates, lipids and DNA. There is evidence that anti-oxidants can protect against free radical which is responsible for reperfusion-induced damage and lipid peroxidation, and may thereby inhibit thrombosis, myocardial damage and arrhythmias during AMI [10]. Total antioxidant capacity (TAC) is a critical tool for assessing redox status [11]. The TAC or related antioxidants may play an important role in protecting against free radicals mediated damage [12]. The role such compounds play in AMI development is important, since their presence may decrease the damage resulting from blood ROS during reperfusion.

The deleterious effects of the free radicals are kept under check by a delicate balance between the rate of their production and the rate of their elimination by these defense systems. When there is an excessive addition of free radicals from exogenous sources added to the endogenous production, the available tissue defense system becomes overwhelmed resulting in oxidative damage to the tissues [13].

Earlier findings reveals that Malondialdehyde (MDA) and Glutathione peroxidase (GPx) are useful parameters in myocardial infarction and their magnitudes depend on the severity and duration of ischaemic. With this view, the present study was carried out to find out the

balance between oxidative stress and antioxidant enzyme in patients with myocardial infarction irrespective of severity and duration of the disease

2. MATERIALS AND METHODS

It was a cross-sectional study carried out in the Department of Biochemistry in collaboration with the Department of Medicine, Regional Institute of Medical Sciences, Imphal-Manipur, India. Fifty acute myocardial infarction patients attending Emergency, Out Patients Department and Intensive Coronary Care unit for treatment during the period from November 2010 to April 2012 were taken as study group, while thirty age and sex matched apparently healthy individuals were taken as controls.

Acute Myocardial Infarction patients were diagnosed based on the European Society of Cardiology and the American College of Cardiology (ESC/ACC) [14-15].

Either one of the following criteria satisfies the diagnosis for an acute, evolving, or recent MI.

1. Typical rise and gradual fall (cardiac troponin) or more rapid rise and fall (CK-MB) of biochemical markers of myocardial necrosis with at least one of the following:

    a. Ischemic symptoms
    b. Development of pathological Q waves on the ECG
    c. ECG changes indicative of ischemia (ST segment elevation or depression) Coronary artery intervention (e.g.,coronary angioplasty).

2. Pathological finding of an AMI

    Patients with Diabetes, valvular heart disease, hepatic disease, neuromuscular disease, septic shock syndrome, renal disease were excluded from the study. No subject (patients or controls) was taking antioxidant or vitamins supplements, lipid lowering drugs or any other drugs known as affecting serum lipid peroxidation and antioxidant values. Demographic data including social, behavioural, medical history, Weight, Height, Body mass index (BMI), Blood pressure were recorded in the performa especially designed for the study.

2.1 Methods of Estimation

Ten ml sample was drawn from antecubital vein of the AMI patients within 6 hours from the complaint of chest pain. For lipid estimation venous blood samples were collected after an overnight fast for 12-14 hrs.

Plasma Malondialdehyde (MDA) was estimated by Beckman DU 640 Spectrophotometer using commercially available kit of Bioxytech, Oxis International, Inc. Portland, USA. Properly stored and accurately diluted standard have a slope within 10% of the expected extinction coefficient, between 0.110 and 0.130 A586/μM MDA. The lower limit of detection is defined as 5.185 standard deviations (n=10) from the blank absorbance at 586 nm. The sensitivity of the MDA-586 method for MDA is shown below [16].

Average A586	0.0102
Standard deviation, A586	0.0017
Detection limit, A586	0.0088
Detection limit, ìM (in reaction mixture)	0.0801

Glutathione Peroxidase (GPx) was estimated by Beckman DU 640 Spectrophotometer Using commercially available kit RANSEL, Randox Laboratories Ltd, UK. The intra assay and inter assay coefficients of variation for GPx were 6.7 percent and 9.9 percent, respectively.The method is linear up to a concentration of 900 U/I. The minimum detectable concentration of glutathione peroxidase with an acceptable level of precision was 74 U/I. [17]

Total cholesterol was estimated by the enzymatic method of Allain et al. [18] modified by Human Gesellschaft fur Biochemical and Diagnostica mbH (Germany).Quantitative estimation of Serum triglyceride was done by the method adopted by Bucolo and Harold [19] modified by Human Gesellschaft fur Biochemical and Diagnostica mbH (Germany). High density lipoprotein cholesterol was estimated by Enzymatic determination of cholesterol in high density ipoprotein fraction prepared by precipitation technique [20]. Low Density Lipoprotein Cholesterol and Very Low Density Lipoprotein cholesterol values in mg/dl were indirectly calculated by using the Friedwald T formula LDL= TC-(HDL+Triglyceride/5) [21].

2.2 Statistical Analysis

Data were expressed as Mean± SD, percentages, differences between the control subjects and patients were determined by student's t-test. Linear regression analysis was performed and $p<0.05$ values was considered statistically significant. Statistical analysis was done using SPSS ver.16

3. RESULTS AND DISCUSSION

The prevalence of AMI is higher among males with 32(64%) cases than females with 18 (32%) cases, the male to female ratio in the study group is 1.7:1 and this variation in sex-wise distribution was statistically insignificant. This finding is comparable with reports of Sytkowski et al. [22] and Shaw et al. [23]. The investigators reported that the incidence of CHD is markedly lower among women than men prior to the age of fifty years after which incidence of CHD increases and approaches that seen among men by the eighth decade. Similar finding was reported by Lawlor et al. [24]; Kannel and Levy [25] that the development of MI in women at older age is due to the protective effects of female sex hormones, but differences in diet and smoking may also be important. However Sonia et al. [26] suggested that the earlier age of acute MI in men can largely be explained by the higher levels of some risk factors men possess at younger ages. It was seen that there is an increased body mass index (BMI) in AMI patients compared to control subjects. Moreover Systolic blood pressure and diastolic blood pressure was significantly high in AMI patients groups as compared with controls. These findings are consistent with the findings of Akosah et al. [27] who reported a significantly high blood pressure and history of hypertension in patients with coronary heart disease as compared to those without coronary heart disease. Moreover higher numbers of smokers (58%) were observed in patients with AMI cases followed by non-smoker (26%) and ex-smokers (16%) (Table 1). These findings are consistent with the findings of Rich-Edwards JW et al. [28], who reported that tobacco use is heavily influenced by the historical context of communities and

cultural norms, and in most societies women have smoked less than men. Tobacco use clearly explains part of the lower rate of MI in younger women compared to that in men.

Table 1. Demographic characteristics of control and AMI patients

Parameters	Control n=30	AMI n=50	t	*P* value
Age(mean±S.D) years	65.4±8.43	59.4±10.39	0.718	0.476
Sex :				
Male (%)	18 (60)	32 (64)	-	-
Female (%)	12 (40)	18 (36)	-	-
Body mass index (mean±S.D),kg/m^2	23.2±1.02	28.26±1.67	14.42	.001
Systolic blood pressure (mm of Hg)	122.5±6.21	145±19.9	5.991	.001
Diastolic blood pressure (mm of Hg)	76.9±4.41	92.5±7.09	10.36	.001
Smoking status: Current smoker (%)	6 (20)	29 (58)	2.65	.01
Ex-smoker (%)	8 (26.7)	13 (26)	-5.70	.001
Non-smoker (%)	16 (53.3)	8 (16)	-13.5	.001

AMI patients had significantly higher level of total cholesterol, triglyceride, LDL-cholesterol and VLDL- cholesterol levels but lower HDL-cholesterol levels than the healthy controls. The difference in the serum lipid profile between control and study groups are statistically significant. Numerous cohort studies and clinical trials have confirmed the association beween a low high density lipoprotein-cholesterol and increased risk of coronary heart disease. Low density lipoprotein –cholesterol is considered as the most important risk factor of coronary heart disease. Its oxidized form promotes foam cells formation which initiates the process of atherosclerosis by accumulating in subendothelial cells leading to fatty streaks and complex fibrofatty or atheromatous plaques formation [28].

Table 2. shows that AMI cases have significantly higher levels of plasma mean MDA (3.94±0.68) as compared to controls group (1.32±0.24) however the whole blood GPx (23.20± 3.90) levels in AMI cases were significantly found to be in a lower levels than the control groups (29.64±1.09). The differences are statistically significant (P <0.05).

Table 2. Comparison of serum lipid profile, MDA and GPx level in controls and AMI cases

Sl. no	Parameter	Control (Mean±SD)	AMI cases (Mean±SD)	t	P value
1	Cholesterol(mg/dl)	172.8±132	248.5±36.5	23.49	0.001
2	TG (mg/dl)	109.8±140	165.4±8.50	19.65	0.001
3	HDL (mg/dl)	61.1±12.9	28.1±2.95	13.71	0.001
4	LDL (mg/dl)	109 ±17.4	175.9 ±11.8	18.44	0.001
5	VLDL (mg/dl)	22.3±8.2	42.8±5.7	12.01	0.001
6	MDA (µmol/L)	1.32±0.24	3.94±0.68	20.04	0.001
7	GPx(U/gHb)	29.64±1.09	23.20±3.90	10.94	0.001

AMI patients have a higher mean±SD plasma MDA level (4.02±0.72 in males and 3.77±0.58 µmol/L in females) than the control group (1.34±0.22µmol/L in males and 1.30±0.26 µmol/L in females) however a lowered mean ±S.D GPx level (23.3±4.2 u/gHb in males and 23.0±3.6 u/gHb in females) than the control group (29.68±1.4 u/gHb in males and 29.57±1.0 u/gHb in females).The differences in the mean of MDA and GPx level between the study group and the control group was found to be statistically significant

(p<.001).It was also seen that both male and female in the study groups had almost similar MDA and glutathione peroxidase levels (Tables 2 & 3). These finding are consistent with the finding of Palanisamy P et al. [29] who reported raised plasma malondialdehyde levels and decreased activity of whole blood glutathione levels in AMI cases when compared with the controls and similar reports have been reported by various investigator [30-32].

Table 3. Comparison of plasma malondialdehyde (MDA) and glutathione peroxidase (GPx) in whole blood (mean ±S.D) of the control and AMI cases by sex

Parameters	Sex	Control	AMI	t	*p* value
MDA	Male	1.34±0.22	4.02±0.72	19.35	0.001
	Female	1.30±0.26	3.77±0.58	15.48	0.001
GPx	Male	29.68±1.4	23.3±4.2	-7.972	0.001
	Female	29.57±1.0	23.0±3.6	-7.453	0.001

Table 4. Results of multiple regression analysis on AMI cases

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	7.506	1.441		5.210	.000
	Age	-.007	.009	-.112	-.812	.421
	Sex	-.301	.196	-.209	-1.530	.133
	Smoking	.253	.125	.278	2.027	.049
	BMI	.110	.046	.321	2.372	.022

a. Dependent Variable: MDA

The multiple regression analysis on AMI cases indicated that both smoking and BMI are independent variables and shows positive correlation with MDA (Table 4).

4. LIMITATION

The sample size was not adequate as the sample was drawn from a single hospital and the duration of study was limited to two years. TAS, oxidized LDL, hsCRP, vitamin C and E were not measured. Comparision of lipid peroxidation in overweight or obese controls with AMI patients were not performed.

5. CONCLUSION

Our study has concluded that AMI patients have increased oxidative stress and a compromised anti-oxidant defense system. Therefore it is recommended that the management strategy for the patients of AMI should include specific antioxidant supplementation along with lowering of lipid peroxidation. Larger study with more biomarker capable of detecting the clinical outcome is warranted.

CONSENT

All authors declare that written informed consent were obtained from the patients (or other approved parties) for publication of this research work and accompanying images.

ETHICAL APPROVAL

The study was approved by the Regional Institute of Medical Sciences (RIMS) ethics committe.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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