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Original Article

Biochemistry Section

A Study on Malondialdehyde as an Oxidative Stress Marker in Patients with Myocardial Infarction at a Tertiary Care Centre

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ABSTRACT

Introduction: Acute Myocardial Infarction (AMI) is the most critical event in cardiovascular disorders and arises as a consequence of myocardial ischemia due to coronary occlusion. Oxygen free radicals have become attractive candidates to explain injuries in the heart with growing appreciation that free radicals such as Malondialdehyde (MDA) which is the end product of lipid peroxidation may accumulate during ischemia at low oxygen tension.

Aim: This study was done to note any changes in the MDA levels in the AMI in comparison with the controls

Materials and Methods: A prospective study was done at Kakatiya Medical College, Waranagal, India between April 2010 to March 2011. Blood samples of 30 patients diagnosed as AMI (admitted within 12 hours after onset of ischemic pain) were collected and subjected to serum MDA, CKMB, Aspartate Transaminase (AST), Lactate dehydrogenase (LDH).

Results: There was significantly increased mean values in serum MDA levels in ($p < 0.001$) in the study group as compared to controls. MDA levels significantly correlated with, CK-MB, AST and LDH.

Conclusion: The MDA values are significantly increased in AMI indicating oxidative stress associated with AMI. Hence, another potential area of treatment to reduce extent of damage. At their best cut off values CK-MB and MDA had high sensitivity and specificity and good discriminatory capacity in identifying AMI. Though, AST and LDH exhibited good sensitivity, they lack specificity to act as good markers.

Keywords: Free radical, Lipid peroxidation, Myocardial ischaemia

INTRODUCTION

Acute myocardial infarction (AMI) arises as a consequence of myocardial ischemia due to coronary occlusion and is the most critical event in cardiovascular disorders [1]. Malondialdehyde (MDA) an end product of lipid peroxidation may accumulate during ischemia at low oxygen tensions [2].

MDA is produced as a byproduct of polyunsaturated fatty acid peroxidation and arachidonic acid metabolism and is highly reactive. MDA readily combines with several functional groups on molecules including proteins, lipoproteins, and DNA [3]. Polyunsaturated fatty acid peroxides further react to form MDA. MDA can be found in most biological samples as a result of lipid peroxidation [4].

Oxidative stress is the most important contributor to the progression of atherosclerosis [5]. Myocardial antioxidants prevent thrombosis, myocardial damage and arrhythmias during AMI by delaying and inhibiting oxidative damage [6].

Pro-oxidant and anti-oxidant imbalance mechanism may lead to lipid peroxidation and tissue damage due to prolonged oxidative stress [7]. As the condition advances from unstable angina to ST-segment elevation myocardial infarction lipid and protein oxidation increases gradually [8]. Myocardial Infarction (MI) with greater elevation of MDA levels may cause peroxidative damage and platelet aggregation [9].

This study was done to note any changes in the MDA levels in the AMI in comparison with the controls. Also, to find out if there is any correlation between the MDA levels with those of CK-MB, AST and LDH.

MATERIALS AND METHODS

A prospective study was done at Kakatiya Medical College, Waranagal, Telangana, India, in the Department of Biochemistry between the period of April 2010 to March 2011 after obtaining Institution Ethics Committee approval. An informed consent was taken from all the patients prior to the study. The subjects were selected on the basis of following inclusion and exclusion criteria.

Inclusion criteria

Cases:

- Blood samples of 30 patients diagnosed as AMI (admitted within 12 hours after onset of ischemic pain)

were collected before the start of treatment and any medication.

- CPK-MB level above 25 IU/L.

Controls:

- Age matched 30 controls.
- Healthy male subjects in the age group of 40 to 65 years, attending the blood banks, and also from other sources.
- CPK-MB level within physiological limits.
- No history or clinical/laboratory evidence of myocardial infarction.

Exclusion criteria:

- Patients with gout
- Patients diagnosed with any acute infections,
- Patients with liver diseases, pulmonary diseases, renal diseases, neoplastic diseases
- Patients with valvular heart diseases,
- Patients with history of smoking
- Patients with diabetes mellitus,
- Obese patients with body mass index >32 kg/m^2.
- Patients with hypertension

Sample collection: 5 ml of blood samples were taken and equally distributed into plain tubes and citrated vials. Clotted blood in the plain tubes was subjected to centrifugation. Care was taken to prevent hemolysis of the blood samples. The clear serum was separated and used for the following biochemical investigations.

Serum MDA

Serum CK-MB

Serum Aspartate Transaminase (AST)

Serum Lactate Dehydrogenase (LDH)

Serum MDA estimation:[10]

Principle: Method is based on the detection of aldehydes formed by the degradation of hydroperoxides including MDA which is used as a standard. These aldehydes form adducts with thiobarbituric acid to form a pink colour complex which has an absorption maxima at 532 nm.

Reagents:

1. Trichloroacetic Acid (TCA): 20%

2. Thiobarbituric Acid (TBA), 0.67% (w/v)

Procedure: The reaction mixture contained 1ml of 0.67% TBA, 500 µL TCA and 100 µL of serum.

This was incubated at 100^{0}C for 20 minutes and centrifuged at 12,000 rpm for 5 minutes. The absorbance of the supernatant was read at 532 nm against a distilled water blank.

MDA was determined by using a molar extinction co-efficient of 1.56X10^5 /M/cm and the values were expressed as nm.

CK-MB level analysis was done by Immuno-inhibition method using the kit manufactured by Trans Asia Biomedicals LTD in technical collaboration with ERBA Diagnostics Mannheim GmbH, Mallaustr, Mannheim/Germany [11].

AST level analysis was done by Kinetic IFCC (International Federation of Clinical Chemistry) method.

LDH level analysis was done by Deutsche Gesellschaft fur Klinische Chemie (DGKC) method using the kit manufactured by Trans Asia Bio-medicals Ltd. [12] .

STATISTICAL ANALYSIS

Statistical packages SPSS version 16, MedCalc 11.0.1.0 were used for the analysis of data. Microsoft Word and Microsoft Excel were used to generate the graphs, tables etc.

RESULTS

There are significantly increased mean values in serum MDA levels in ($p<0.001$) in the study group as compared to controls as seen in the [Table/Fig-1].

Mean±SD values of serum CKMB for the control group and the study group were 11.8±2.2 and 123±27 respectively. There are significantly increased mean values in serum CK-MB levels in ($p<0.001$) in the study group as compared to controls.

Mean±SD values of serum AST for the control group and the study group were 26.5±7.2 and 54.7±14.2. Mean±SD values of serum LDH levels were 270.1±99.9. There are significantly increased mean values in serum AST and LDH levels ($p<0.001$) in the study group as compared to controls.

In this study the MDA levels are significantly correlated with, CK-MB, AST and LDH.

At their best cut off values CK-MB and MDA had high sensitivity and specificity and good discriminatory capacity in identifying AMI. Though, AST and LDH exhibited good sensitivity, they lack specificity to act as good markers [Table/Fig-2-4].

Parameter	Controls n = 30	Study group n = 30	Comparison
	Mean ± SD	Mean ± SD	p-value
Serum MDA (nmoles/L)	214.6±43.2	463.0±70.1	p <0.001

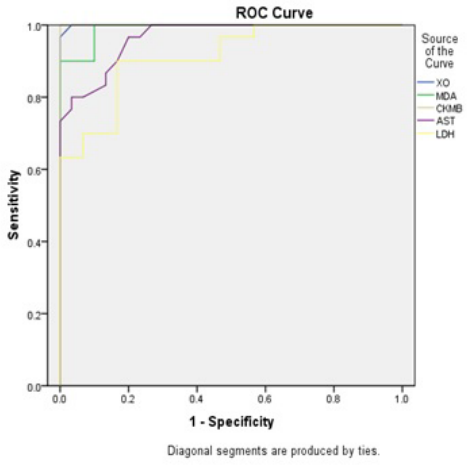
[Table/Fig-1]: Comparison of serum malondialdehyde values between the control group and the study group.

Parameters	CK-MB	AST	LDH
MDA	*0.856	*0.726	*0.682

[Table/Fig-2]: Correlation (r) values between MDA with CK-MB, AST, LDH in the study group using pearson correlation.
*correlation is significant at the 0.01(2- tailed)

Parameters	Best cut off value	Sensitivity	Specificity	AUROC
MDA (n/moles)	262	88%	90%	0.990
CK-MB (U/L)	86	96.7%	100%	1.000
AST (U/L)	31	96.7%	23.3%	0.965
LDH (U/L)	379	90%	16.7%	0.912

[Table/Fig-3]: Sensitivity, Specificity and AUROC of MDA, CK-MB, AST and LDH in the study group.



[Table/Fig-4]: Comparison of Receiver Operator Characteristics (ROC) of MDA, CK-MB, AST and LDH.

DISCUSSION

In this study, we analyzed the levels of MDA, CK-MB, AST, LDH. For this, we collected samples from 30 patients of AMI and 30 age and sex matched apparently healthy controls.

Increased level of MDA in AMI patients was highly significant as compared to controls. Similar findings were observed in other studies [13-18].

Dev Sarkar P et al., study showed increased levels of MDA in high alcoholic Coronary Artery Disease (CAD) patients as compared to non-alcoholic CAD and healthy controls, because alcoholics seem to have still greater degree of oxidative stress. The estimation of lipid peroxidation along with lipid profile in the CAD patients is very useful as it may serve as a useful monitor to judge the prognosis of the patient [14].

Gerittsen WB et al., study showed significant differences in the lipid peroxidation parameter MDA between the groups investigated in favour of OPCAB surgery. Parameters of oxidative stress are significantly increased during and after CABG with the use of ECC [17].

Raghuvanshi R et al., reported highly significant levels of MDA in the blood of patients with myocardial infarction has been found and it may also be used as the marker of the ischemic myocardial syndrome, but increase in MDA levels is much less than that of xanthine oxidase. Moreover, the measurement of MDA levels is complicated and time consuming as compared to the assay of xanthine oxidase activity [18].

Jain AP et al., reported plasma levels of MDA and nitrite were significantly elevated in the patients of AMI compared to the control group (7.29 ± 3.28 v/s 4.57 ± 0.63 nmol/ml and 12.85 ± 8.71 v/s 0.97 ± 0.25 µM respectively), thereby indicating that oxygen free radicals cause endothelial damage in them [15].

Peroxidation of lipids is a chain of reaction which is catalyzed invivo by heme compounds and lipoxygenases found in platelets, leucocytes, etc., Lipid peroxides formed in this reaction degraded to form a characteristic product such as MDA. Peroxidation provides continuous supply of the radicals which causes damage to tissues in vivo causing atherosclerosis, cancer, inflammatory disease, aging, etc., [19].

Thus, MDA which is an end product of lipid peroxidation, is commonly used as a marker of oxidative stress.

Free radical mediated peroxidation of unsaturated fatty acids and auto-oxidation is reflected by MDA [20]

LIMITATION

A small sample size was one of the short comings of the study.

CONCLUSION

The MDA values are significantly increased in AMI indicating oxidative stress associated with AMI. Hence, it is another potential area of treatment to reduce extent of damage. The oxidative stress markers MDA showed good correlation with CK-MB, AST and LDH, confirming ROS mediated damage to the cardiac myocyte membrane.

CK-MB, and MDA are good markers in identifying AMI. They all can be used together as multiple markers to increase their efficiency as markers of AMI. The AST and LDH though exhibited good sensitivity, lack specificity. Hence they should not be used as individual markers.

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