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ORIGINAL ARTICLE

Cardiac Enzymes, Total Thiols And Lipid Peroxidation In Patients With Acute Myocardial Infarction

BABU SURESH*, JEEVAN KUMAR SHETTY**, MUNGLI PRAKASH***

ABSTRACT

Background: Oxidative stress has been implicated in the pathogenesis of acute myocardial infarction. In the current work ,we have measured malondialdehyde (MDA), total thiols, total creatine kinase (CK), creatine kinase-MB isoenzyme (CK-MB) and aspartate aminotransferase (AST) in electrocardiogram (ECG) proven acute myocardial infarction (AMI) patients at 12 hours after the onset of chest pain and also in healthy controls.

Methods: Blood samples from 25 AMI patients and 25 age and sex matched healthy controls were obtained for the measurement of cardiac enzymes like total CK, CK-MB and AST by using an automated analyzer. Serum MDA and total thiols were determined by using spectrophotometric methods.

Results: We have found a significant increase in MDA, total CK, CK-MB and AST ($p< 0.001$) and a significant decrease in total thiols ($p<0.001$) in AMI patients as compared to the healthy controls. MDA correlated negatively with total thiols ($r = - 0.573$, $p<0.01$) and positively with CK-MB ($r = 0.845$, $p<0.01$) and AST ($r = 0.676$, $p<0.01$) in the AMI patients.

Conclusions: Reactive oxygen species play a role in the pathogenesis of atherosclerosis, thus leading to acute coronary events and their levels are further elevated by the ischaemic event itself.

Key Words: MDA, total thiols, myocardial infarction, oxidative stress, cardiac enzymes.

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Introduction

Acute myocardial infarction (AMI) is one of the major causes of mortality and morbidity in the world.[1] The most common cause of AMI is atherosclerotic coronary artery disease (CAD) with the erosion or rupture of a plaque, thus causing transient, partial or complete arterial occlusion.[2] () Previous studies have shown that reactive oxygen species (ROS) cause the initiation and progression of atherosclerosis, thus leading to coronary artery disease. [3] During AMI, two distinct types of damage occur to the heart: ischaemic injury and reperfusion injury. The heart can tolerate a brief exposure to ischaemia, as the temporary protective mechanisms like anaerobic glycolysis, fatty acid utilization, increase in glucose uptake and

decreasing contractility of heart muscles. Persistent ischaemia can develop a severe ATP deficit and myocardial cell death.[4]

During ischaemia, ROS can be produced both by the endothelial cells and the circulating phagocytes, and they are capable of damaging macromolecules; including nucleic acids, proteins, lipids, lipoproteins and carbohydrates.[5] On interaction with unsaturated lipids, ROS are capable of initiating the self-perpetuating chain reactions of lipid peroxidation in the membranes. Malondialdehyde (MDA), a lipid peroxidation end product, is considered as one of the markers of cell membrane damage.[6] The major antioxidant in the body fluids is the cysteine-SH which is bound to protein, a majority of it being found on albumin and glutathione (GSH). These –SH groups (total thiols) play a major role along with other antioxidants in the body to ameliorate the lipid peroxidative effects of ROS.[7]

In the current work, we have measured total thiols which are major antioxidants in body fluids, and MDA which is an important marker of lipid peroxidation, along with cardiac enzymes like total CK, CK-MB and AST in AMI patients at 12 hours after the onset of chest pain, to compare their levels with those of age matched healthy controls. We have also tried to establish a relationship between oxidative stress markers and cardiac enzymes.

Materials and Methods

Subjects and Samples

The study was carried out in the Department of Biochemistry, JJM Medical College, Davangere, India. The study group consisted of 25 AMI patients and 25 age and sex matched healthy controls. The mean age and sex of the patients was 54±9 years, and 20 males/5 females, and that of the controls was 45±17 years, and 17 males/ 8 females, respectively. The patients who were recruited from the Bapuji and Chigateri Government hospitals were brought to the emergency room with a history of chest pain. Patients with chest pain were diagnosed to have AMI according to the clinical criteria- chest pain which lasted for more than 3 hours, ECG changes (ST elevation of 2mm or more in at least two leads) and elevation in total CK and CK-MB.

Informed consent was obtained from all the subjects who were involved and ethical clearance was obtained from the Institutional Ethics Committee (IEC). Blood samples were drawn into plain vacutainers from the antecubital veins of AMI patients immediately after admission. Similarly, samples were also obtained from age and sex matched healthy controls. Total CK, CK-MB, AST, MDA and total thiol levels were measured in all the obtained samples after proper processing.

Reagents

Special chemicals like 5' 5' dithio-bis (2-nitrobenzoic acid) (DTNB), reduced glutathione (GSH), and standard MDA were obtained from Sigma Chemicals, St Louis, MO, USA. All other reagents were of the analytical grade.

Biochemical Determinations

Measurement of cardiac enzymes:

Reagent kits for total CK, CK-MB and AST were obtained from Merck, India**.** Cardiac enzymes like total CK, CK-MB and AST were measured by an enzymatic assay by using a Ciba Corning 550 Express automated analyzer. [8], [9], [10]

The Total thiol assay:

The reaction mixture contained 900 µL of 2 mM Na_2 EDTA in 0.2 M Na_2HPO_4, 20 µL of 10 mM DTNB in 0.2 M Na_2HPO_4 and 100 µL of serum. The reaction mixture was incubated at room temperature for 5 minutes and the absorbance was read at 412nm. Appropriate sample and reagent blanks were prepared simultaneously and the respective absorbance was noted. Corrected absorbance values were used to calculate serum total thiols by using the molar extinction coefficient of 1600 M^{-1} cm^{-1} and the values were expressed as µM. The calibration curve was produced by using GSH dissolved in phosphate buffered saline. [11]

MDA assay:

The reaction mixture contained 1 mL of 0.67% thiobarbituric acid (TBA), 500 µL of 20% Tri

carboxylic acid (TCA) and 100 µL of serum. This was incubated at 100°C for 20 minutes and was centrifuged at 12,000rpm for 5 minutes. The absorbance of the supernatant was read at 532 nm. MDA was determined by using a molar extinction coefficient of 1.56 x 10^5 M^{-1} cm^{-1} and the values were expressed as nM. [12]

Statistical Analysis

The results were expressed as the mean ± standard error of the mean (SEM). p values <0.05 were considered to be statistically significant. Statistical analysis was performed by using the Statistical Package for Social Sciences (SPSS-16, Chicago, USA). The independent sample t test was used to compare the mean values between the cases and the controls. Pearson correlation was applied to correlate between the parameters.

RESULTS

As shown in [Table/Fig 1], we have found a significant increase in MDA, total CK, CK-MB and AST (p< 0.001), and a significant decrease in total thiols (p<0.001) in AMI patients as compared to the healthy controls.

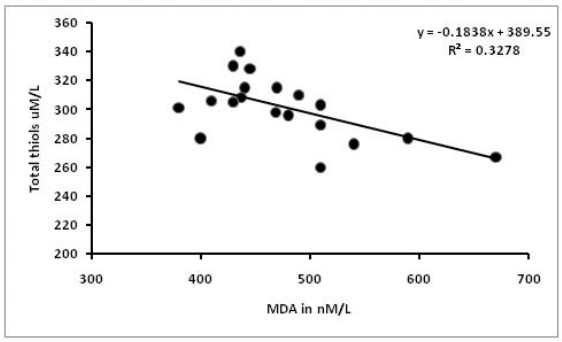
[Table/Fig 1]: Total thiols, MDA, total CK, CK-MB and AST levels in healthy controls and myocardial infarction patients at 12 hours after the onset of chest pain (Values expressed in mean ± SD).

	Controls (n = 25)	AMI Cases (n = 25)
Creatinine Kinase (U/L)	110 ± 18	754 ± 539**
CK-MB (U/L)	15 ± 4	194±200**
AST (U/L)	26 ± 6	69±54**
MDA (nM/L)	225 ± 40	472± 63*
Total thiols (uM/L)	376 ± 64	302± 20**

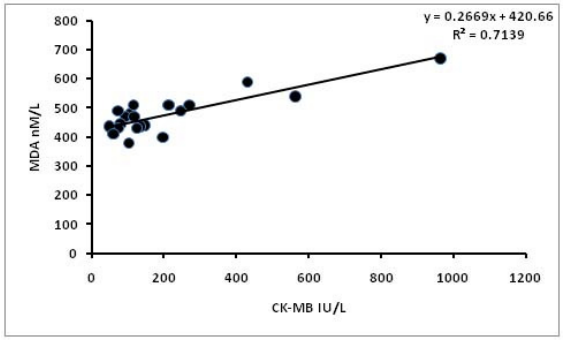
**p< 0.001 compared to healthy controls

On applying Pearson's correlation, MDA was found to correlate negatively with total thiols (r = - 0.573, p<0.01) [Table/Fig 2], and positively with CK-MB (r = 0.845, p<0.01) [Table/Fig 3] and AST (r = 0.676, p<0.01) in AMI patients.

[Table/Fig 2]: Correlation graph between MDA and Total thiols in AMI patients



[Table/Fig 3]: Correlation graph between CK-MB and MDA in AMI patients



DISCUSSION

We have found a significant increase in the cardiac enzymes in AMI patients which rose in parallel to the extent of myocardial injury. The characteristic pattern of the rise in the serum cardiac enzymes was: they started to increase 4-6 hours after injury, reaching peak concentrations after 12-24 hours and returning to the baseline after 48-72 hrs. [13] The rise in the CK-MB levels was seen in all the AMI patients and they were elevated about an average of 8 times than the normal. This may indicate the extent of cardiac muscle damage during the ischaemic event, which can further damage the adjacent tissue by generating free radicals. Several previous studies have shown the presence of oxidative stress and the increased generation of reactive oxygen species during myocardial injury. [14]

One of the best markers of lipid peroxidation in serum is MDA, which indicates the amount of membranes which are being damaged by the reactive oxygen species. Being a lipid peroxidation product, the elevation of MDA in AMI patients is an indicator of increased

oxidative stress. [14] We have found significant elevation in MDA levels after myocardial infarction as compared to the healthy controls. This finding further substantiates the role of free radicals in damaging the myocardial membrane. There is growing evidence that an increase in free radicals is relevant to atherosclerotic plaque formation and activation. [6] Furthermore, it has been shown that lipid peroxidation and MDA generation are enhanced by the ischaemic event itself. In ischaemia, the ATPs are drastically reduced and are degraded to hypoxanthine and then into uric acid by xanthine oxidase. During this process, enormous amounts of superoxide radicals are formed, which can stimulate the Haber-Weiss reaction by generating ROS, which enhance the lipid peroxidation process. [15] We have also observed that increased MDA levels after reperfusion, correlated positively with the cardiac marker enzyme, CK-MB, which may again explain the ROS mediated damage to the myocyte membranes, thereby increasing the release of the cardio specific marker, the CK-MB fraction. [16]

We have found a decrease in total thiols in AMI patients, thus indicating an increased consumption of thiols due to the increased generation of ROS due to ischaemia and reperfusion. The decrease in total thiols correlated negatively with MDA, thus indicating an increased consumption of thiols while neutralizing an enormous increase in MDA levels during ischaemia. Total thiols are important antioxidants and a part of them in plasma is derived from proteins, especially albumin. They constitute the foremost defense system that limits the biomembrane damage which is associated with free radicals. [17] It has been demonstrated that the plasma antioxidant capacity decreases and the oxidative/antioxidative balance shifts to the oxidative side in patients with AMI. [5] Total thiols being important antioxidants, a decrease in their levels may be the reason for increased lipid peroxidation in patients with AMI. [6]

In conclusion, ROS is responsible for the pathogenesis of AMI and their production is enhanced during ischaemia, due to decreased antioxidant defense mechanisms.

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