Online Journal of Health and Allied Sciences
Peer Reviewed, Open Access, Free Online Journal
Published Quarterly : Mangalore, South India : ISSN 0972-5997
Volume 9, Issue 3; Jul-Sep 2010

Original Article:

Total Thiols and MDA Levels in Patients with Acute Myocardial Infarction Before and After Reperfusion Therapy

Suresh Babu,
Department of Biochemistry, JJM Medical College, Davangere, Karnataka, India,
Jeevan K Shetty,
Department of Biochemistry, Kasturba Medical College, Manipal University, Karnataka, India,
Mungli Prakash,
Department of Biochemistry and Genetics, St. Mathews University, School of Medicine, Grand Cayman, Cayman Island, BWI.

Address For Correspondence:
Dr. Jeevan K Shetty,
Assistant Professor,
Department of Biochemistry,
Kasturba Medical College,
Manipal- 576104, INDIA.
E-mail: drjkshetty1978@yahoo.com

Citation: Babu S, Shetty JK, Prakash M. Total thiols and MDA levels in Patients with Acute Myocardial Infarction Before and After reperfusion therapy. *Online J Health Allied Scs.* 2010;9(3):6
URL: http://www.ojhas.org/issue35/2010-3-6.htm
Open Access Archives: http://cogprints.org/view/subjects/OJHAS.html and http://openmed.nic.in/view/subjects/ojhas.html

Submitted: Jul 19, 2010; Accepted: Sep 18, 2010; Published: Oct 15, 2010

Abstract:
Background: Reactive oxygen species have been implicated in the pathogenesis of ischemic and reperfusion injury. In the current work we have measured malondialdehyde (MDA), total thiols, total CK, CK-MB and AST in ECG proven acute myocardial infarction (AMI) patients immediately after admission and 24 hours after administration of thrombolytic agent streptokinase, and in healthy controls. **Methods:** Blood samples from 44 AMI patients and 25 age and sex matched healthy controls were obtained and analyzed for MDA, total thiols using spectrophotometric methods and cardiac enzymes CK, CK-MB and AST using automated analyzer. **Results**: We have found significant increase in MDA, CPK, CK-MB, AST ($p< 0.001$) and significant decrease in total thiols ($p<0.001$) in AMI patients after thrombolytic therapy compared to values at admission, and healthy controls. MDA correlated negatively with total thiols ($r = - 0.333$, $p<0.05$) and positively with CK-MB ($r = 0.491$, $p<0.01$) in AMI patients after thrombolytic therapy. **Conclusions**: Reperfusion following thrombolytic therapy increases reactive oxygen species with concomitant decrease in antioxidant total thiols.
Key Words: MDA; Total thiols; Myocardial infarction; Reperfusion injury

Introduction:

In acute myocardial infarction (AMI), two distinct types of damage occur to the heart: ischemic injury and reperfusion injury. The first results from the initial loss of blood flow and the second upon the restoration of oxygenated blood flow. The heart can tolerate a brief exposure to ischemia as the inherent mechanisms to preserve energy levels prevent injury.(1) Reperfusion of ischemic myocardium is, therefore, a prerequisite for cellular survival. Reperfusion followed by AMI has several benefits such as significant increase in recovery of left ventricular functions.(2) Along with beneficial effects, reperfusion has also been attributed to production of large amounts of reactive oxygen species (ROS).(3) Increased generation of ROS may be responsible, at least in part, for irreversible peroxidative damages to myocardial tissue, which may persist for months following thrombolytic therapy.(4) Among the ROS, malondialdehyde (MDA), a lipid peroxidation end product, is considered as one of the marker of cell membrane damage.(5) The major antioxidant in the body fluids is the cystein-SH bound to protein, majority of it 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. (6,7)

In the current work, we have measured major antioxidant total thiols, and important lipid peroxidation end product MDA, along with cardiac enzymes total CK, CK-MB, AST in AMI patients immediately after admission and 24 hours after thrombolytic therapy to compare their levels with age matched healthy controls. We have also tried to establish relationship between oxidative stress markers with 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 forty four AMI patients and twenty five age and sex matched healthy controls. Mean age and sex of patients was 55 ± 11 years, and 33 males/11females, and that of controls 47 ± 15 years, and 16 males/09 females, respectively. Patients were recruited from Bapuji and Chigateri government hospitals, who were brought to emergency room with history of chest pain within last six hours of onset. They were diagnosed to have AMI according to clinical criteria, chest pain which lasted for up to 3 hours, ECG changes (ST elevation of 2mm or more in at least two leads). All AMI patients were treated with streptokinase 1,500,000 IU for 1 hour within six hours of onset of pain.

Informed consent was obtained from all the subjects involved and ethical clearance was obtained from institutional ethical clearance committee. Blood samples were drawn into plain

vacutainers from the antecubital veins of AMI patients immediately after admission and 24 hours after initiation of thrombolytic therapy. Similarly, samples were also obtained from age and sex matched healthy controls. Total CK, CK-MB, AST, MDA, 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 cheAMIcals, St Louis, MO, USA. All other reagents were of analytical grade.

Biochemical Determinations

Measurement of cardiac enzymes:

Cardiac enzymes CPK, CK-MB and AST were measured using enzymatic assay using auto analyzer.(8-10)

Total thiol assay:

Reaction mixture contained 900 µL 2 mM Na_2 EDTA in 0.2 M Na_2HPO_4, 20 µL 10 mM DTNB in 0.2 M Na_2HPO_4 and 100 µL of serum. Reaction mixture was incubated at room temperature for 5 minutes; absorbance 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 using the molar extinction coefficient 1600 M^{-1} cm^{-1} and values expressed as µM. The calibration curve was produced using GSH dissolved in phosphate buffered saline.(11)

MDA assay:

Reaction mixture contained 1 mL 0.67% thiobarbituric acid (TBA), 500 µL 20% Tri carboxylic acid (TCA) and 100 µL serum. Incubated at 100ºC for 20 minutes; centrifuge at 12,000rpm for 5 minutes. Absorbance of supernatant read at 532 nm. MDA was determined by using molar extinction coefficient 1.56 x 10^5 M^{-1} cm^{-1} and values expressed as nM. (12)

Statistical Analysis

The results were expressed as mean ± standard error of mean (SEM). A $p<0.05$ was considered statistically significant. Statistical analysis was performed using the statistical package for social sciences (SPSS-16, Chicago, USA). One way analysis of variance (ANNOVA) was used to compare the mean values in three groups, followed by multiple comparison post hoc tests. Pearson correlation was applied to correlate between the parameters.

Results:

As shown in Table 1 we have found significant increase in MDA, total CK, CK-MB, AST ($p< 0.001$) and significant decrease in total thiols ($p<0.001$) in AMI patients after thrombolytic therapy compared to values at admission and healthy controls. There is significant increase in levels of MDA ($p<0.001$) and decrease in total thiols ($p<0.001$) in AMI patients immediately after admission compared to controls. On applying Pearson's correlation total thiol levels in serum correlated negatively with MDA ($r = - 0.333$, $p<0.05$) (Figure 1). As depicted in Figure 2, there was significant positive correlation between CK-MB and MDA ($r = 0.491$, $p<0.001$).

Table 1: Total thiols, MDA, Creatinine kinase, CK-MB and AST levels in healthy controls and myocardial infarction patients at admission and 24 hours after streptokinase therapy (Values expressed in mean ± SD).

Tests	Controls (n = 25)	AMI Cases (n = 44)	
		On admission	24 hours after Streptokinase
Creatinine Kinase (U/L)	108 ± 27	188 ± 125**	608 ± 216#
CK-MB (U/L)	17 ± 2	38±22**	151 ± 64#
AST(U/L)	24 ± 5	40±38**	80 ± 30#
MDA (nmoles/L)	215 ± 51	362±53**	1542 ± 353#
Total thiols (µmoles/L)	384 ± 70	306± 24**	196± 29#

**$p< 0.001$ compared to healthy controls; #$p<0.001$ compared to AMI on admission

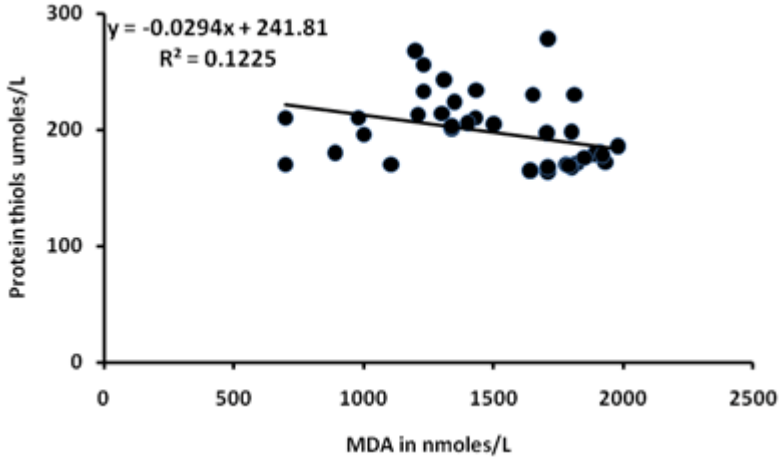

Figure 1: Correlation between MDA and Total thiols in AMI patients 24 hours after thrombolytic therapy

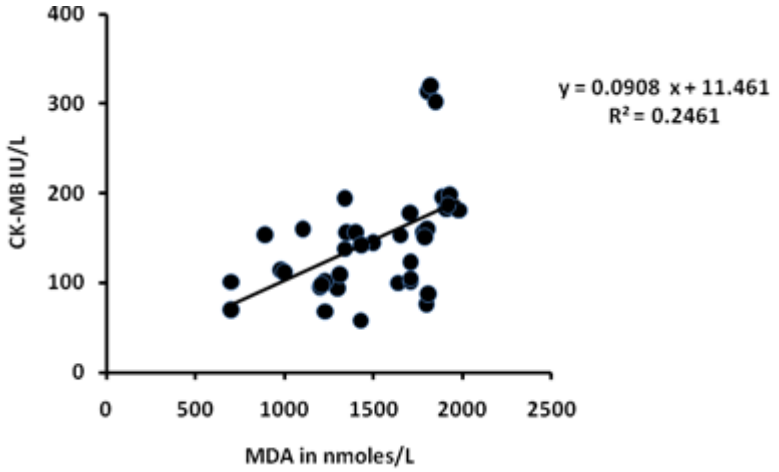

Figure 2: Correlation between MDA and CK-MB in AMI patients 24 hours after thrombolytic therapy

Discussion:

We have found increase in the MDA levels immediately after myocardial infarction compared to healthy controls. This may be due to oxidative stress induced by acute ischemic injury and similar observation was published by many previous authors.(6,13) We have also observed significant increase in MDA levels 24 hours after reperfusion, which possibly explain increased oxygen availability to ischemic site generating increased amount of ROS, thereby causing further injury to cell membrane. This may possibly explain the reperfusion injury observed by previous authors.(14,15)

We have also observed that increased MDA levels after reperfusion correlated positively with cardiac marker enzyme CK-MB, 24 hours after thrombolytic therapy, which may again explain the ROS mediated damage to myocyte membrane thereby increasing the release of cardiospecific marker CK-MB fraction.(1) Available literature explains that oxidative stress followed by reperfusion therapy may be due to variety of factors such as (1) Enhanced generation of ROS due to sudden massive increase in oxygen supply; (2) Reduced levels of antioxidants available, (3) Enhanced consumption, leakage or destruction of antioxidants, (4) Leakage of electrons from the disrupted mitochondrial electron transport chain and (5) Phagocytic recruitment and activation.(16) All of above effects together initiate lipid peroxidation in cell membrane, damage membrane proteins or cause DNA fragmentation. These processes may result in a loss of contractile function of cardiac myocyte and lead to severe myocardial cell damage collectively termed as reperfusion injury.(17)

We have found decrease in total thiols following reperfusion, which correlated negatively with MDA. The major part of thiols in plasma is derived from proteins, especially albumin, and they are susceptible to oxidation during reperfusion. Significant low level of total thiols during myocardial infarction and after reperfusion may indicate increased consumption of these thiol groups to neutralize increase levels of ROS in these states.(18) Decreased availability of thiols may also affect other biochemical function of the cell where thiols play an important role for the catalysis process.(19)

In conclusion the cardiac ischemia induces ROS production and subsequent reperfusion can result in toxic ROS overproduction with concomitant decrease in antioxidants.

References:

1. Misra MK, Sarwat M, Bhakuni P, Tuteja R, Tuteja N. Oxidative stress and ischemic myocardial syndromes. *Med Sci Monit*. 2009;15(10):RA209-19.
2. Ozmen D, Boydak B, Mutaf I, Zoghi M, Kumanlioglu K, Guner I, Bayindir O. The state of lipid peroxidation and antioxidants following thrombolytic therapy with rt-PA and streptokinase in acute myocardial infarction. *Jpn Heart J*. 1999 May;40(3):267-73.
3. Budas GR, Churchill EN, Mochly-Rosen D. Cardio protective mechanisms of PKC isozyme-selective activators and inhibitors in the treatment of ischemia-reperfusion injury. *Pharmacol Res.* 2007;55:523–36.
4. Hori M, Nishida K. Oxidative stress and left ventricular remodelling after myocardial infarction. *Cardiovasc Res*. 2009;81:457–64.
5. Kharb S, Singh V, Ghalaut PS, Singh GP. Oxidative stress after acute myocardial infarction: effect of thrombolytic treatment. *J Assoc Physicians India.* 2000;48(6):578-80.
6. Gururajan P, Gurumurthy P, Nayar P, Chockalingam M, Bhuvaneshwari S, Babu S, Sarasabharati A, Victor D, Cherian KM. Lipid profile and non-enzymic antioxidant status in patients with acute coronary syndrome in South India. *Heart Lung Circ*. 2010;19(2):75-80.
7. Prakash M**,** Shetty JK**,** Sudeshna Tripathy, Pannuri Vikram, Manish Verma. Serum paraoxonase activity and protein thiols in patients with hyperlipidemia. *J Hainian Medical College*. 2009;15(2):111-13.
8. Horder M, Elser RC, Gerhardt M. Approved Recommendation on IFCC Methods for the Measurement of Catalytic Concentration of Enzymes. Part 7. IFCC Method for Creatine Kinase. *Eur J Clin Chem Clin Biochem*. 1991;29:435–56.
9. Guzy PM: Creatine phosphokinase-MB (CPK-MB) and the diagnosis of myocardial infarction. *West J Med.* 1977;127:455-60.
10. Klauke R, Schmidt E, Lorentz K. Recommendations for carrying out standard ECCLS procedures (1988) for the catalytic concentrations of creatine kinase, aspartate aminotransferase, alanine aminotransferase and f-glutamyltransferase at 37°C. *Eur J Clin Chem Clin Biochem*. 1993;31:901–09.
11. Motchnik AP, Frei B, Ames NB. Measurement of antioxidants in human blood plasma: Protein thiols. In: Packer L, editor. Oxygen radicals in biological systems. *Methods in Enzymology,* Academic Press: California; 1994. p. 234(D):273-4.

12. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem*. 1979;95(2):351-8.
13. Pasupathi P, Rao YY, Farook J, Saravanan G, BakthavathsalamG. Oxidative Stress and Cardiac Biomarkers in Patients with Acute Myocardial Infarction. *Eur Jour of Sci Res*. 2009;27(2):275-85.
14. Levy Y, Bartha P, Ben-Amotz A, Brook JG, Dankner G, Lin S, Hammerman H. Plasma antioxidants and lipid peroxidation in acute myocardial infarction and thrombolysis. *J Am Coll Nutr*. 1998;17(4):337-41.
15. Ohlin H, Gustavsson CG, Pantev E, Thorvinger B, Ohlin AK. Transient release of lipid peroxidation products as a non-invasive marker of successful reperfusion after thrombolysis for myocardial infarction. *Br Heart J*. 1995;73(3):223-6.
16. Mimic-Oka J, Simic DV, Simic TP. Free radicals in cardiovascular diseases. *Med. Biol* 1999;6:11-22.
17. Jacobson MD, Reactive oxygen species and programmed cell death. *Trends Biochem Sci*. 1996;243:81-119.
18. Bhakuni P, Chandra M, Misra MK. Levels of free radical scavengers and antioxidants in post-perfused patients in myocardial infarction. *Cur Sci* 2005;89(1):168-70.
19. Miyagawa W, Kennedy DO, Nakatani T, Ohtani S, Kim M, Yuasa IM. Protective effect of green tea polyphenols against the cytotoxicity of 1-4 napthoquinone in isolated rat hepatocytes. *Biosci. Biotechnol Biochem*. 1997;61:1901-05.