

Decreased Levels Of Erythrocyte Glutathione In Patients With Myocardial Infarction

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Abstract

Background: Although experimental and clinical studies have demonstrated that evidence of oxidative stress in myocardial damage caused by reperfusion, defined as an imbalance between pro-oxidants and antioxidants. Glutathione (GSH) plays an important role in autoxidation of oxygen free radical (OFR) involved in diseases such as atherosclerosis, rheumatoid arthritis, and reoxygenation injury.

Aim of the Study: The present study, to determined erythrocyte GSH levels in patients with myocardial infarction (MI).

Material and Methods: Erythrocyte GSH levels were determined in 50 patients with MI and 45 age matched healthy controls. Erythrocyte GSH levels were measured by using Beutler method, also lipid profile was analyzed enzymatically. Malonaldehyde (MDA) levels were estimated by thiobarbituric acid reaction. Results are expressed as Mean±SD. Sigma stat version 3.0 was used for statistical analysis. P-value of less than 0.001 and 0.05 was considered to indicate statistical significance.

Results: Glutathione levels were significantly decreased ($p < 0.001$) in MI as compared to control. Malonaldehyde levels were significantly higher ($p < 0.001$) in MI as compared to control. Also, total cholesterol and triglycerides were significantly higher ($p < 0.05$) in MI subjects as compared to control. Significant correlation between MDA and GSH levels ($r = -0.94$, $p < 0.001$) were also found.

Conclusion: The Results suggest that decreased in GSH levels may be associated with enhanced protective mechanism to oxidative stress in MI.

INTRODUCTION

Evidence of oxidative stress in myocardial damage caused by reperfusion, defined as an imbalance between pro-oxidants and antioxidants, has been documented in a number of experimental and clinical studies (1,2,3,4). Glutathione (GSH), a cysteine containing tripeptide is the most abundant nonprotein thiol in the mammalian cells. It plays an important role in autoxidation of oxygen free radical (OFR) involved in diseases such as atherosclerosis, rheumatoid arthritis, reoxygenation injury and regulation of cellular events (including gene expression, DNA and protein synthesis, cell growth, and immune response (5). Among the enzymatic systems of protecting the cell against OFR injury, GSH, GSH peroxidase (GSHP), GSH reductase (GSSGR) and GSH transferase (GST) play a crucial role (6). Although experimental studies have demonstrated that reduced

glutathione is involved in cellular protection from deleterious effects of OFRs in ischaemia and reperfusion (7). With this in view, the main aim of the study is to determine erythrocyte GSH levels in patients with myocardial infarction (MI).

MATERIAL AND METHODS

STUDY DESIGN AND PATIENTS

This study was performed at a single center. Patients admitted in Intensive Cardiac Care Unit (ICCU) of Lokamanya Tilak Municipal Medical College and General Hospital with MI. 50 patients with MI and 45 age matched healthy volunteers were taken as a control (Control group consisted of clinically normal subjects without any infectious disease or chronic ailment) for this study. The L.T.M. Medical College and General Hospital, ethics committee gave approval and informed consent was

obtained from every patient.

INCLUSION CRITERIA

Smoking habit, blood pressure and family history of MI were recorded after clinical confirmation of MI. All the patients had their first episode of MI with diagnostic criteria: chest pain, specific abnormalities for MI on electrocardiogram, elevated serum creatine phosphokinase (CPK) and creatine phosphokinase (CPK-MB) enzyme levels.

EXCLUSION CRITERIA

Patients with diabetes mellitus, taking lipid lowering drugs or antioxidant vitamin supplements were excluded.

BIOCHEMICAL INVESTIGATION

5 ml fasting Venous blood sample was collected after overnight fasting in biochemistry department of L.T.M.M.C. Samples were collected in EDTA bulb (For GSH estimation) and Plain bulb (for MDA and Lipid profile). Samples were processed immediately in order to avoid GSH oxidation.

The level of reduced glutathione (GSH) in erythrocytes was determined by a method of Beutler (8). Erythrocyte was deproteinated by addition of trichloroacetic acid (TCA). DTNB [5,5'-dithiobis(2-nitrobenzoic acid)] was added to supernatants cleared by centrifugation (10 min, 3000 g/min). The formation of 5-thio-2-nitrobenzoic acid, which is proportional to total glutathione concentration, was monitored at 412 nm at 25°C against reagent controls.

Lipid profile [cholesterol (9), triglyceride (10) (TG) and HDL-cholesterol (9)] was analyzed by enzymatic kit method.

Determination of Malondialdehyde (MDA) levels were estimated by thiobarbituric acid reaction (11). Using sulphuric and phosphotungstic acid first precipitated the proteins in 0.2 ml serum and then the levels of MDA were measured in these samples. Precipitate obtained incubated with TBA in a water bath at 95°C for 60 minutes in an environment with oxygen and pH=3.4. The colored complex that occurred was refrigerated to room temperature. Then the complex was taken into n-butanol phase. At the end, the complex of MDA- (TBA) 2 was measured by using Shimadzu UV-1201V spectrophotometry at 532 nm. 11 TEP (1,1,3,3-tetraethoxypropane) was used as standard MDA.

STATISTICAL ANALYSIS

The data from patients and controls were compared using student's t-test and values were expressed as means \pm

standard deviation (SD). Sigma stat version 3.0 was used for statistical analysis. P-value of less than 0.001 and 0.05 was considered to indicate statistical significance.

RESULTS

Demographic data are shown in Table 1. Demographic data showed that in patients, average age of 47 ± 14 years with weight of 69.3 ± 20.2 kg. and in control, average age of 49 ± 10 years with weight of 64.2 ± 15.8 kg.

Figure 1

Table 1: Demographic data in patients and Healthy control

Variables	Control	MI
Patients	45	50
Age, years	49 ± 10	47 ± 14
Weight, kg	64.2 ± 15.8	69.3 ± 20.2

Values Expressed as Mean \pm SD.
MI = Myocardial infarction

GSH and MDA levels in patients and healthy control are shown in Table 2. GSH levels were significantly decreased ($p < 0.001$) in patients with MI than in the controls. MDA levels were significantly elevated in MI patients as compared to control ($p < 0.001$).

Figure 2

Table 2: GSH and MDA levels in patients and healthy control

Variables	GSH ($\mu\text{mol/L}$)	MDA ($\mu\text{mol/L}$)
Control (n = 45)	95.2 ± 11.5	2.35 ± 0.45
MI (n = 50)	$42.8 \pm 14.5^*$	$6.40 \pm 0.85^*$

Values expressed as Mean \pm SD
* $p < 0.001$ as compared to Control.
MI = Myocardial infarction
GSH = Glutathione reduced
MDA = Malondialdehyde

Cholesterol, TG and HDL-cholesterol levels in patients and healthy control are shown in Table 3. Total cholesterol and triglycerides were higher in MI subjects as compared to control ($p < 0.05$). Also, significant differences ($p < 0.05$) were seen in HDL-C levels between MI and controls. A statistically significant negative correlation was observed between rise in MDA and fall in GSH levels ($r = -0.94$, $p < 0.001$).

Figure 3

Table 3: Cholesterol, TG and HDL-cholesterol levels in patients and healthy control

Variables	Cholesterol (mg/dl)	TG (mg/dl)	HDL-cholesterol (mg/dl)
Control (n = 45)	161±18.5	135±20.3	48.2±9.8
MI (n = 50)	181±25.6●	149±19 ●	38.6±9.0●

Values expressed as Mean±SD

● p<0.05 as compared to Control.

MI = Myocardial infarction

TG = Triglyceride

HDL-cholesterol = High-Density Lipoprotein - Cholesterol

DISCUSSION

Erythrocytes are the first to react to increased activity of free radical oxidation and to exhaust their compensatory potential. Previous studies on erythrocyte antioxidant capacity and human disease relation showed that some changes in activities of the antioxidant enzymes in the cell might occur (12). Involvement of OFRs in the pathophysiology of inflammation, ischaemia and in reperfusion damage in a number of organs and tissues has been reported (1,13). Demonstration of an enhanced OFRs production, which produces cellular damage, including peroxidation or isomerization of lipids, confirms these findings (14,15,16,17). In the present study, a significant increase in MDA levels was observed in patients with MI. The plasma concentration of MDA has been reported to be elevated in patients with MI in many studies (18,19,20). OFRs are generated particularly in the early stage of MI and GSH is involved in the reduction of hydrogen peroxide radicals, resulting in a decrease in GSH levels during that period (5,20). In the present study, low GSH levels were observed as compared to control. Simic (21) demonstrated that MI patients had higher MDA and conjugated dienes and reduced activities of erythrocyte antioxidant enzymes and lower concentration of GSH. Also, Blaustein et al (22) demonstrated that GSH is important in protecting the myocardium against OFR injury and that a reduction in cellular GSH content would impair recovery after short periods of ischaemia. Decreased GSH levels may be associated with an enhanced protective mechanism to oxidative stress in AMI. Ondregjickova et al (23) has demonstrated that increase in level of glutathione disulfide and decrease in level of GSH occurs in all parts of myocardium during coronary occlusion and these changes are maintained during reperfusion in canine heart. The importance of our findings is that it confirms the existence of an abnormal balance between the oxidative and protective mechanisms in MI patients.

CONCLUSION

Patients with MI showed a decreased in GSH levels.

Measurements of erythrocyte GSH can be helpful in the estimation of oxidative stress in the course of MI. These finding suggest that decreased in GSH levels may be associated with enhanced protective mechanism to oxidative stress in MI.

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