Erythrocyte Glutathione Level in Patients of Acute Myocardial Infarction

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Abstract:
Myocardial infarction is an imbalance between pro-oxidants and antioxidants. Increase oxidative stress leads lipid peroxidation and malondialdehyde (MDA) is the stable end product of lipid peroxidation. But erythrocyte glutathione (GSH) plays an important role in auto-oxidation of oxygen free radicals (OFR), this study was conducted to determine erythrocyte antioxidant level by measuring erythrocyte glutathione (GSH) in patients of acute myocardial infarction (AMI). GSH level was measured by Ellman method, plasma malondialdehyde (MDA) level was estimated by Yagi method. 25 AMI patients and 25 healthy controls of 40-70 years in both sexes were included according to inclusion and exclusion criteria. Erythrocyte glutathione level was significantly decreased (p < 0.001) and plasma MDA level was significantly increased (p < 0.001) in AMI patients in comparison to healthy control. On the other hand, total cholesterol, triglyceride and LDL-C were significantly higher (p < 0.001) and HDL-C was significantly lower (p < 0.01) in AMI patients as compared to control. This study found a significant correlation between MDA and GSH levels (r = -0.94, p < 0.001).

In AMI GSH level is reduced to scavenge the ROS. As reduced antioxidant level may be associated with enhanced protective mechanism against oxidative stress, erythrocyte GSH level may be use as an important cardiac marker in AMI.

Key Words: Acute myocardial infarction, erythrocyte glutathione, malondialdehyde

Introduction:
Acute Myocardial Infarction (AMI) is a health problem worldwide. In spite of all efforts in prevention and management of this disease, it remains a major challenge to the health managers and scientists. It is associated with increased production of oxygen free radicals (OFRs) which are highly reactive, toxic and important mediators for AMI.¹ ² Therefore excess OFRs lead to an imbalance between pro-oxidant and antioxidant defense mechanism in the body. They depress cardiac functions by extensive necrosis, myocytolysis and cellular edema and ultimately cause cell death.³

Human body has an inherent synergistic and multilevel defense mechanism in the form of antioxidants⁴. They govern the balance between free radicals production and elimination; keep the body away from the deleterious effects of OFRs. However any shift in this critical balance causes oxidative stress that leads to cellular damage. Glutathione, a cysteine containing tripeptide, is a powerful and most important endogenous antioxidant. It has several physiological functions: it maintains SH groups of proteins in a reduced state and integrity of red blood cells, repairs DNA, participates in amino acid transport and important immune responses, forms bioactive molecules, acts as a coenzyme in several enzymatic reactions.⁵ ⁶ By detoxifying oxygen free radicals it prevents cellular damage against oxidative stress.⁶ Among enzymatic protection, GSH peroxidase (GSHP), GSH reductase (GSHR) and GSH transferase (GST) also play important role against oxidative stress⁷. Experimental study demonstrated that in ischemia and reperfusion, reduced glutathione (GSH) provided cellular protection against oxidative stresses⁸. On the other hand, erythrocytes are first to react and exhaust their compensatory potential in oxidative stress as their membranes are able to lipid peroxidation due to their high content of polyunsaturated lipids⁹. Moreover, oxygen radicals are produced continually in erythrocytes by hemoglobin autoxidation which accelerates oxidative damage. Therefore the present study has been designed to estimate the level of reduced GSH in erythrocytes in the patients of acute myocardial infarction.
Methods:
Study design and study population: This prospective type observational study was conducted in the department of Pharmacology, BSMMU in collaboration with National Institute of Cardiovascular Disease (NICVD), Dhaka, during the period of July 2010 to December 2010. 40-70 years of total 25 AMI patients (male: 19; female: 6) and 25 healthy controls (male: 15; female: 10) were studied depending on some inclusion and exclusion criteria. The study was approved by central ethical committee, BSMMU and a written informed consent was obtained from all the participants.

Inclusion criteria: All AMI patients had been admitted to the coronary care units (CCU) of NICVD, Dhaka. The diagnosis of AMI was based on prolonged chest pain, characteristic electrocardiogram changes and elevated creatine kinase isoenzyme MB (CK-MB) and troponin T within 12 hours after the onset of pain. Hypertension was defined as a diastolic blood pressure >90 mmHg, systolic blood pressure >140 mmHg, or history of taking antihypertensive drugs. Patients had total cholesterol level of >220 mg/dL or triglycerides concentration >150 mg/dL, or receiving lipid lowering drugs were defined as having dyslipidemia. Diabetes mellitus was diagnosed if fasting plasma glucose concentration was >120 mg/dL or if patients had the history of taking hypoglycemic agents. Age and sex matched healthy controls were free from any cardiac diseases like MI, valvular disease or other chronic disease like tuberculosis, malignancy etc.

Exclusion criteria: Patients with impaired renal functions, liver functions, antioxidant vitamin supplements or drugs that influence the pro and antioxidative balance such as quinidine, propranolol, allopurinol etc. were excluded.

Blood collection and erythrocyte hemolysate preparation: With all aseptic precaution blood samples were collected by venous puncture from AMI patients after arrival into the hospital and all healthy controls. Then plasma was separated by centrifugation at 3500 rpm for 15 min. The plasma was collected with the simultaneous removal of buffy coat. The packed cells were washed thrice with cold physiological saline. A known volume of erythrocytes was lysed with hypotonic phosphate buffer (pH 7.4). The hemolysate was separated by centrifugation at 4500 rpm for 15 min and the samples were stored at -10°C temperature for further analysis.

Biochemical investigation: Troponin T was measured by immuno assay analyzer. Lipid profile (total cholesterol, triglyceride, HDL-C, LDL-C) and CK-MB were estimated by enzymatic kit method.

Estimation of erythrocyte GSH: Erythrocyte GSH content was determined by the method of Ellman. 10

Erythrocyte hemolysate was deproteinized by trichloroacetic acid (TCA), 5,5-dithiobisnitrobenzoic acid (DTNB) was added. The absorbance was read at 412 nm.

Estimation of lipid peroxidation: Lipid peroxides were estimated by measurement of thiobarbituric acid reactive substances in plasma by the method of Yagi. 11 The pink chromogen produced by the reaction of thiobarbituric acid with malondialdehyde. The absorbance of clear supernatant was measured against reference blank at 535 nm.

Statistical analysis: Statistical analysis was done by SPSS (Statistical Package for Social Science) software for windows version 15. All data were expressed as mean ± SD. Student t-test was done for comparing data between control group and study (AMI patients) group.

Results:
The demographic characteristics of control and all AMI patients were shown in Table-I. In control group the mean age ± SD was 51.13 ± 6.74 years, while in AMI patients mean age ± SD was 54.15 ± 7.69 years respectively.

Control and study groups consisted of both sexes. In control group all subjects were normotensive and devoid of family history of coronary arterial diseases (CAD). All females were non-smoker while males in both control and study group were either smoker or non-smoker or ex-smoker.

Table I

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Controls (n = 25)</th>
<th>AMI Patients (n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean ± SD) in years</td>
<td>51.13 ± 6.74</td>
<td>54.15 ± 7.69</td>
</tr>
<tr>
<td>Sex: Male/Female</td>
<td>15/10</td>
<td>19/6</td>
</tr>
<tr>
<td>Risk factors:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>-</td>
<td>14 (56%)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>-</td>
<td>22 (88%)</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>-</td>
<td>19 (76%)</td>
</tr>
<tr>
<td>Smoking</td>
<td>S = 7 (28%)</td>
<td>S = 10 (40%)</td>
</tr>
<tr>
<td>Ex. S = 4 (16%)</td>
<td>Ex. S = 7 (28%)</td>
<td></td>
</tr>
<tr>
<td>NS = 14 (56%)</td>
<td>NS = 8 (32%)</td>
<td></td>
</tr>
<tr>
<td>Family H/O CAD</td>
<td>-</td>
<td>3 (12%)</td>
</tr>
</tbody>
</table>

Continuous variables are presented as mean ± SD and other variables are shown as percentage of patients

AMI = Acute Myocardial Infarction, CAD = Coronary Arterial Disease
Erythrocyte GSH and plasma MDA levels in healthy control and AMI patients were shown in Table – II and Figure - 1. In AMI patients erythrocyte GSH level was significantly decreased (p<0.001) as compared to control group. But plasma MDA level was significantly increased (p<0.001) in AMI patients as compared to control group. A statistically significant negative correlation was observed between fall in GSH level and rise in MDA level (r = -0.94, p<0.001).

**Table-II**

*Erythrocyte GSH and plasma MDA levels in healthy control and AMI patients*

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Unit</th>
<th>Controls</th>
<th>AMI Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocyte GSH</td>
<td>(mg/gm of Hb)</td>
<td>1.70 ± 0.34</td>
<td>1.21 ± 0.15***</td>
</tr>
<tr>
<td>Plasma MDA</td>
<td>μmol/l</td>
<td>2.62 ± 0.37</td>
<td>5.29 ± 0.65***</td>
</tr>
</tbody>
</table>

Values were expressed as mean ± SD
AMI = Acute Myocardial Infarction; GSH = Reduced glutathione; MDA = Malondialdehyde
*** = significant at p d" 0.001; ** = significant at p d" 0.01; * = significant at p d" 0.05; ns = not significant at p > 0.05

**Fig. -1:** *Erythrocyte GSH and plasma MDA levels in control and AMI patients*

Total cholesterol, TG, HDL-C and LDL-C levels in control and AMI patients were shown in Table – III.

Cholesterol, TG and LDL-C were significantly higher (p<0.001) in AMI patients as compared to control. Significant difference (p<0.01) was observed in HDL-C level.

**Table III**

*Total cholesterol, TG, HDL-C and LDL-C levels in healthy control and AMI patients*

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Unit</th>
<th>Controls</th>
<th>AMI Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cholesterol</td>
<td>mg/dl</td>
<td>162 ± 11.23</td>
<td>238 ± 19.17***</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>mg/dl</td>
<td>137 ± 21.23</td>
<td>177 ± 21.26***</td>
</tr>
<tr>
<td>LDL-C</td>
<td>mg/dl</td>
<td>92 ± 9.14</td>
<td>155 ± 8.32***</td>
</tr>
<tr>
<td>HDL-C</td>
<td>mg/dl</td>
<td>43 ± 5.07</td>
<td>30 ± 10.15***</td>
</tr>
</tbody>
</table>

Values were expressed as mean ± SD
AMI = Acute Myocardial Infarction; LDL-C = Low Density Lipoprotein; HDL-C = High Density Lipoprotein
*** = significant at p d" 0.001; ** = significant at p d" 0.01; * = significant at p d" 0.05; ns = not significant at p > 0.05

**Discussion:**

It is predicted that by the year 2020, Coronary Artery Disease (CAD) would persist as the major threat to human life.\(^\text{12}\) The most important form of CAD is myocardial infarction (MI) which results from atherosclerotic lesion of coronary artery with rupture of plaque causing arterial occlusion.\(^\text{13}\) Persistence of this condition for prolong period causes ischemia with necrosis of myocytes, leads to death.\(^\text{14,15}\) Recently, it has been recommended that in MI hypoxia or ischemia followed by reoxygenation or reperfusion causes increased production of oxygen free radicals (OFRs)\(^\text{16}\) that leads to a condition referred as oxidative stress where the balance between pro-oxidant and antioxidant is impaired.

In this study we found that erythrocyte GSH level was significantly reduced and plasma MDA level was significantly increased in AMI patients as compared to control group. Our results were in accordance with the many studies.\(^\text{17-20}\) Patil et al.,\(^\text{18}\) found that MDA and ceruloplasmin levels were significantly increased and GSH level was significantly decreased in AMI patients as compared with control. They also observed a negative correlation between rise in MDA and fall in GSH levels in both diabetic and non-diabetic AMI patients, Vishnu Priya et al.,\(^\text{19}\) also found that erythrocyte MDA and serum homocysteine levels were significantly increased and antioxidant levels such as reduced GSH, vitamin E, C levels were significantly decreased in the patients of CAD as compared to controls. GSH is a powerful and important endogenous antioxidant that plays an important role in auto-oxidation of OFRs. Usually these OFRs are generated in the early stages of MI. Which causing oxidative stress leads to increase lipid peroxidation. As a result MDA level is increased in MI, as it is the universal indicator of lipid peroxidation. But GSH is involved in the reduction of hydrogen peroxide radicals, So, in MI reduced GSH level.
indicates antioxidant defense mechanism is severely impaired. Because this glutathione system is most important protective system against oxidative damage. So the findings of the study confirm the existence of imbalance between oxidative and protective mechanisms in the patients of AMI.

Limitation of the study: Due to unavailability of resources such as manpower, logistic support, financial support this study was done in a single centre with small sample size.

References: