Oxidative Stress and Antioxidant Status in Patients of acute Myocardial Infarction before and after Regular Treatment

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Abstract:
In acute myocardial infarction (AMI), generation of free radicals contribute to its pathogenesis. We evaluated the extent of oxidative stress and its amelioration in AMI patients after regular treatment and assessed its association with risk factors like dyslipidemia and diabetes mellitus (DM). Plasma MDA level increased (p<0.001) and erythrocyte GSH and plasma vitamin E levels decreased (p<0.001) in AMI patients. On the 5th day of regular treatment MDA level reduced (p<0.001) and erythrocyte GSH and vitamin E levels increased (p>0.001). The rise in MDA and fall in vitamin E levels were significant (p<0.001) in patients with both dyslipidemia and DM in comparison to patients without dyslipidemia or DM. It is concluded that an imbalance exists between oxidant and antioxidant molecules in AMI patients which shifts towards oxidative side and treatment restores this balance. There may be some association between oxidative stress in AMI and risk factors like dyslipidemia and diabetes mellitus.

Keywords: Oxidative stress, Malondialdehyde, Reduced glutathione, Vitamin E, Cardiovascular risk factors.

Introduction:
Acute myocardial infarction is one of the leading causes of mortality and morbidity in the world. AMI generally occurs following an abrupt impediment of coronary blood flow after a thrombotic occlusion of a coronary artery as a result of atherosclerosis. This vascular injury is facilitated by some factors hypertension, dyslipidemia, diabetes etc. Besides these factors, reactive oxygen species (ROS) may play an important role in the pathogenesis of AMI. Myocardial ischemia, a decrease in the blood supply to the heart leads to reperfusion in the myocardium, where interaction between molecular oxygen and the reduced respiratory chain causes formation of ROS. These ROS may have several different deleterious effects on heart. The effect of increased oxidative stress leads to the oxidative damage of proteins, lipids, DNA and enzymes of myocardial cells that involved in energy production. These factors contribute to myocardial cell damage, extensive necrosis and cellular oedema. In addition, increasing ROS leads to lipid peroxidation of membranes and loss of its integrity, resulting in necrosis and cell death. Lipid peroxidation leads to generation of a variety of aldehydic products of which malondialdehyde (MDA), a stable end product, is frequently used as a marker of ROS production. The deleterious effect of ROS is balanced by the antioxidant action of non-enzymatic as well as enzymatic antioxidants. Vitamins and reduced glutathione (GSH) are important non-enzymatic antioxidant defenses. Vitamin E has a strong antioxidant capacity which plays an important role in maintaining cell membrane integrity by interacting with membrane phospholipids and thereby limiting lipid peroxidation by ROS. On the other hand, GSH, the most important endogenous antioxidant, maintains SH groups of proteins in reduced state that detoxifies ROS and prevents ROS induced cellular damage. There is evidence that antioxidants can protect against free radical defense and may thereby inhibit thrombosis and myocardial damage during AMI. In AMI oxidative stress is developed because of excessive production of ROS and outstripping endogenous antioxidant mechanism so antioxidant status is the critical tool for assessing redox status.

AMI is a multifactorial disease. Recent studies have demonstrated an association between increased oxidative stress and diabetes, hypertension, smoking etc. which are well known risk factors for atherosclerosis. Lipid profile has been found to be deranged in AMI patients. Increased oxidative stress leads to generation of ROS, causes modification of LDL that initiates further generation of ROS. However in diabetic patients, hyperglycemia increases the production of ROS by auto-oxidation of lipoproteins, proteins and glucose and reduces the availability of nitric oxide (NO) which maintains antioxidant capacity of endothelial cells. Therefore the present study is designed to evaluate the extent of oxidative stress in AMI and to assess antioxidant protection against ROS in AMI patients after regular treatment. In addition the study will also evaluate an
association between oxidative stress and risk factors for atherosclerosis like dyslipidemia and diabetes mellitus.

**Materials and Methods:**
This prospective type of observational study was conducted in the department of Pharmacology, Bangabandhu Sheikh Mujib Medical University (BSMMU) and National Institute of Cardiovascular Diseases (NICVD), Dhaka from July 2010 to May, 2011.

**Study design:**
The study consisted of 72 patients (52 males and 16 females) of mean age group 53.15 ± 7.59 years with the diagnosis of AMI admitted into Coronary Care Unit (CCU) of NICVD. Control group consisted of 18 healthy subjects, recruited from Matuail Health Complex attending for health check up. The whole population was grouped as follows:

- **Group I or control**: 18 healthy subjects
- **Group II or study**: 72 AMI patients
  - **Group IIa**: 18 AMI patients without diabetes mellitus or dyslipidemia
  - **Group IIb**: 18 AMI patients with dyslipidemia
  - **Group IIc**: 18 AMI patients with diabetes mellitus
  - **Group IId**: 18 AMI patients with both diabetes mellitus and dyslipidemia

Diagnostic criteria of patients: The diagnosis of AMI was based on a history of prolonged ischemic chest pain, characteristic ECG changes (ST elevation of 2mm or more in at least 2 contiguous leads) and elevated creatine kinase isoenzyme MB (CK-MB) and troponin T within 8 hours after the onset of pain. All the patients received conventional therapy for AMI which included Inj. streptokinase, Inj. morphine, Inj. prochlorperazine, Tab. clopidogrel, Tab. atorvastatin. Patients who already received antihypertensive, antidiabetic and lipid lowering agents should be continued according to needs. General patients characteristics are shown in Table I. Hypertension was defined as a diastolic blood pressure > 90 mm of Hg and systolic blood pressure ≥ 140 mm of Hg or self reported use of antihypertensive drugs. The subjects who had total cholesterol level of < 200 mg/dL, triglycerides < 150mg/dL, LDL-C < 160 mg/dL and HDL-C > 35 mg/dL were defined as normolipidemic patients, patients treated with insulin or oral hypoglycemic agents were considered as diabetic. Statistical analysis: Statistical analysis was done by SPSS software (SPSS 15.0). The quantitative variables were expressed as mean ± SD. ANOVA followed by Bonferroni test was done to measure level of significance for statistical analysis. P value < 0.05 was considered to indicate statistical significance.

**Results:**
The demographic characteristics of control and all AMI patients are shown in Table I. Incidence of hypertension was more common in AMI patients with both DM and dyslipidemia. Blood glucose level of AMI patients with DM or dyslipidemia or with both DM and dyslipidemia was significantly high (p<0.001) as compared to control (Table II). Compared with controls, the level was also higher.
Plasma lipids showed a higher concentration (p<0.001) of total cholesterol (TC), triglyceride (TG) and low density lipoproteins (LDL-C) in patients with dyslipidemia and with both dyslipidemia and DM. LDL-C level was also high (p<0.001) in AMI with DM. Total cholesterol level was significantly higher (p<0.01) in AMI patients with DM. The level of LDL-C was also higher (p<0.01) in AMI patients without dyslipidemia or DM as compared to controls. The difference in TG concentration in AMI patients without DM or dyslipidemia or with DM as compared to controls was not significant. On the other hand the level of HDL-C was lower (p<0.01) in patients with dyslipidemia and with both DM and dyslipidemia when compared to healthy control subjects. The change in HDL-C concentration was not significant in patients without DM or dyslipidemia and in patients with DM when compared to controls.

Table III illustrates the level of lipid peroxidation and antioxidant status in different groups of AMI patients and control.
control subjects. Lipid peroxidative marker, MDA, was significantly higher (p<0.001) in all groups of AMI patients as compared to control. The activity of erythrocyte antioxidant GSH and plasma concentration of vitamin E levels declined (p<0.001) in all group of AMI patients when compared to normal subjects. Significant difference (p<0.001) in plasma MDA level was observed between group IId versus group Ia. However no significant change (p>0.05) in MDA, erythrocyte GSH and vitamin E level was observed in group IIb as compared to group Ia. MDA level increased significantly (p<0.001) in group Ic versus group Ia. Significant increase in MDA level was observed when group IId was compared with group Ia (p<0.001). Significant reduction in vitamin E level was found in group Ic versus group Ia (p<0.05) and group IId versus Ia (p<0.01).

Table IV illustrates the level of lipid peroxidation and antioxidant status before starting regular treatment and on the 5th day of treatment in all AMI patients and control subjects. Before starting regular treatment plasma MDA level was significantly higher (p<0.001) and erythrocyte GSH and plasma vitamin E levels were significantly low (p<0.001) in AMI patients when compared to controls. After regular treatment, on the 5th day, plasma concentration of MDA reduced significantly (p<0.001) in AMI patients. However, MDA level did not return to the normal value and its difference from control value was significant (p<0.05). On the 5th day of regular treatment non-enzymatic markers of antioxidants, erythrocyte GSH and plasma vitamin E, levels increased significantly (p<0.001) in AMI patients as compared to patients before starting regular treatment, but the rise was not up to the control level.

### Table III

**Plasma MDA, plasma vitamin E and erythrocyte GSH levels in AMI patients and control**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>AMI Patients (72)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group I</td>
<td>AMI without DM or Dyslipidemia</td>
</tr>
<tr>
<td></td>
<td>(n = 18)</td>
<td>Group Ia</td>
</tr>
<tr>
<td>Plasma MDA (µmol/l)</td>
<td>2.51 ± 0.42</td>
<td>3.79 ± 0.96***</td>
</tr>
<tr>
<td>Plasma vitamin E (mg/l)</td>
<td>8.26 ± 0.65</td>
<td>4.89 ± 0.78***</td>
</tr>
<tr>
<td>Erythrocyte GSH (mg/gm of Hb)</td>
<td>1.31 ± 0.11</td>
<td>0.91 ± 0.09***</td>
</tr>
</tbody>
</table>

Values were expressed as mean ± SD; ANOVA followed by Bonferroni t test was done to measure level of significance
- a Group Ia/ Iib/ Iic/ IId (study groups) as compared to Group I (control)
- b Group Iib as compared to Group Ia
- c Group Iic as compared to Group Ia
- d Group IId as compared to Group Ia

*** = significant at p < 0.001; ** = significant at p < 0.01; * = significant at p < 0.05; ns = not significant at p > 0.05

### Table IV

**Plasma MDA, plasma vitamin E and erythrocyte GSH levels before and after treatment in AMI patients and control**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control n = 18</th>
<th>AMI Patients n = 72</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before Treatment</td>
<td>On the 5th day of Treatment</td>
</tr>
<tr>
<td></td>
<td>(At baseline)</td>
<td></td>
</tr>
<tr>
<td>Plasma MDA (µmol/l)</td>
<td>2.51 ± 0.42</td>
<td>4.76 ± 2.72***</td>
</tr>
<tr>
<td>Plasma vitamin E(mg/l)</td>
<td>8.26 ± 0.65</td>
<td>5.43 ± 1.93***</td>
</tr>
<tr>
<td>Erythrocyte GSH (mg/gm of Hb)</td>
<td>1.31 ± 0.11</td>
<td>0.93 ± 0.09***</td>
</tr>
</tbody>
</table>

Values were expressed as mean ± SD
ANOVA followed by Bonferroni t test was done to measure level of significance
- a before treatment group compared to control group
- b after treatment group compared to before treatment group

*** = significant at p < 0.001; ** = significant at p < 0.01; * = significant at p < 0.05; ns = not significant at p > 0.05
Discussion:
A decrease in blood supply to the heart due to atherosclerosis, thrombosis or coronary artery spasm is known to induce myocardial ischemia. Reperfusion of ischemic myocardium may restore oxygen supply. However, sudden massive increase in oxygen supply causes a burst of oxygen consumption with consequent generation of reactive oxygen species (ROS). Excess production of ROS causes inhibition of antioxidant activity, which may cause modification of cellular lipids, proteins and DNA of myocardium, result in severe myocardial damage. In the present study lipid peroxidation product, MDA was significantly increased (p<0.001) in AMI patients as compared to control which is indicative of elevated oxidative stress in AMI patients. Our results are consistent with other researchers findings where they showed significant increase in the lipid peroxidation products in blood of AMI patients following an acute event. The same result was found by Senthi et al., in patients with cardiogenic shock complicating AMI. Increased lipid peroxidation is thought to be a consequence of oxidative stress which occurs when the dynamic balance between pro-oxidant and antioxidant mechanism is impaired. Lipid peroxidation may alter intrinsic membrane properties due to physicochemical changes of oxidized lipids or cause polymerization of membrane components. The increased level of MDA suggests that lipid damage observed in our study may be a result of the AMI pathology itself.

Antioxidants constitute the foremost defense system that limit the toxicity associated with free radicals. A reason for increased lipid peroxidation of patients with AMI may be a poor enzymatic and non enzymatic antioxidant defense system. Reduced glutathione (GSH) is one of the important endogenous non-enzymatic antioxidants that provides sulphhydryl (SH) group for direct scavenging reactions. GSH acts both as substrate in the scavenging reaction, catalyzed glutathione peroxidase (GPx) and as a scavenger of peroxyl radicals. Our data showed that GSH level was significantly lowered in AMI patients as compared to control and a negative correlation was observed between GSH and MDA levels in AMI patients (r = -0.515). The finding of the present study is in accordance with a number of studies. Erythrocyte GSH level has been found to be lowered significantly in AMI patients as compared to control. A negative correlation between GSH and MDA has been shown in both diabetic and non diabetic AMI patients. The decreased level of GSH and negative correlation of GSH and MDA levels in our study implies that glutathione system, is severely impaired in AMI patients. In our study, we found significantly low level of plasma vitamin E level in AMI patients compared with control. This indicates severe damage to antioxidant system which is unable to combat oxidative stress. Vitamin E is an important membrane constituent of cardiac muscle which haults lipid peroxidation by trapping the peroxyl radical and stabilizes lipid bilayer of cell membranes, where it interacts with phospholipases to reduce membrane rearrangements. It may provide protection against ischaemic myocardial damage by eliminating pro-oxidants and scavenging free radicals.

The low vitamin E level found in our study is in accordance with other studies. Some studies showed that total antioxidant capacity (TAC) has been decreased in AMI patients. Decrease in the levels of GSH and vitamin E in our study may be due to increased turn over for preventing oxidative damage in these patients, suggesting an increased defense against oxidative damage in AMI patients.

In this study, on the 5th day of regular treatment, we found significant decrease in plasma MDA level (p<0.001) in AMI patients, However the level did not reach to control value. We also found significant increase (p<0.001) in erythrocyte GSH and plasma vitamin E level in AMI patients on the 5th day of regular treatment. LoPresti et al., found significant reduction of lipid peroxidation and NO metabolite concentration and increase of total antioxidant capacity (TAC) in young AMI patients after 12 months in comparison with initial stage. However each of parameters remained statistically different in comparison to normal controls. In our study on the 5th day of treatment we found significant difference of MDA level as compared to control but the difference of erythrocyte GSH and plasma vitamin E level was not significant in comparison to control. It may be that TAC takes longer period to be restored.

Numbers of risk factors are associated with the pathogenesis of AMI. Increase in the concentration of plasma lipids is frequently observed in patients with AMI which contributes to the development of vascular disease. Cholesterol has been considered to be an important factor in the development of atherosclerosis. HDL-C provides protection against it by reverse transport of cholesterol. In our study, we found significant elevation of cholesterol and LDL-C levels in AMI patients with dyslipidemia or both dyslipidemia and DM as compared to control. There was significant decrease in HDL-C in the same group of patients. In patients with only DM, LDL-C level increased significantly. We found that the rate of lipid peroxidation was significantly high in AMI patients with or without risk factors as compared to control. MDA level was...
significantly different in patients with DM or both dyslipidemia and DM as compared to AMI patients with no risk factors. Plasma vitamin E and GSH levels decreased in AMI patients with or without risk factors. The decreased was more significant in AMI patients with risk factors than without risk factors. The finding of this study is in conformity with the previous observations. It can be predicted that oxidative stress in AMI patients is higher among the patients having more risk factors than the patients having no or less risk factors.

The findings of our study suggest that in AMI patients oxidative stress occurs as a result of an imbalance between oxidants and antioxidants. The magnitude of imbalance is greater in AMI patients with risk factors like dyslipidemia or diabetes mellitus. Amelioration of oxidative stress after regular treatment suggests that such an imbalance between oxidants and antioxidants may be involved in the pathogenesis of AMI.

Limitation of the study:
Due to the unavailability of the resources such as manpower, financial support and time limitation the study was done with small sample size.

Reference: