Abstract

Destabilization of atherosclerotic plaque in the coronary artery is the result of Oxidative Stress (OS) that damage the myocardial tissues in Acute Coronary Syndrome (ACS). This study investigated the activities of certain circulatory and cellular antioxidant- and glutathione-associated enzymes in ACS patients in comparison to a control group. Standard assay methods were followed to evaluate the activities of Superoxide Dismutase (SOD), catalase, Glutathione-S-transferase (GST), Glutathione Peroxidase (GPx) and Glutathione Reductase (GR). For data analysis, the categorical variables were measured in percentages, and continuous variables were expressed in means and standard deviations. The ACS patients had significantly higher activities of circulatory SOD, GPx and GST compared to the controls (4.36 ± 2.28 U/mL, 49.20 ± 14.12 U/mL and 5.02 ± 3.03 U/mL versus 2.87 ± 1.28 U/mL, 21.53 ± 10.80 U/mL and 3.03 ± 1.99 U/mL, respectively) but their catalase and GR activities were significantly lower. While the catalase activities in the erythrocyte and leukocyte lysates were similar in both groups, the leukocyte SOD activity was significantly lower in patients. A significant positive correlation was found between the GR and catalase activities in patients. The circulatory enzymes SOD, GPx and GST are over-expressed in controlling excessive OS, while the reduced activities of catalase and GR could be the consequence, suggesting therapeutic potentials of using enzymes in reducing OS-mediated endothelial injury in ACS patients.

Keywords: Acute coronary syndrome (ACS), Superoxide dismutase (SOD), Catalase, Glutathione-associated enzymes, Oxidative stress (OS)

1. Introduction

Cardiovascular Disease (CVD) is the major cause of death worldwide, which has been about 17.7 million in 2015, accounting 31% of all deaths (WHO, 2016). Coronary Artery Disease (CAD) is the formation of...
atherosclerotic plaque in the coronary artery while Acute Coronary Syndrome (ACS) is the set of signs and symptoms that develop when this plaque ruptures as there is a lack of sufficient blood flow to the myocardium. ACS is the umbrella term of different types of myocardial ischemia including Unstable Angina (UA), Non ST-Elevation Myocardial Infarction (NSTEMI) and ST-Elevation Myocardial Infarction (STEMI). Partially blocked coronary artery causes NSTEMI and UA; on the other hand, STEMI is the result of full blockage of coronary artery (Overbaugh, 2009).

Oxidative Stress (OS) is the disruption of fine balance between Reactive Oxygen Species (ROS) and antioxidants. Increase in OS has been linked to the development of vascular dysfunction, inflammation, atherosclerosis, and diabetic cardiomyopathy. Ischemic and reperfusion injury that occurs during myocardial infarction produces ROS at a significant amount (Zweier et al., 1987). It has been reported that enzymatic antioxidants—Superoxide Dismutase (SOD), catalase and Glutathione Peroxidase (GPx), along with the non-enzymatic antioxidants, like reduced glutathione (GSH), controls the OS (Park et al., 2009).

SOD, one of the most primitive antioxidant enzymes found in human and other mammals, has three isomeric forms (Miao and Clair, 2009); all isoenzymes function to the removal of superoxide anion and suppress OS. It has been found that there is a marked increase in SOD3 activity in atherosclerotic lesion (Fukai et al., 2002). Catalase functions in the decomposition of hydrogen peroxide, which is derived from SOD activity or from any other sources, and thus protects the cellular components from oxidative damage (Chelikani et al., 2004). The antioxidant enzyme GPx utilizes GSH to reduce OS induced by ROS by catalyzing the reduction of lipid hydroperoxides to their corresponding alcohols and hydrogen peroxide to water (Stocker and Keaney, 2005). Glutathione-S-transferase (GST) also lowers OS by conjugating GSH to a variety of electrophilic compounds as the first step in detoxification pathways. There are reports that disruption in GST activity may increase the OS (Wu et al., 2004). Both GPx and GST produce oxidized glutathione (GSSG) and the restoration of GSH is crucial for the glutathione redox metabolism. Glutathione Reductase (GR) catalyses the reduction of GSSG by NADPH and thus plays a crucial role in the maintenance of the cellular antioxidant status.

The erythrocytes are the major oxygen carrier in the circulation, and one of the most common sites of ROS production. One study found that anemia could affect the clinical outcomes in ACS patients (Keough-Ryan et al., 2005). In erythrocytes, SOD1 plays the major role as enzymatic antioxidant to control the level of ROS (Fukai et al., 2002) along with catalase and GPx. Therefore, increase in OS may have some effect on the activity of enzymatic antioxidants.

An increase in blood leukocytes has been reported to be an independent risk factor and prognostic indicator of future cardiovascular outcomes (Madjid et al., 2004) and a number of studies found that neutrophil number increases during the onset of ACS (Furman et al., 2004; Choudhury et al., 2019). Upregulation of neutrophil adhesion molecules on their surface attach them to the receptors on endothelial cells resulting in occlusion of the capillary, thereby increasing release of ROS (Lefer, 1999) which contribute to the development of OS.

Though there is a growing interest about the contribution of OS in the development of ACS, the complete scenario on the activity of different antioxidant- and glutathione-associated enzymes in the ACS patient remains to be found out. Therefore, this study intends to evaluate the activities of SOD, catalase, GPx, GST and GR in the circulation as well as SOD and catalase activities in the erythrocytes and leukocytes of ACS patients compared to a control group in an attempt to investigate their role in controlling OS.

2. Materials and methods

2.1. Ethical clearance

This study was approved by the Ethical Review Committee of the Faculty of Biological Sciences, University of Dhaka, Bangladesh. Each individual was informed about the objectives and significance of the study. Only the full consenting volunteers were enrolled.

2.2. Study subjects

A total of 185 participants comprising of 100 patients suffering from ACS admitted in the coronary care unit and progressive coronary care unit of the Dhaka Medical College Hospital were enrolled. Expert physicians diagnosed ACS by examining the characteristic electrocardiogram and troponin changes. Exclusion criteria included those suffering from diabetes mellitus, impaired renal and liver functions, and other chronic inflammatory conditions. A total of 85 subjects from five different locations of the local community were
enrolled as the control group who did not have CVD or any other diseases known to develop OS. Simple random and availability sampling was applied to collect samples.

### 2.3. Sample collection

About 10 mL of peripheral venous blood was collected from each participant, 5 mL taken in lavender capped tube containing Ethylenediaminetetraacetic acid (EDTA) for plasma collection, and the rest in a glass tube for serum collection. Serum and plasma were separated and stored in small aliquots at -20 °C.

### 2.4. Preparation of erythrocyte lysate

An aliquot of 200 µL of fresh blood was taken, the cells were washed with excess of normal saline and the pellet was resuspended by 800 µL of ice cold nanopure water. The lysed cells were centrifuged and the supernatant was collected and used immediately for enzyme assays.

### 2.5. Preparation of leukocyte lysate

The erythrocytes were allowed to sediment from a blood sample by adding dextran and the leukocytes were collected, washed twice, and then leukocyte lysis buffer (Boston BioProducts) was added to the pellet. The lysed cells were then centrifuged and the supernatant was collected.

### 2.6. Superoxide dismutase assay

SOD activity was assayed by the method of Marklund and Marklund (1974). Briefly, 50 µL of serum/cell lysate was added to 2.85 mL of Tris-EDTA (Sigma-Aldrich) and 100 µL of pyrogallol (Merck). The absorbance was followed for 5 min at 420 nm.

### 2.7. Catalase assay

The catalase (CAT) activity was assayed using the method described by Goth (1991) with a slight modification. A sample of 50 µL serum/cell lysate was incubated with 0.25 mL of 65 µmol H₂O₂ in 1 mL of 60 mM phosphate buffer at 37 °C.

### 2.8. Assay of Glutathione Peroxidase

Plasma GPx activity was measured according to the method described by Flohé and Günzler (1984). Briefly, 50 µL of test plasma, 200 µL of 0.1M phosphate buffer and 125 µL of GSH (Sigma-Aldrich) were mixed and incubated at 37 °C for 10 min. The reaction was initiated by adding H₂O₂, allowed for 1 min and then was stopped by adding TCA (Merck). The reaction supernatant was mixed with DTNB (Sigma-Aldrich) and absorbance was recorded at 420 nm.

### 2.9. Assay of Glutathione-S-transferase

Serum GST was estimated according to the procedure described by Prabhu et al. (2005).

### 2.10. Assay of Glutathione Reductase

GR activity was determined according to the method of Manso and Wroblewski (1958). Briefly, 40 µL of GSSG was added to a reaction mixture containing plasma (200 µL), phosphate buffer (920 µL) and 40 µL of NADPH (Sigma-Aldrich), and followed at 340 nm.

### 2.11. Statistical analyses

Data analyses were carried out using the Statistical Package for Social Sciences (version 17.0 for Windows, SPSS Inc., USA). The statistical methods used were Student’s t-test, Mann-Whitney U and chi-squared tests, and Spearman correlation (as applicable, mentioned in Tables/Figures). The mean ± SD values were calculated for each parameter. Figures were done using GraphPad Prism (version 8.3.1). The results were considered significant when the p-value was <0.05.

### 3. Results

#### 3.1. Baseline characteristics of the studied subjects

The baseline characteristics including age, gender, body-mass index, systolic blood pressure, diastolic blood pressure, pulse rate, and smoking habit of the studied subjects have been recorded in questionnaire forms and the values have been compared (Table 1). Among the enrolled patients, 72% had STEMI, 24% had NSTEMI and 4% had UA. The ACS patients had a mean duration of chest pain of 14.74 ± 21.10 hrs before hospitalization and mean cardiac troponin level of 13.16 ± 20.36 ng/mL.
3.2. Evaluation of SOD activity

The mean SOD activity in serum of the ACS patients was significantly higher than in the control subjects (Figure 1). The mean serum SOD activity in patients with STEMI was 4.41 ± 2.33 U/mL, NSTEMI was 4.04 ± 2.07 U/mL, and UA was 5.71 ± 2.93 U/mL, which did not vary significantly.

### Table 1: Baseline characteristics of the ACS patients and control subjects

<table>
<thead>
<tr>
<th>Variables</th>
<th>ACS patients (N = 100)</th>
<th>Control subjects (N = 85)</th>
<th>Statistics (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male gender (%)</td>
<td>89.00</td>
<td>87.06</td>
<td>ND</td>
</tr>
<tr>
<td>Age (years)</td>
<td>51.39 ± 8.00</td>
<td>46.45 ± 8.86</td>
<td>NS*</td>
</tr>
<tr>
<td>BMI (kg/ m²)</td>
<td>22.94 ± 1.99</td>
<td>24.14 ± 3.92</td>
<td>NS*</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>125.10 ± 24.39</td>
<td>124.01 ± 9.32</td>
<td>NS*</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>82.11 ± 16.24</td>
<td>81.99 ± 8.15</td>
<td>NS*</td>
</tr>
<tr>
<td>Pulse (beats/ minute)</td>
<td>84.36 ± 19.26</td>
<td>78.90 ± 11.23</td>
<td>NS*</td>
</tr>
<tr>
<td>Smokers/ Non-smokers (%)</td>
<td>77/ 23</td>
<td>47/ 53</td>
<td>&lt;0.01**</td>
</tr>
</tbody>
</table>

Note: *NS = Not significant (t-test); **Chi-squared test, χ² = 17.72; ND = Not Done; BMI = Body Mass Index; DBP = Diastolic Blood Pressure; SBP = Systolic Blood Pressure; and ACS = Acute Coronary Syndrome.

3.3. Evaluation of CAT activity

In the patients, the mean serum catalase activity was significantly lower than in the control subjects (Figure 2). The mean CAT activity in the STEMI, NSTEMI, and UA patients were 4.41 ± 2.33 U/ mL, NSTEMI was 4.04 ± 2.07 U/ mL, and UA was 5.71 ± 2.93 U/ mL, which did not vary significantly.

Figure 1: Comparison of serum Superoxide Dismutase (SOD) activity between the studied groups. The Acute Coronary Syndrome (ACS) patients had significantly higher SOD activity than the controls (p < 0.01, Mann-Whitney U test).

3.3. Evaluation of CAT activity

In the patients, the mean serum catalase activity was significantly lower than in the control subjects (Figure 2). The mean CAT activity in the STEMI, NSTEMI, and UA patients were 51.61 ± 24.64 U/ mL, 46.97 ± 31.47 U/ mL, and 58.84 ± 29.27 U/ mL, respectively, which did not vary significantly.
3.4. Evaluation of glutathione-associated enzymes

The mean plasma GPx activity in the patients was significantly higher than in the control subjects. Similarly, the mean serum GST activity in the ACS patients was significantly higher than in the controls. On the contrary, the mean plasma GR activity in the ACS patients was significantly lower than in the control subjects. These results are shown in Figure 3. Further analysis of the data showed GR activity was higher in the UA patients (120.0 U/mL/min) compared to the other two subtypes of ACS (STEMI, 83.9 U/mL/min and NSTEMI, 69.3 U/mL/min) patients.
3.5. Assessment of SOD and CAT in erythrocyte and leukocyte lysates

The mean SOD and catalase (CAT) activities in erythrocyte and leukocyte lysates of the studied groups are presented in Table 2, which shows the leukocyte SOD activity was significantly lower in the patients.

<table>
<thead>
<tr>
<th>Cell lysate enzyme</th>
<th>ACS patients (N = 50)</th>
<th>Control subjects (N = 50)</th>
<th>Statistics*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocyte SOD (U/mg Hb)</td>
<td>1.05±0.64</td>
<td>0.82±0.61</td>
<td>NS</td>
</tr>
<tr>
<td>Erythrocyte CAT (kU/mL)</td>
<td>89.47±28.71</td>
<td>88.04±20.20</td>
<td>NS</td>
</tr>
<tr>
<td>Leukocyte SOD (U/mg protein)</td>
<td>1.15±1.22</td>
<td>1.81±1.52</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Leukocyte CAT (U/million cells)</td>
<td>25.60±8.89</td>
<td>23.87±7.42</td>
<td>NS</td>
</tr>
</tbody>
</table>

Note: *t*-test; NS = Not Significant; CAT = Catalase; SOD = Superoxide Dismutase; and ACS = Acute Coronary Syndrome.

3.6. Correlation between enzyme activities

There was a negative correlation between SOD and catalase activities in the patients, a weak or no direct correlation between SOD and GR in the STEMI patients only, but a significant positive correlation was found between the circulatory GR and catalase activities of the ACS patients (Figure 4).

4. Discussion

In this study, the effect of OS have been investigated on a number of enzymatic antioxidant biomarkers at the fluid and cellular levels in the ACS patients and the findings have been compared with those in the non-CVD control subjects. Since OS also develops in diabetes mellitus, chronic kidney disease, liver dysfunction and other chronic inflammatory diseases, individuals with such clinical conditions have been excluded from this study to avoid false positive results. Analyses of the baseline characteristics of the study participants showed smoking was a risk factor for the development of CVD, as observed previously (Kamruzzaman et al., 2019).
The blood samples were collected from ACS patients already admitted in the hospital and under anti-hypertensive drugs; so their blood pressure at the time of blood collection may not reflect the actual blood pressure prior to infraction or during infraction, which may be the reason for not having a significantly higher blood pressure data in the ACS patients. This is to mention here that the control subjects enrolled in the present study were not age-matched with the patients. It may be recalled that one study found the activity of antioxidant enzymes not to vary significantly in different age groups (Vitai and Góth, 1997).

In this study, the serum SOD activity of the ACS patients was found significantly higher than controls, which was consistent with the findings of Horiuchi et al. (2004) in which the SOD activities were found significantly higher in patients with acute myocardial infarction, which was suggested to be due to upregulation of extracellular SOD gene (SOD3) in the vascular wall to compensate the increased OS in ACS patients. However, a Chilean study showed the serum SOD activity was decreased in patients with chronic heart failure (Alcaino et al., 2008). Interestingly, another study found that the infract size in ACS significantly decreased in animal models after SOD treatment (Miao and Clair, 2009).

The present study found a significantly lower catalase activity in the ACS patients which was similar to the findings of a Turkish study on CAD patients, in which the serum catalase activity decreased significantly with the increase in disease severity (Serdar et al., 2006). In another study on APO-E knockout mice, it was found that the SOD1 and catalase acted synergistically in inhibiting atherogenesis induced by benzo(a)pyrene (Yang et al., 2009).

The potentials of the antioxidant enzyme GPx had been investigated in a previous study that found GPx activity had some association with the onset of CAD (Schnabel et al., 2005). The present study found significantly higher GPx activity in the ACS patients compared to the control subjects, which was in agreement with the findings of García-Pinilla et al. (2006).

One of the major findings of the present study was significantly lower GR activity in the patients, which was consistent with a recent study by Shahzad et al. (2018). Further analysis of the current data revealed that among the subtypes of ACS, those with UA had the highest GR activity, which supported another study that showed UA patients had higher GR activity than the STEMI and NSTEMI patients (Zuzak et al., 2017).

Serum GST has been observed to be an informative marker in CVD patients. One study suggested that increased GST activity acted as a protective mechanism to combat increased OS (Pahwa et al., 2017). The present study found significantly higher serum GST activity in the ACS patients than the controls; however in another study in India, GST activity was found lower than the controls (Shahzad et al., 2018).

In this study, the erythrocyte SOD activity in ACS patients was insignificantly higher than the controls which were consistent with other studies in the CAD patients (Serdar et al., 2006; Park et al., 2009); however, a significantly lower activity was also reported in CAD patients (Ahmed et al., 2018). In this study, the erythrocyte catalase activity of ACS patients was similar to the controls, while other studies reported significantly lower activity in CAD patients (Park et al., 2009; Ahmed et al., 2018). This variation in findings could be due to plaque rupture and disease severity in ACS. This study found the mean leukocyte SOD activity to be significantly lower than the controls, and in some cases it was below detectable level. No report on leukocyte SOD activity in ACS patients was found in the literature to compare the present findings.

This study found two glutathione-associated enzymes GPx and GST both utilizing GSH, to have significantly higher activity in ACS patients that caused fast depreciation of the GSH pool in the circulation. Further, a significantly lower GR activity in the patient group might be due to extreme activity of the enzyme to replenish the GSH pool and thus getting exhausted over time. A positive correlation between GR and catalase activities portrays that both the enzymes function synergistically while GR activity is dependent upon the level of GSH in the circulation, whose lower level further increases OS in ACS patients.

5. Conclusion
In controlling increased OS in ACS, there is over-expression of circulatory SOD, GPx and GST, and concomitant reduction in catalase and GR activities resulting impaired cardioprotection by antioxidant—and glutathione—enzymes, implying therapeutic potentials of using SOD and GR in reducing OS-mediated endothelial injury in patients with ACS.

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Conflicts of interest
The authors declare no conflicts of interest in publishing this article.

References


