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Role of complete blood count, antioxidants, and total antioxidant capacity in the pathophysiology of acute coronary syndrome

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Abstract

Acute Coronary Syndrome (ACS) resulting from sudden rupture of arterial plaques with exposure of highly thrombogenic fatty deposits in the coronary arteries is one of the most common causes of mortality worldwide, needs further attention. In this case control study, a total of 138 patients with ACS and 134 non-ACS controls were enrolled. The Red Blood Cell (RBC) count, hemoglobin, and hematocrit were significantly lower in patients. The White Blood Cell (WBC) and platelet counts, and neutrophil to lymphocyte ratio were significantly higher in patients ($11.76 \pm 2.49 \times 10^6$ cells/mL, $343.80 \pm 113.76 \times 10^9$ cells/L, and 3.88 ± 2.24, respectively), which strongly indicated inflammatory responses. The serum and RBC lysate reduced glutathione (GSH), antioxidant vitamins - C, D and alpha-tocopherol, total bilirubin, and Total Antioxidant Capacity (TAC) were significantly lower in ACS patients (3.78 ± 1.53 and 384.21 ± 184.86 μmol/L, 0.30 ± 0.22 mg/dL, 49.46 ± 47.11 ng/mL, 2.66 ± 3.67 μg/mL, 0.51 ± 0.27 mg/dL and 533.61 ± 232.47 μmol/L, respectively). Their serum GSH showed significant positive correlation with vitamin C and hemoglobin, and significant negative correlations were found between WBC count and serum GSH, and platelet count with TAC. Neutrophils and platelets play pivotal roles in mediating inflammatory responses, and significant reduction of antioxidants cause increased oxidative stress in patients with ACS, indicating that administration of antioxidant vitamins could reduce oxidative stress and inflammatory conditions.

Keywords: *Acute Coronary Syndrome (ACS), Complete Blood Count (CBC), Reduced glutathione, Antioxidant vitamins, Bilirubin, Total Antioxidant Capacity (TAC)*

1. Introduction

Acute Coronary Syndrome (ACS) is the single-most important contributor to the causes of deaths among the group of disorders of the heart and blood vessels that are associated with Cardiovascular Diseases (CVD) (Benjamin *et al.*, 2019). Like other Asian countries, Bangladesh has been experiencing an advanced epidemiologic transition from acute infectious and parasitic diseases to non-communicable diseases, which

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is attributed to the significantly massive increase in deaths due to CVD (Karar *et al.*, 2009). Considering the increasing trend of heart disease related mortality, it is indispensable to further investigate novel risk factors for the pathogenesis of ACS and to look for potential management approaches to reduce the burden of ACS in this population.

The most suggestive underlying cause of ACS is atherosclerosis, a silent progressive plaque formation process in the coronary arteries, initiated by chronic and acute overproduction of Reactive Oxygen Species (ROS) under the oxidative stress condition resulting in thrombus formation (Madamanchi *et al.*, 2005; and Sanchis-Gomar *et al.*, 2016). The degree of arterial blockage caused by the thrombus determines the amount of myocardial damage and distinguishes the three types of ACS: Unstable Angina (UA) or partial occlusion with no myocardial damage; Non-ST Elevation Myocardial Infarction (NSTEMI) due to partial occlusion with myocardial damage, and ST-Elevation Myocardial Infarction (STEMI) caused by complete occlusion with myocardial damage.

It has been acknowledged previously that atherosclerosis also involves inflammatory mechanisms where leukocyte recruitment and expression of pro-inflammatory cytokines characterize the early atherogenesis process (Libby, 2012). In addition, a recent study indicated that nearly all the cellular components of the Complete Blood Count (CBC) are involved in the pathogenesis of atherosclerosis which in turn plays a role in the aetiology of CVD (Lassale *et al.*, 2018).

Reduced glutathione (GSH) acts as a first line of defense against oxidative stress which is oxidized in the form of oxidized glutathione to regulate and maintain cellular redox status. Previous studies reported that lower plasma levels of GSH were associated with chronic illness including heart disease, arthritis, diabetes, and malignancies, suggesting that GSH has a protective effect against such diseases (Julius *et al.*, 1994). Later, it has been shown that deficiency of GSH is closely associated with cardiac abnormalities and heart failure (Damy *et al.*, 2009), suggesting the possibility of blood GSH levels to be an important new biomarker to diagnose asymptomatic patients with CVD.

Growing evidence suggests that antioxidant vitamins reduce the risk of CVD outcomes; and vitamin E (the preferentially absorbed form in humans is alpha-tocopherol), and vitamin C (also known as ascorbic acid), both are involved in the non-enzymatic detoxification against ROS (Karajibani *et al.*, 2010). A prospective epidemiological study demonstrates that with alpha-tocopherol intake, there is a reduction in subsequent nonfatal myocardial infarction (MI) in individuals with a previous history of MI (Tribble, 1999). Another study suggests that vitamin C deficiency is associated with a higher risk of mortality from ACS and it may slightly improve endothelial function and lipid profiles in some groups, especially those with low plasma ascorbic acid levels (Moser and Chun, 2016).

Vitamin D, a fat-soluble prohormone, has anti-oxidative properties to inhibit iron-dependent lipid peroxidation in liposomes (Wiseman, 1993). Recent studies found association of vitamin D with CVD risk factors like hypertension, obesity, diabetes mellitus, and dyslipidemias (Wang *et al.*, 2016; and Jeong *et al.*, 2017). Bilirubin is a byproduct of heme degradation which arises from the breakdown of RBC. It has been proposed that bilirubin, within the reference range, can be protective against atherosclerosis through antioxidant activity and suppression of low-density lipoprotein cholesterol oxidation (Neuzil and Stocker, 1994; and Lin *et al.*, 2010). The Total Antioxidant Capacity (TAC) is used to evaluate the antioxidant status of tissue or body fluids, which is measured by the Ferric Reducing Ability of Plasma (FRAP).

Given the utility of the components of CBC and, the anti-oxidative properties of GSH, bilirubin as well as antioxidant vitamins and their protective role in reducing the risk of cardiovascular events, it is worthwhile to investigate whether the levels and correlations of these parameters can be adopted for effective intervention therapy to prevent adverse outcomes in ACS patients.

2. Materials and methods

2.1. Ethical clearance

The study was performed in accordance with the Helsinki declaration and approved by the local Institutional Ethical Review Committee. Informed consent was obtained from all the participants before their enrolment.

2.2. Study subjects

A total of 272 participants comprising of 138 patients suffering from ACS admitted in the coronary care unit and progressive coronary care unit of a tertiary care hospital were enrolled. The diagnosis of ACS was made by expert physicians according to clinical criteria of characteristic electrocardiogram, and troponin changes. Exclusion criteria included diabetes, renal failure, malignancy, or other chronic diseases. A total of 134 healthy subjects from four different locations of the local community were enrolled as the control group who did not have any prior history of CVD, diabetes mellitus, or any other inflammatory disorders. Simple random and availability sampling was applied to collect samples.

2.3. Data and blood sample collection

The demographic and anthropometric data of the studied subjects including age, gender, body weight, height, resting blood pressure, pulse rate, breathlessness, previous hypertension, smoking status, and family history of CVD was recorded in a preformed questionnaire form. About 10 mL of peripheral venous blood was drawn from each subject, 5 mL taken in purple capped tube containing Ethylenediamine Tetraacetic 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 until analyzed.

2.4. Complete blood count measurement

The components of the CBC including Red Blood Cells (RBC), total White Blood Cells (WBC), and platelets were counted following appropriate dilutions of the blood using an improved Neubauer hemocytometer; the differential WBC counts were done by staining a smear of blood with Giemsa's stain and counting at least 200 cells under the high power optics of an Olympus microscope; hemoglobin was determined by the potassium ferricyanide reagent (Randox Laboratories Ltd., United Kingdom); hematocrit and mean corpuscular volume (MCV) were also determined. The neutrophil to lymphocyte ratio (NLR) was calculated from the differential WBC data.

2.5. Preparation of RBC lysate

An aliquot of 200 μL of fresh blood was taken, the cells were washed with an 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.

2.6. Assay of reduced glutathione

The serum and red cell lysate GSH levels were measured using 5-5'-di-thiobis [2-nitrobenzoic acid] (DTNB) with a slight modification of the method described by Ellman (1959).

2.7. Assay of ascorbic acid

Serum ascorbic acid levels were measured using di-nitro phenyl hydrazine, according to the method of Lowry *et al*. (1945).

2.8. Assay of vitamin D and alpha-tocopherol

The levels of vitamin D and alpha-tocopherol in the plasma samples were determined according to the method of Brabcova *et al*. (2013), using a C18 High Performance Liquid Chromatography (HPLC) column by measuring the absorbance at 265 nm and 295 nm, respectively.

2.9. Assay of the total antioxidant capacity

The TAC was measured by the FRAP assay (Rubio *et al*., 2016). The ferric ion (Fe^{3+}) when comes in contact with an antioxidant at an acidic pH, is reduced to a ferrous ion (Fe^{2+}) which can form a blue colored complex with 2,4,6-tripyridyl-S-triazine. The FRAP values were obtained by comparing the absorbance change at 593 nm in test reaction mixtures.

2.10. Assay of bilirubin

Serum bilirubin level was determined quantitatively using a total bilirubin assay Kit (Randox Laboratories Ltd., UK). Albumin bound bilirubin was released by a detergent and then reacted with 2,4-dichloroaniline to form a colored product whose absorbance was measured at 546 nm.

2.11. Statistical analysis

Data analyses were carried out using the Statistical Package for Social Sciences (SPSS Inc., version 19.0 for Windows, USA). The *t*-test was used to compare the continuous variables, chi-squared test was used to compare categorical variables, and Spearman correlation was used to analyze correlations of different variables. For each parameter, the mean ± SD values were computed. The results were considered significant when the value of p was < 0.05.

3. Results

3.1. Demographic and baseline characteristics

A total of 272 subjects, aged 30-70 years, were enrolled in this study of whom women represented less than 6% of the total studied patient and control groups; their data were computed with the men. Among the patients, 99 (71.7%) had STEMI, 35 (25.4%) had NSTEMI and 04 (2.9%) had UA. The demographic and baseline characteristics of studied subjects are summarized in Table 1. The mean age of the patients was 50.91 ± 9.70 years and that of the control group was 48.10 ± 9.54 years. The ACS patients had mean cardiac troponin values of 18.60 ± 23.96 ng / mL, ranging from 0.03 to 100 ng / mL. Significant differences in the proportion of current smokers, hypertension, breathlessness, and family history of CVD were observed between the ACS patients and control groups, while non-significant variations in the body mass index and pulse rate were observed between the study groups.

Table 1: Demographic and baseline characteristics of control subjects and ACS patients

Variables	Control subjects (*N* = 134)	ACS patients (*N*=138)	*p*-value*,#
Body mass index (kg / m^2)	23.42 ± 3.24	24.21 ± 2.33	0.187*
Gender (Female / Male)	08 / 126	08 / 130	0.496*
Age (years)	48.10 ± 9.54	50.91 ± 9.70	0.017*
Systolic blood pressure (mmHg)	125.71 ± 5.89	122.79 ± 20.83	0.116*
Diastolic blood pressure (mmHg)	80.93 ± 9.38	79.86 ± 14.64	0.469*
Pulse (beats per min)	81.81 ± 9.05	84.38 ± 16.07	0.105*
Hypertension, *n* (%)	14.63	46.34	<0.001#
Breathlessness, *n* (%)	14.63	48.08	<0.001#
Current smokers, *n* (%)	20.00	51.19	<0.001#
Family history of CVD, *n* (%)	2.13	28.85	<0.001#

Note: *n* (%) = Percentage of the subjects; *p*-values were derived from: * *t*-test, # Chi-square test; and ACS = Acute Coronary Syndrome.

3.2. Evaluation of complete blood count

Upon comparison of the CBC between the ACS patients and control subjects, significantly lower RBC count, hematocrit, and hemoglobin were found in patients whilst the MCV did not vary significantly (Table 2). The total WBC count was significantly higher in ACS patients. The mean neutrophil count in patients, 72.16 ± 9.28%, was significantly higher than that of the control subjects, and 58% of the patients had more than 70%

neutrophils. The patients had significantly higher NLR than the control group (Table 2). The platelet count was also significantly higher in ACS patients and platelet aggregation was observed under the microscope in every patient sample.

Table 2: Comparison of complete blood count of the control subjects and ACS patients

Variables	Control subjects (*N* = 134)	ACS patients (*N* = 138)	*p*-value*
RBC count (×10^9 cells/mL)	5.32 ± 0.85	4.82 ± 0.82	0.003
WBC count (×10^6 cells/mL)	7.37 ± 1.77	11.76 ± 2.49	<0.001
Neutrophil (%)	56.90 ± 4.36	72.16 ± 9.28	<0.001
Lymphocyte (%)	35.80 ± 4.40	23.11 ± 8.39	<0.001
Monocyte (%)	3.52 ± 1.61	2.94 ± 1.58	0.075
Eosinophil (%)	3.63 ± 1.60	1.66 ± 1.25	<0.001
Basophil (%)	0.15 ± 0.41	0.06 ± 0.22	0.137
Neutrophil to lymphocyte ratio	1.63 ± 0.33	3.88 ± 2.24	<0.001
Platelet count (×10^9 cells/L)	273.02 ± 62.18	343.80 ± 113.76	<0.001
Hemoglobin (g/dL)	14.11 ± 1.54	13.36 ± 1.85	0.029
Hematocrit (%)	49.17 ± 3.55	46.10 ± 5.49	0.002
Mean corpuscular volume (fL)	94.00 ± 14.74	96.32 ± 14.69	0.454

Note: RBC = Red Blood Cells; WBC = White Blood Cells; * = *p*-value derived from *t*-test; and ACS = Acute Coronary Syndrome.

3.3. GSH levels in serum and red cell lysate

The levels of reduced GSH in both serum and red cell lysate of ACS patients were significantly lower compared to the controls (Table 3). Among the three ischemic subsets, significantly lower values than controls were found in patients with STEMI and NSTEMI in both serum (3.77 ± 1.44 and 3.97 ± 1.61 μmol/L, respectively) and red cell lysate (400.45 ± 158.95 and 345.19 ± 257.34 μmol/L, respectively) (Figure 1). In patients with UA, reduced GSH levels displayed larger depletion in serum (2.99 ± 2.17 μmol/L) compared to red cell lysate (349.67 ± 39.74 μmol/L).

Table 3: Status of antioxidant defense systems in controls and ACS patients

Measurements	Control subjects (*N*)	ACS patients (*N*)	*p*-value*
Ascorbic acid (mg/dL)	0.59 ± 0.24 (100)	0.30 ± 0.22 (100)	< 0.001
Alpha-tocopherol (μg/mL)	8.05 ± 7.09 (40)	2.66 ± 3.67 (40)	< 0.001
Vitamin D (ng/mL)	75.85 ± 41.42 (40)	49.46 ± 47.11 (40)	0.010
Serum GSH (μmol/L)	8.06 ± 1.69 (100)	3.78 ± 1.53 (100)	< 0.001
Red cell GSH (μmol/L)	575.42 ± 102.87 (40)	384.21 ± 184.86 (40)	< 0.001
Bilirubin (mg/dL)	0.66 ± 0.20 (60)	0.51 ± 0.27 (60)	0.001
TAC (μmol/L)	1091.62 ± 267.27 (60)	533.61 ± 232.47 (60)	< 0.001

Note: *N* = Number of subjects. GSH = Reduced glutathione; TAC = Total Antioxidant Capacity; and * *t*-test.

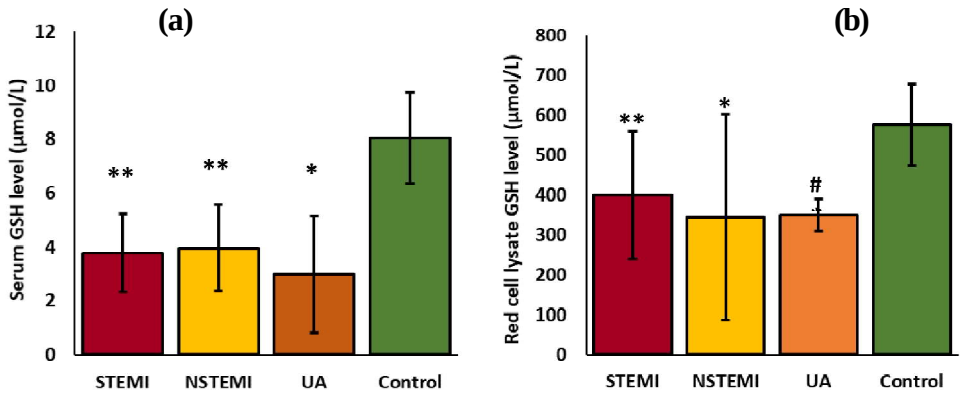

Figure 1: Comparison of the levels of (a) serum GSH, and (b) red cell lysate GSH among various ischemic subgroups of ACS patients and controls. Patients with ST-Elevation Myocardial Infarction (STEMI), Non-ST Elevation Myocardial Infarction (NSTEMI) and Unstable Angina (UA) had significantly lower GSH (reduced glutathione) in serum and red cell lysate than the respective controls; * $p < 0.05$, # $p < 0.01$ and ** $p < 0.001$.

3.4. Evaluation of total antioxidant capacity

It was found that the TAC of the ACS patients varied from 136.0 to 1228.8 μmol / L compared against 645.1 to 1707.2 μmol / L in the control subjects, and the mean TAC was significantly lower in the ACS patient group (Table 3).

3.5. Levels of ascorbic acid, alpha-tocopherol, and vitamin D

It was noted that the ACS patients had significantly lower serum ascorbic acid levels than the control subjects (0.30 ± 0.22 and 0.59 ± 0.24 mg / dL, respectively) (Table 3). Further analysis of the data revealed that the mean ascorbic acid levels were significantly lower in patients with STEMI (0.28 ± 0.21 mg / dL), NSTEMI (0.38 ± 0.25 mg / dL), and UA (0.12 ± 0.02 mg / dL) compared to the controls (Figure 2). The plasma alpha-tocopherol levels in ACS patients showed significantly lower values compared to the controls, and vitamin D levels were also found to be significantly lower in the patients than in controls (Table 3).

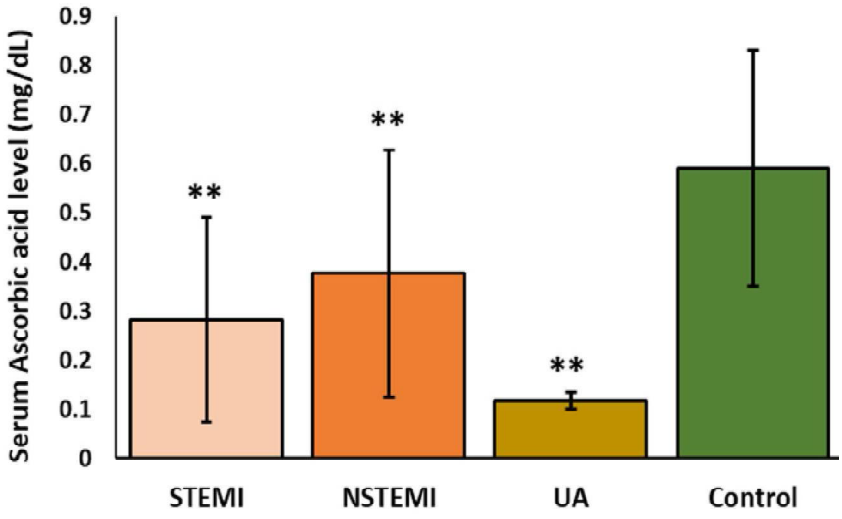

Figure 2: Comparison of the levels of serum ascorbic acid in various ischemic subgroups of ACS patients and controls. The results showed that all three subgroups of patients [with ST-Elevation Myocardial Infarction (STEMI), Non-ST Elevation Myocardial Infarction (NSTEMI) and Unstable Angina (UA)] had significantly lower values than controls; and ** $p < 0.001$.

3.6. Comparison of bilirubin levels

The mean serum total bilirubin level in ACS patients, 0.51 ± 0.27 mg / dL, was found to be significantly lower than in the controls, 0.66 ± 0.20 mg / dL (Table 3).

3.7. Correlation of antioxidants and complete blood count

In ACS patients, a significant positive correlation was found between serum GSH and ascorbic acid levels (*r* = 0.39, *p* = 0.002) (Figure 3a). The total WBC counts exhibited a significant positive correlation with red cell GSH (*r* = 0.41, *p* < 0.05), and a strong negative correlation with serum GSH (*r* = – 0.41, *p* = 0.01) (Figure 3b) as well as with serum ascorbic acid (*r* = –0.33, *p* < 0.05). The RBC count of the patients showed a strong significant positive correlation with TAC (*r* = 0.40, *p* = 0.01) (Figure 3c) while the platelet count showed a negative corelation with the TAC (*r* = –0.32, *p* = 0.05) (Figure 3d), and a strong significant association was noted between the hemoglobin and serum GSH levels (*r* = 0.54, *p* < 0.001).

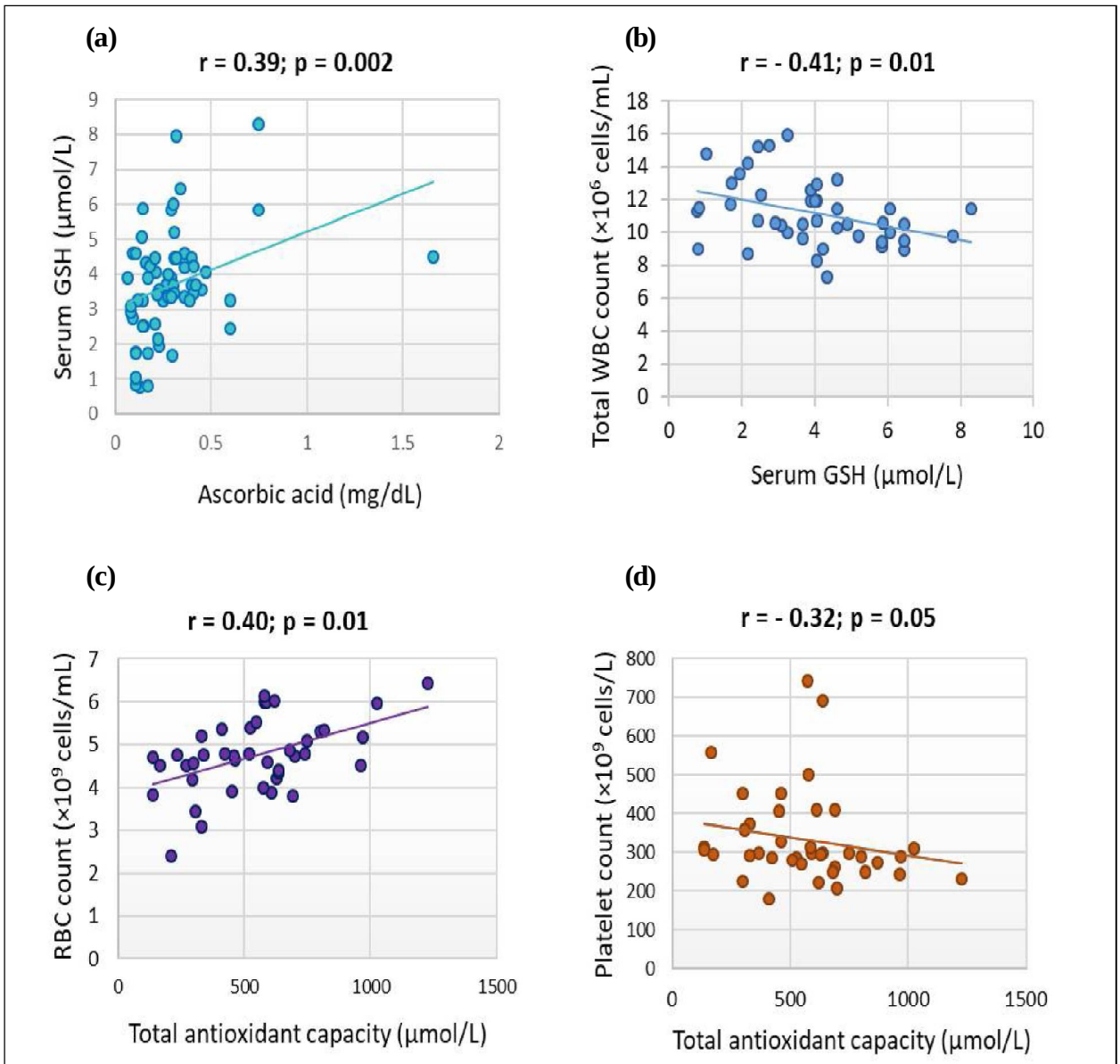

Figure 3: Significant correlations between the levels of (a) Ascorbic acid and serum GSH; (b) Total WBC count and serum GSH; (c) RBC count and total antioxidant capacity; and (d) Platelet count and total antioxidant capacity in patients with acute coronary syndrome [Spearman correlations].

4. Discussion

This study demonstrated that the components of the CBC are associated with the development of ACS; and identified that reduced levels of the antioxidative defensive systems are directly or indirectly associated with ACS. As reported by other workers (Messner and Bernhard, 2014; and Messerli *et al*., 2017), the present study found smoking and previous history of hypertension to be significantly higher in patients with ACS who were screened to exclude those suffering from diabetes mellitus, and any other chronic inflammatory disorder to avoid false-positive results. In contrast to a recent investigation (Ferdausi *et al*., 2020), this study found a significant association between breathlessness and family history of CVD in the development of ACS. However, the patients enrolled in this study were already hospitalized and being treated with antihypertensive drugs; thus, the observed blood pressure and pulse rate might not be the same as the time of MI attack.

In this study, the RBC count, hemoglobin and hematocrit values were found to be significantly lower in ACS patients compared to the control subjects. On the other hand, no significant difference was observed in the MCV of the studied groups. A low level of hemoglobin, hematocrit, and RBC count clinically indicates anemia (Tefferi *et al*., 2005), and findings of this study suggest that the prevalence of anemia was significantly higher in the patients. It has been shown that chronic anemia has adverse effects on myocardial and large arterial remodeling and anemia has been marked as a risk factor for CVD (Sarnak *et al*., 2002).

This study found associations of higher leukocyte count and ACS, and increased neutrophils was attributed to the higher count of total WBC among ACS patients, as observed previously (Rana *et al*., 2007; and Choudhury *et al*., 2019). The differential WBC counts further revealed significant associations of decreased eosinophil and lymphocyte counts with ACS, which was consistent with the findings of a recent cohort study showing lower counts of these cells in the general population were associated with the increased short-term incidence of heart failure and coronary death (Shah *et al*., 2016). Another important finding of this study was a significantly higher neutrophil to lymphocyte ratio in ACS patients compared to the controls, which was consistent with previous findings (Choudhury *et al*., 2019).

A novel finding of the present study is significantly increased platelet counts and their aggregation in ACS patients, which has been reported in a few population-based studies that reported a significantly higher coronary heart disease mortality among subjects with the highest platelet counts (Thaulow *et al*., 1991; and Lassale *et al*., 2018). It is well recognized that excessive activation and aggregation of platelets play a key role in thrombotic vascular occlusion at the ruptured coronary atherosclerotic plaque, leading to acute ischemic episodes, the ACS (Lange and Hillis, 2004; and Falk *et al*., 2013). This study observed multiple lumps of aggregated platelets in whole blood on microscopic analysis in the ACS samples, confirming the high levels of platelet aggregation in ACS pathophysiology.

The status of antioxidant vitamins and GSH in ACS patients as well as in various subtypes of ACS has been explored in this study. It was found that decreased serum levels of GSH and ascorbic acid were associated with increased risk of ACS, and patients with STEMI, NSTEMI and UA were found to have significantly lower GSH and ascorbic acid levels compared to the controls. This study found a significantly lower level of alpha-tocopherol in ACS patients, suggesting that high alpha-tocopherol intake could inhibit the pathogenesis of ACS. Several studies also found a moderate reduction in the risk of coronary disease by increasing the intake of alpha-tocopherol as a supplement (Karajibani *et al*., 2010; and Myung *et al*., 2013). In the present study, a significantly decreased level of plasma vitamin D was found in ACS patients, indicating its deficiency might be a potent risk factor for ACS, as observed previously (Aljefree *et al*., 2016).

Serum bilirubin has a protective effect against CVD (Lin *et al*., 2010), and acts as an endogenous regulator of inflammation by disrupting adhesion molecule-mediated leukocyte migration (Vogel and Zucker, 2016). In this study, ACS patients had a significantly lower level of serum bilirubin compared to the controls, which was consistent with the observation of a recent study by Xu *et al*. (2019), indicating its protective role against ACS. The major findings of this study include depletion of GSH in serum and RBC along with a reduced TAC in ACS patients, which suggest a cellular oxidative stress condition. The present study found serum levels of GSH to be positively associated with ascorbic acid, and negatively correlated with leukocyte count suggesting a synergistic role of these antioxidants in controlling cellular oxidative stress.

Further, this study found a significant positive correlation between RBC count and TAC. The underlying mechanisms include oxidative stress leading to an imbalance of free radicals and antioxidants that impairs membrane fluidity and reduction of the life span of RBC, implying antioxidants may improve RBC survival in

the body (Mozos, 2015). Besides, the present study found a negative correlation between platelet count and TAC, while serum GSH was directly associated with hemoglobin levels in ACS patients. These associations may be attributed to the fact that reduced GSH play important roles in protecting blood platelets and hemoglobin from oxidative damage.

4.1. Limitations of the study

This study has several limitations. First, it is a small-scale study which may not reflect the full picture obtained for a complete population. Second, the patients are about three years older than the control subjects, which is due to the difficulties in finding age-matched controls with the stringent inclusion criteria of the study. Thirdly, to a certain extent, the results may be affected by the influence of lifestyle, food habit, and medical treatment facilities of the studied subjects. Finally, due to a shortage of sample volumes all assays couldn't be performed on some samples.

5. Conclusion

This study found ACS to be strongly associated with elevated WBC, platelet and neutrophil counts as well as NLR. Besides, other components of the CBC namely RBC count, hematocrit, and hemoglobin have been found to be associated with ACS. These parameters are inexpensive, easy to interpret, and widely available tools for the management and prognosis of ACS in patients. Furthermore, the present study indicated that ACS is associated with lower levels of antioxidant vitamins, reduced GSH, bilirubin, and impaired TAC. The antioxidant vitamins C, D and alpha-tocopherol strengthen the antioxidant defense system against oxidative damage, and therefore, dietary supplementation of these vitamins might act as a primary preventive intervention for developing atherosclerosis, the core mechanism responsible for the CVD. Monitoring the CBC with the status of antioxidant vitamins, GSH, and TAC can improve our ability to predict ACS risks.

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Ethical approval and consent to participate

This study was approved by the Ethical Review Committee (ERC) of the Faculty of Biological Sciences, University of Dhaka, Bangladesh (Ref. 56 / Biol. Sci. / 2017-2018). All subjects from both the patient and control groups gave full verbal consent to be included in this investigation, which was approved by the ERC.

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Conflicts of interest

The authors declare no conflicts of interest.

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