ISSN NO. 2320-5407

*Journal homepage: http://www.journalijar.com*

*INTERNATIONAL JOURNAL OF ADVANCED RESEARCH*

**RESEARCH ARTICLE**

# Association of AST, ALT, ALB and Total Protein with Beta-thalassemia in Bangladeshi Population

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## *Manuscript Info*

***Manuscript History:***

Received: 12 November 2014
Final Accepted: 22 December 2014
Published Online: January 2015

***Key words:***

Aspertate transaminase, Alanine transaminase, Beta-thalassemia**,** Hemoglobin, β-globulin

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## *Abstract*

Beta-thalassemia is one of the most common hereditary hematologic disorders characterized by severely impaired β-globulin synthesis. Our objective of the study was to estimate the AST, ALT, ALB and total protein levels to evaluate the heart and liver functions in beta-thalassemia patients. The study comprises a total of 65 subjects including patients with beta-thalassemia (n=35) and healthy volunteer (n=30) matched by age and sex. Hemoglobin (Hb) analysis was done using HPLC. Serum total protein (TP) and albumin (ALB) were determined by Biolis 24i Automated Clinical Analyzer. Serum AST (aspertate transaminase) and ALT (alanine transaminase) were determined by enzymatic method. The AST and ALT levels were significantly ($p<0.001$, respectively) increased in beta-thalassemia patients when compared to the controls. On the other hand, there were no significant differences found in TP and ALB levels in patients with beta-thalassemia. There were negative correlations of Hb level with AST ($r = -0.4$; $p<0.05$) and ALT ($r = -0.5$; $p<0.01$) levels while no correlations with TP ($r = 0.3$; $p>0.05$) and ALB ($r = 0.1$; $p>0.05$) levels were found among patients. Our findings indicate that abnormal AST and ALT levels exist in patients with β- thalassemia. The increased in AST and ALT levels indicate that patients with β- thalassemia are in increased risk of heart and liver dysfunction.



## INTRODUCTION

Thalassemia is a group of conditions in which there are a reduced rate of synthesis of one or more of the globin chains leading to imbalanced synthesis of globin chains, production of defective hemoglobin and the red blood cells damage or their precursors which results from the effects of the globin subunits that are produced in excess. Beta-thalassemia major (BTM) is a common health problem in the Africa, Middle East, Southeast Asia and the Indian subcontinent. BTM is considered to be a hereditary severe anemia resulting from defects in beta-globin synthesis (Modell et al., 2001; Rund and Rachmilewitz, 2005). Beta-thalassaemia major is the most prevalent type of thalassaemia as it is common in certain individuals. In its homozygous state it produces severe anemia (Widad et al., 2003). Around 190 million people across the world have genetic mutations associated with different hemoglobinopathies, and more than 90 million of them carry defective genes leading to thalassaemia (Ambekar et al., 2001; Das et al., 2004). In thalassemia, the imbalance of globin chain synthesis leads to red blood cells damage resulting in destruction of red blood cells in the bone marrow (ineffective erythropoiesis) and peripheral circulation (hemolysis) (Noguchi et al., 2004). For many patients with chronic anemia, regular transfusions of red blood cells represent life saving therapy. Regular blood transfusions have dramatically extended life expectancy in thalassemia major (Lanzkowsky, 2000).

Iron overload causes most of the mortality and morbidity associated with thalassemia. There are many reports on complications of β-thalassemia in different organs (Low, 2005; Al-Rimawi et al., 2005; Angelopoulos et al., 2006; Cetin et al., 2003; Asma et al., 2003). The accumulation of Iron in the liver, heart, and multiple endocrine glands results in severe damage to these organs, with variable endocrine organ failure. Profound anemia and excess iron deposition leads to dysfunction of cardiovascular, reticuloendothelial, and other organ systems (Muncie and Champbell, 2009). However, the most serious complication of iron overload is life-threatening cardiotoxicity. Cardiac events due to iron overload are still the primary cause of death (Papanikolaou et al., 2005). Iron-chelating therapy is largely responsible for doubling the life expectancy of patients with thalassemia major: it has been proven to prevent liver and heart damage and causes normal growth and sexual development in children with thalassemia, and increase life span (Low, 2005). In the last 30 years, conventional treatment of β-thalassaemia major, based primarily on regular blood transfusions and iron chelation therapy with desferrioxamine (DFO) and know a day with desferizirox (exjade) has markedly improved the prognosis of the disease. Use of parenteral DFO reduces or prevents iron accumulation and iron-mediated organ damage, resulting in a consistent decrease of morbidity and mortality (Wong and Richardson, 2003). Our over all aim of this study was to estimate the AST, ALT, ALB and total protein levels to evaluate the heart and liver functions in beta-thalassemia patients in Bangladesh.

## Materials and Methods

### Study Subjects

The study was a case-control study conducted on 65 subjects. The case group comprises 35 patients with beta-thalassemia major (18 men, 17 women; age range from 8 to 34 years). The patients were recruited from the outpatient department of Ibn Sina Medical College Hospital, Dhaka. The patients were clinically diagnosed as suffering from beta-thalassemia and treated using different therapeutic regimens. The beta-thalassemia was diagnose by electrophoresis. Patients with other haemostatic abnormalities (leukaemia, lymphoma, sepsis and vasculitis), kidney disease, alcohol intake, pregnancy were excluded from the study. A total of 30 healthy volunteers (15 men, 15 women; age range from 15 to 35 years) with no history of beta-thalassemia or other chronic diseases were recruited in this study.

All participants were given an explanation of the nature of the study and informed consent was obtained. They completed a structured questionnaire covering information on age, gender, medical and family history of chronic diseases. This study was approved by the ethical committee of Dhaka University. All the analyses were done in the Biochemistry laboratory, Ibn Sina Medical College Hospital and Department of Biochemistry and Molecular Biology, University of Dhaka, Dhaka-1000, Bangladesh.

### Sample Collection

About 5.0 mL of venous blood was drawn from each individual following all aseptic precautions with the help of a trained person, using a disposable syringe. The blood sample was taken in an EDTA coated tube (2.0 mL) and a plain tube (3.0 mL) for the estimation of study parameters. The serum was separated by centrifugation at 3000 rpm for 10 minutes and kept at -20°C until analysis.

### Clinical Analysis

Hemoglobin analysis was done using HPLC by D-10 (BioRad, Marnes La Coquette, France). Serum total protein (TP) and albumin (ALB) were determined by Biolis 24i Automated Clinical Analyzer (Tokyo Boeki Machinery Ltd., Japan).

Serum AST (aspertate transaminase) was determined by enzymatic method described by Sampson et al. (1980) and ALT (alanine transaminase) was determined by enzymatic method described by Hafkenscheid and Dijt, (1979).

### Statistical Analysis

All the results were expressed as mean ± SEM. The statistical analysis of the data was carried out with Statistical Package of Social Science (SPSS), version 17 and Graph pad Prism version-5. The comparisons between two groups were tested by unpaired t-test. A 95% confidence interval was used. p values less than 0.05 were considered as statistically significant. Correlation between two continuous outcomes among patients was evaluated using Pearson correlation coefficient.

## Results

Statistically significant differences among patients and controls are indicated in Table 1 and in Fig. 1 along with their significant values.

As shown in Table 1, the Hb level was significantly ($p<0.001$) lower among the patients compared to the controls. On the other hand, the AST and ALT levels were significantly ($p<0.001$, respectively) higher in patients while ALB and TP levels were not significantly different (Table 1).

**Table 1 Different parameters in study subjects.**

| Parameters | Control (n=30) | Patients (n=35) | p value |
|---|---|---|---|
| **Hb (g/dL)** | 12.50 ± 0.2 | 9.50 ± 0.2 | <0.001 |
| **AST (U/L)** | 17.60 ± 0.8 | 34.16 ± 2.5 | <0.001 |
| **ALT (U/L)** | 16.44 ± 1.6 | 46.60 ± 4.0 | <0.001 |
| **ALB (g/dL)** | 4.65 ± 0.1 | 4.59 ± 0.1 | 0.6 |
| **TP (g/dL)** | 7.70 ± 0.1 | 7.85 ± 0.3 | 0.6 |

Results are expressed as mean ± SEM. Unpaired t-test was done as the test of significant. $p<0.05$ was taken as the level of statistical significant.

Fig. 1 shows the correlations of Hb level with different parameters among the patients. There were significantly negative correlations ($r = -0.4$, $p<0.05$) of Hb with AST and ALT levels while ALB and TP have no significant correlations ($r = 0.4$, $p<0.05$) with Hb level.

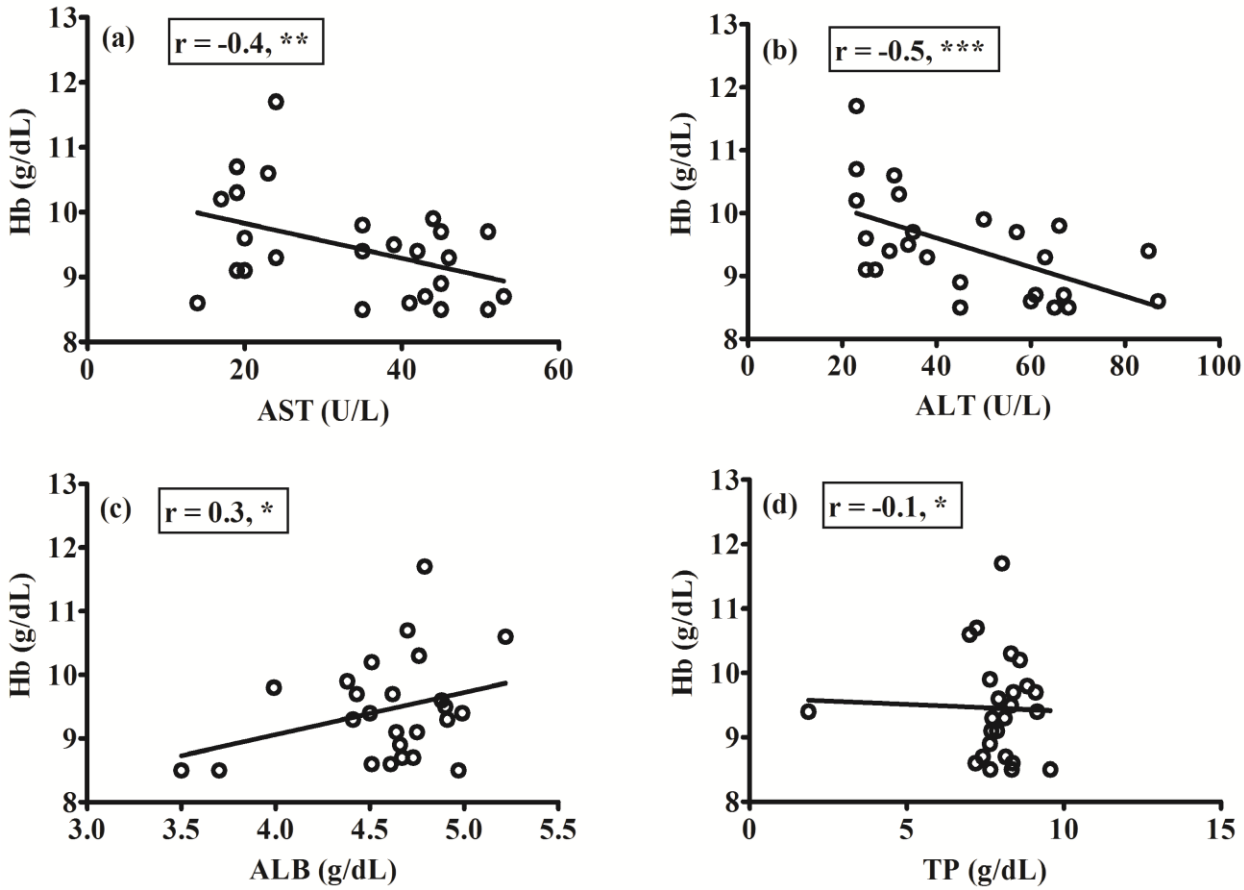


**Figure 1** Correlation of Hb with (a) AST, (b) ALT, (c) ALB and (d) TP. r; correlation coefficient. ***; $p<0.01$, **; $p<0.05$, *; $p>0.05$.

## Discussion

Patients with beta-thalassaemia major are prone to metabolic complications with different organ dysfunction. Though the actual mechanism is not clear, beta-thalassemia is considered to be related to anaemia and iron overload, in addition to lipid peroxidation, oxidative stress and free radical release (Walter et al., 2008). The organ failure is the most important cause of mortality and morbidity due to iron deposition in patients with beta-thalassaemias. In our study, we investigated the AST, ALT, ALB and total protein levels to evaluate the heart and liver functions in beta-thalassemia patients in Bangladesh.

Our results revealed a significant increase in serum AST and ALT levels in patients compared to controls (Table 1). There were significantly negative correlations of Hb with AST and ALT levels (Fig. 1a and 1b). This result is in agreement with Mansi et al. (2013) who reported increased levels of AST and ALT levels in beta-thalassemic patients. Awadallah et al. (2012) also found increased levels of AST and ALT levels in beta-thalassemic patients. ALT is an enzyme found primarily in the liver but also in the heart and other tissues, it is often used in diagnosing liver function (Okuda et al., 2011). The mean ALT activity in thalassemic patients was reported to be increase and this increase in ALT was generally transient and occurred more commonly in patients with hepatitis C (Cohn et al., 2000). ALT activity was elevated in all thalassemic patients, which may due to the symptoms of liver damage (Dobrowska et al., 2001). AST is an enzyme found primarily in the liver, heart, kidney, pancreas, and muscles, elevated in tissue damage especially heart and liver, decreased levels can be found in vitamin B deficiency and pregnancy. Liver fibrosis and cirrhosis are well known complications of thalassemia. Transaminases are expressed as multiplied by the upper level of the normal range to identify the role of iron overload in the natural history of liver fibrosis (Harmatz et al., 2000).

Total protein is considered as the most abundant compounds in serum. The proteins are involved in enzymes, hormones and antibodies as well as osmotic pressure balance. The possible cause of decreased serum total protein secondarily decreased synthesis of protein by the liver. Albumin is the major constituent of serum protein (usually over 50%). It is manufactured by the liver and it helps in osmotic pressure regulation, nutrient transport and waste removal (Walter et al., 2008). Abnormal TP and ALB levels are the indicators of liver malfunction. In our current study, we found no significant difference in TP and ALB levels in beta-thalassemic patients when compared to healthy controls (Table 1). There was no significant correlation of Hb with TP and ALB among beta-thalassemic patients (Fig. 1c and 1d). Awadallah et al. (2012) also reported no association of TP and ALB with beta-thalassemic patients.

In summary, the increased in AST and ALT levels indicate that patients with β- thalassemia are in increased risk of heart and liver dysfunction. Physicians should be aware of heart and liver function during the treatment of beta-thalassemia patients. However, our study has a small sample size resulting in low power to detect minor to modest associations, therefore further study with large sample size is required.

## Acknowledgement

Thanks to the study subjects for their participation in this study.

## Conflict of interest

No competing financial interests exist.

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