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A Correlative Study Of Serum Bilirubin And Liver Enzymes With Serum Ferritin In Beta Thalassaemia Major

Patel S.A[1], Siddiqui A.M [2], Kareem I.[3]
[1,2,3]*(Department of Biochemistry , Dr. S.C.GMC, Nanded, India)*
Corresponding Author: Siddiqui A.M [2],

Abstaract :
Background: *Beta-thalassemia is one of the most common hereditary hematologic disorders characterized by severely impaired β-globulin synthesis. Liver is the earliest site of iron deposition in transfusion dependent β - thalassemia major and iron induced liver injury is the common cause of morbidity. Objectives of our study were*

1. *To estimate serum bilirubin ,AST and ALT in beta thalassaemia major patients and compare it with controls.*
2. *Estimation of serum ferritn in beta thalassaemia major patients and its correlation with serum bilirubin and liver enzymes AST (Aspartate transaminase) and ALT (Alanine transaminase) in beta thalassemia major patients*

Methods: *The study comprises of 70 subjects. The study subjects were distributed into two groups, the group - A (40 cases) and group – B (30 age and sex matched healthy controls). Subjects in age group 2 to 14 years were selected. Cases who are regularly transfused with >5 blood transfusions and more than one year of oral chelation therapy were included in the study*
Results: *The mean levels of serum bilirubin , AST and ALT in controls were 0.58 ± 0.18, 19.50 ± 1.7 and 22.33 ± 1.4 respectively. In cases controls the mean levels of serum bilirubin , AST and ALT were 2. 50 ± 0.24 , 45.68 ± 1.6 and 50.27 ± 1.3 respectively. Serum ferritin was estimated in 40 cases. The mean level was 2402 ± 1292ng/ml, the minimum level was 653 ng/ml and maximum level was 6015 ng/ml. We found a highly significant correlation between serum ferritin and serum bilirubin beyond serum ferritin levels 1000ng/ml ml (Pearson's correlation co-efficient r= + 0.53). In case of AST and ALT high statistically significant correlation with serum ferritin was found beyond 2000 ng/ml(Pearson's correlation co-efficient r= + 0.62).*
Conclusion : *Iron overload , jaundice and raised liver enzymes are common findings in beta thalassaemia major patients suggesting an increased risk of liver dysfuction. Derangements in liver function begin when serum ferritin levels are above 1000ng/ml. Hence we recommend that liver functions should be carefully monitored in patients with transfusion dependent β-thalassemia major especially when serum ferritin levels are above 1000 ng/ml*

Keywords**:** *ALT (Alanine transaminase) ,AST (Aspartate transaminase), Beta thalassaemia major ,bilirubin , ferritin , iron overload.*

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I. Introduction

Thalassaemia is the one of the most common hereditary disorder in the world including India. Thalassaemia is a major health problem all overthe world but this is particularly in thedeveloping countries where the resources arelimited.[1] Thalassemia refers to a group of genetic disorders characterized by insufficient production of hemoglobin, wherein there is a defect in the synthesis of hemoglobin. Thalassemia can be classified according to the genes affected. There are two main types of thalassemia, alpha and beta, named after the two protein chains that make up normal hemoglobin.[2] Beta thalassaemia is caused by decreased production of beta-globin chain. Its homozygous variant is called Beta thalassaemia major which usually produce severe chronic hemolytic anemia. [3] Beta thalassaemias occur widely in a broad belt, ranging from the Mediterranean and parts of north and West Africa through the Middle East and Indian subcontinent to South East Asia.[4] Hence It is sometimes called Mediterranean anemia.

Children who suffer from this disease need a lifelong repeated blood transfusion to maintain their hemoglobin level around 12g/dl. But unfortunately this results in increase serum iron level leading to its accumulation in various tissue. [5] Therefore progressive iron overload has become the major complication of treatment .[6] Moreover there is increased absorption of iron from gut . This excess gastrointestinal iron absorption persists despite massive increases in total body iron load. Hepcidin is a small peptide that inhibits iron absorption in the small bowel. Hepcidin levels normally increase when iron stores are elevated.But

paradoxically its levels were found to be inappropriately low in patients thalassaemia major.[7]The combination of iron overload and increase outpouring of catabolic iron from the reticuloendothelial system surpass the iron binding capacity of transferrin, resulting in the emergence of toxic non transferrin bound plasma iron (NTBI). NTBI promotes the formation of free hydroxyl radicals and accelerates the peroxidation of membrane lipids. It affects almost all systems of body such as endocrines, liver and heart. But liver is the earliest site of iron overload involving both hepatocytes and reticuloendothelial cells. Progressive lipid peroxidation and TGFbeta-1 expression resulted from iron over load may promote hepatic injury , fibrogenesis eventually cirrhosis.[8,9] So thalassaemia patients must be
routinely checked for liver function.

In beta thalassamia major there is an increase of serum iron level, transferrin saturation and ferritin level. Ferritin is an intracellular ,high molecular weight iron containing storage protein present mainly in the reticuloendothelial cells of liver, spleen, bone marrow and other tissue of the body. Its two major functions are to remove excess iron from cells converting it into a harmless soluble form, and to provide a mobilizable reserve of iron which can be drawn when needed. Clinically significant concentration is found in serum and the level of serum ferritin reflects total body iron stores.[10, 11] Serum ferritin concentration reflects iron storages in health and also in certain diseases.[12] Most of the ferritin is intracellular but the measurement of circulating serum ferritin reflects the level of the body iron store.[13, 14] To judge the extension of liver cell damage caused by iron accumulation, a very sensitive indicators for liver function, serum bilirubin and liver enzymes such as AST , ALT were assessed. All these parameters are raised in transfusion dependent β-thalassemia major patients. Though liver biopsy is gold standard test to know iron overload state in liver but it is invasive method and T2 MRI is best non-invasive method of determining liver iron.[15] Relatively simpler way of knowing the liver damage is by estimation of liver enzymes which are raised due to oxidative injury and direct toxic effect of iron on liver cells.[9] This study was planned to study the correlation ofserum bilirubin and liver enzymes (SGOT and SGPT) with serum ferritin levels in children with transfusion dependent β-thalassemia major.

II. Materials And Methods

A cross sectional comparative study was carried out in the department of Biochemistry, Dr. Shankarrao Chavan Government Medical College, Nanded. Total 70 subjects were included in this study. The study subjects were distributed into two groups, the group - A (40 cases) and group – B (30 age and sex matched healthy controls). Inclusion and exclusion criteria were considered. The inclusion criteria were a. Diagnosed case of thalassaemia major who have received transfusion more than five times and chelation therapy started for more than 1 year, b. Age: two to fourteen years c. Sex: both sexes. Exclusion criteria were a. Below two years, b. Other hemolytic disorders, c. Acute systemic illness and d. Hepatitis B or Hepatitis C positive patients. The patients who fulfill the inclusion and exclusion criteria were selected for the study. Blood samples were collected in a plain sterile bulb after blood transfusion and kept for an hour for clotting and then centrifuged. Supernatant clear serum was taken for the estimation of serum ferritin , serum bilirubin , AST and ALT of the patients. In controls serum bilirubin, AST and ALT were done and compared with cases. Furthur serum ferritn was estimated in cases and correlation was done between serum ferritin and serum bilirubin ,AST and ALT. Serum ferritin was measured by immunoturbidimetric latex assay of Proton Diagnostics (Gen X series). All the investigations were done on Erba Chem 7 semi-autoanalyzer. Serum level of bilirubin >1.0 mg/dl, AST > 40 IU/L and ALT > 35 IU/L were considered abnormal.

Institutional ethical committee permission and written consent was taken from the parents of children. Data were entered in SPSS software (version 11.5) used to analyze data. Data were expressed as mean ± SD. To compare the values ofserum bilirubin , AST and ALT OF cases and controls unpaired Student's "t" test was used. p value less than 0.05 was taken as significant. For correlation between serum bilirubin , AST and ALT with serum ferritin in cases Pearson's correlation test was used.

III. Results

The parameters serum bilirubin , AST and ALT were estimated in cases and controls and were compared (Table 1). In controls the mean levels of serum bilirubin, AST and ALT were 0.58 ± 0.18, 19.50 ± 1.7 and 22.33 ± 1.4 respectively. In cases controls the mean levels of serum bilirubin, AST and ALT were 2.50 ± 0.24 , 45.68 ± 1.6 and 50.27 ± 1.3 respectively. The differences were statistically significant as shown in Table 1.

Table 1: Different parameters in study groups

Parameters	Control (n=30)	Cases (n=40)	p value
Serum Bilirubin mg/dl	0.6367 ± 0.03	2.970 ± 0.32	< 0.0001
AST(IU/L)	19.50 ± 1.68	63.83 ± 3.94	< 0.0001
ALT(IU/L)	22.33 ± 1.36	61.83 ± 4.25	< 0.0001

Serum ferritin was estimated in 40 cases. The mean level was 2402 ± 1292ng/ml, the minimum level was 653 ng/ml and maximum level was 6015 ng/ml. Out of 40 cases 16 patients had serum ferritin levels between 1000 to 2000 ng/ml , 12 pateints had levels between 2000 to 3000 ng/ml and 9 had very high levels >3000 ng/ml (Table 2) . only 3 had serum ferritin level <1000 ng/ml while maximum patients had high ferritin levels inspite of chelation (Table 2). We correlated serum ferritin levels in cases with serum bilirubin , AST and ALT respectively. We found a high correlation between serum ferritin and serum bilirubin beyond serum ferritin levels 1000ng/ml. In case of AST and ALT high statistically significant correlation with serum ferritin was found beyond 2000 ng/ml

Table 2 : Serum bilirubin , AST and ALT at different serum ferritin levels

S. Ferritin ng/ml (No)	Serum bilirubin	p value	AST	p value	ALT	p value
< 1000 (3)	1.067 ± 0.2517	>0.05	44.33 ± 4.041	>0.05	40.00 2.000	>0.05
1000-2000 (16)	1.663 ± 0.3557	<0.05	49.38 8.913	>0.05	47.61 4.666	>0.05
2000- 3000(12)	2.725 ± 0.4615	<0.05	61.50 9.357	< 0.05	55.94 8.760	< 0.05
>3000(9)	6.256 ± 1.371	< 0.0001	100.2 18.97	< 0.0001	90.22 23.25	< 0.05

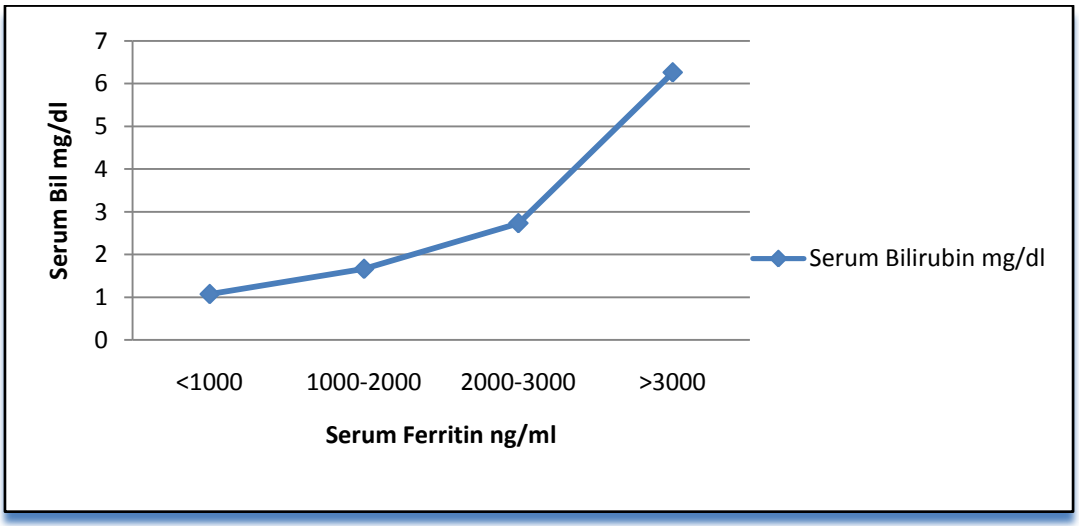

Figure 1: Trend of Serum bilirubin with rising Serum ferritin

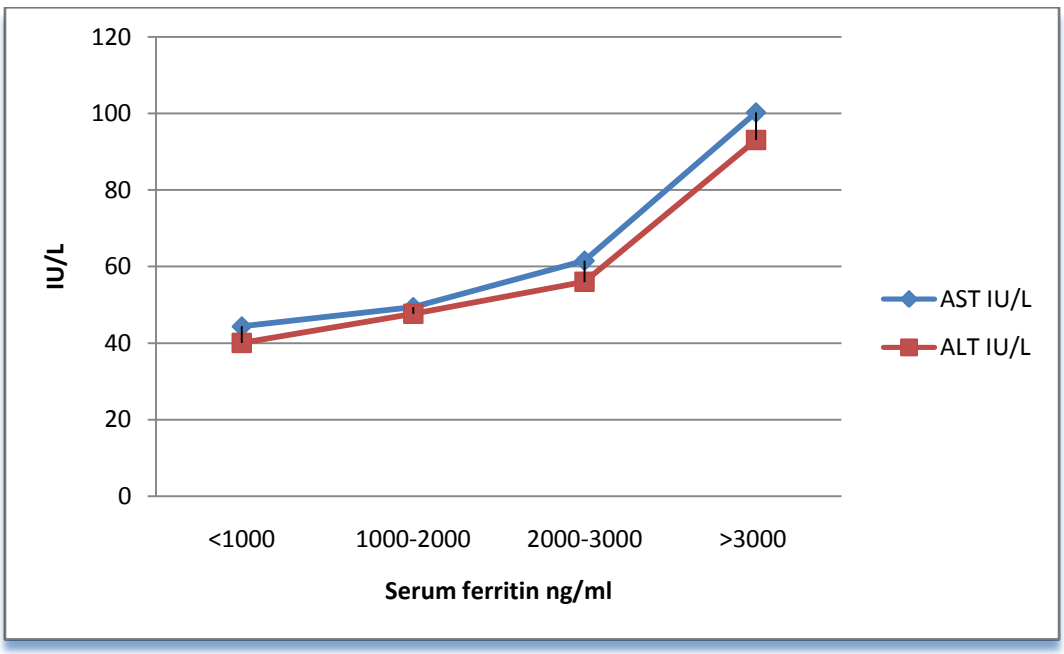

Figure 2: Trend of AST and ALT with rising Serum ferritin

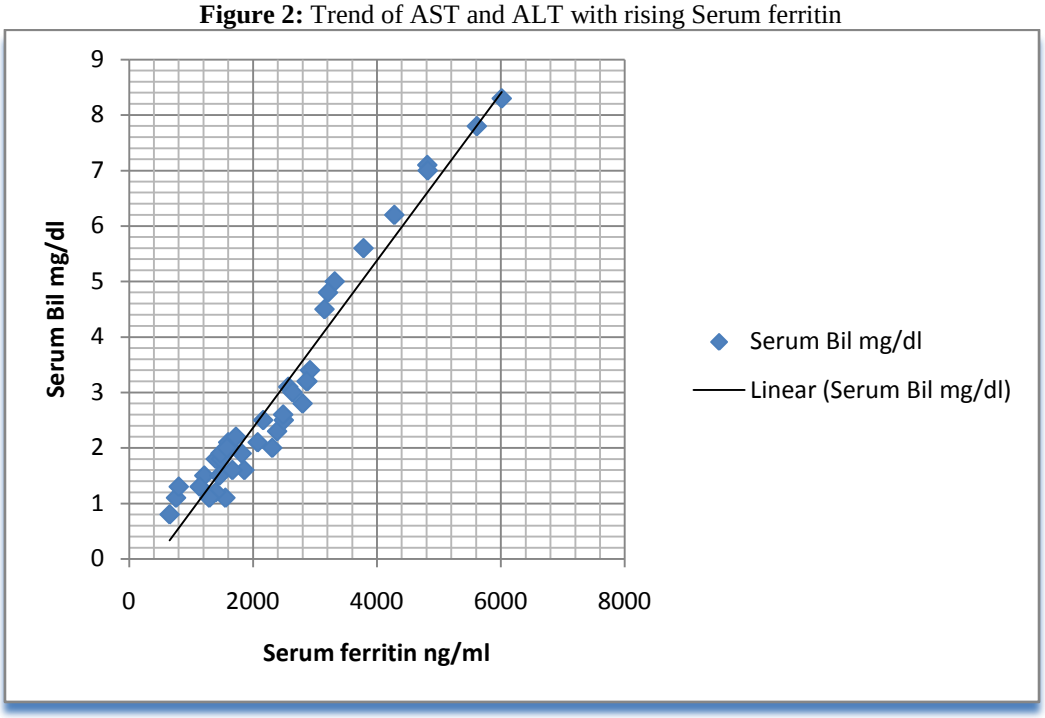

Figure 3: Correlation of Serum ferritin with Serum bilirubin

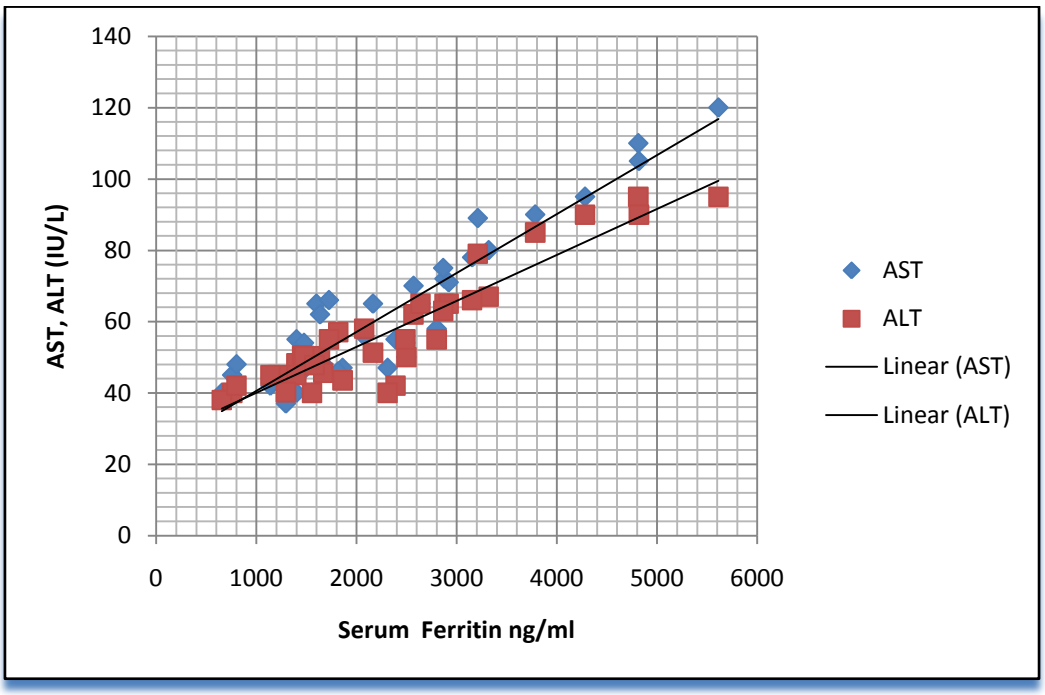

Figure 4: Correlation of Serum ferritin with AST and ALT

IV. Discussion

Among the multiple organs damaged in beta thalassaemia major , liver remains the earliest. The damage is a result of iron overload due to repeated blood transfusions and increased intestinal absorption. This leads to progressive lipid peroxidation and TGFbeta-1 expression causing fibrosis and cirrhosis in liver. This study was therefore conducted to know the hepatotoxic potential of iron overload on liver by measuring serum bilirubin , liver enzymes like AST and ALT. Our study showed significantly high (<0.05) serum bilirubin in patients compared to controls. Silmilar results were found by N.Sultana et al.[16] Serum bilirubin increases in beta thalassaemia major due to hemolysis of RBCs.

We also found raised AST and ALT in beta thalassaemia patients compared to controls.Our results are in agreement with Md. Bayejid Hosen et al[17] , Mansi et al[18], Sedigheh shams et al[19]and Awadallah et al.[20] ALT and ASTactivity are elevated in beta thalassemic patients due to the symptoms of liver damage .[21]

Chekir KA et al conducted a study on 56 thalassemic children and determined various metabolic parameters. The study suggested that in beta thalassemia first organ impaired was liver. Plasma thiobarbituric acid reactive substances (TBARS) were significantly raised leading to deranged liver functions.[22] Similar finding were seen by Ameli M et al by study conducted in Iran . In their study they found that mean serum ALT (Alanine aminotransferase) was significantly high in thalassemic children with high serum ferritin and high transfusion index.[23] Asharaf soliman et al observed during a study that some disturbances occur in liver functions in hepatitis negative thalassemia patients with iron overload. Use of Desferroxamine is associated with decrease in liver enzymes.[24]

Among the cases serum ferritin was measured and correlated with serum bilirubin ,AST and ALT . Majority of patients showed high serum ferritin levels despite of chelation therapy. A positive correlation was found between serum bilirubin and serum ferritin above 1000ng/ml (Pearson's correlation co-efficient r= + 0.53). In case of liver enzymes positive correlation was found for serum ferritin levels above 2000 ng/ml (Pearson's correlation co-efficient r= + 0.62). Our results are similar to Rameshwar et al [25] who found positive correlation of serum ferritin with AST and ALT above 2000 ng/ml of serum ferritin levels. Barton JC et alnoted similar results as serum ferritin increases liver enzymes also increases.[26] Limitations of our study are that we take into consideration the total number of blood transfusions and duration of chelation therapy in cases which would have given us more accurate correlative results. Also the sample size is small, so a further study with large sample size is required.

V. Conclusion

From present study it is concluded that β-thalassemics had hepatic dysfunctions in the form of raised serum bilirubin and abnormal liver enzymes. The effects are more pronounced when serum ferritin level crosses 2000ng/ml due to potential toxic effect of iron overload on hepatocytes. Proper management of thalassaemia patient can decrease morbidity and mortality and they can survive with a good quality of life up to third or fourth decade. Hence we recommend that liver functions should be carefully monitored in patients with transfusion dependent β-thalassemia major especially when serum ferritin levels are above 1000 ng/ml

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