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Original Article

Association of Iron Overload with Hepatic Function Abnormalities in Transfusion Dependent Beta-Thalassemia Major Children- Insights from a Thalassemia Center in Karachi

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Abstract

Objective: To determine iron overload induced liver function abnormalities in transfusion dependent beta thalassemia major patients and to analyze relationship between serum ferritin levels with liver enzymes.

Methodology: This cross-sectional study was conducted in Baqai Medical University Karachi and Muhammadi Thalassemia Center Karachi from December 2021 to May 2022. Total 100 transfusion dependent beta-thalassemia major patients were included. Lab investigations for serum ferritin and liver function tests were performed on Roche Cobas c111 and e411 analyzers.

Results: Serum ferritin, Bilirubin, ALT, and ALP levels from blood samples of 100 patients with ages ranging from 2 to 16 years were analysed. Females constituted 51 % of the samples. Ferritin levels were not significantly different between genders ($p=0.1429$). Patients were divided into two groups according to ferritin levels ($<2500\text{ng/mL}$) and ($>2500\text{ng/mL}$). Median(IQR) for ALT level of group 1 and 2 was 47.0 (34.0–67.0) and 164.0 (89.0–200.0) respectively, whereas median(IQR) ALP level for each group was 125.0 (92.5–161.5) and 200.0 (172.0–224.0) respectively. There was a significant, strong positive correlation of rise in ALT($r=0.675$) and ALP($r=0.677$) as ferritin levels rose ($>2500\text{ng/mL}$). Bilirubin levels were high in patients with ferritin levels ($<2500\text{ng/mL}$). There was a weak and non-significant correlation of rise in bilirubin levels, with iron overload ($>2500\text{ng/mL}$).

Conclusion: Elevated serum ferritin levels indicate iron overload induced derangement of the liver functions in transfusion dependent beta-thalassemia major children. There is a strong positive association between increased serum ferritin levels and increased liver enzymes levels.

Keywords: Beta Thalassemia Major, Iron Overload, Liver Function Tests, Serum Ferritin.

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Introduction

Beta thalassemia major is an inherited, autosomal recessive hemoglobinopathy, characterized by defective globin synthesis, resulting in ineffective erythropoiesis and causing severe, transfusion dependent anemia.¹ Globally, millions of individuals are affected by thalassemia, and approximately 1.5% of the world's population are carriers of beta thalassemia.² According to the available data from multiple studies, more cases of clinically severe thalassemia are expected to be diagnosed over the course of the next two decades.³ Unfortunately, Pakistan is among the countries with the highest incidence rate of thalassemia; an estimated 5,000 to 9,000 new cases are diagnosed annually, therefore, thalassemia is a major health concern in our country.¹

Patients with beta thalassemia major are usually

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diagnosed between the ages of six months to two years and typically present with severe hemolytic anemia, lethargy, fatigue, delayed developmental milestones, jaundice, failure to thrive, hepatosplenomegaly, and thalassemic facies (chipmunk facies).⁴ Therefore, in transfusion dependent beta thalassemia major individuals, frequent blood transfusions from early childhood are mandatory to avoid the complications of severe anemia, maintain adequate hemoglobin levels and to improve quality of life while prolonging survival.⁵ Although transfusion therapy enhances lifespan significantly, it also increases iron overload in vital organs.⁶ The body absorbs 200–250 mg of iron from each unit of transfused blood; however, humans lack an efficient physiological system to eliminate excess iron, which leads to gradual accumulation of iron in the liver, heart, endocrine glands, and other organs.⁷

The liver is the main organ in the human body for storing iron, and in patients with transfusion dependent beta thalassemia major, the adverse effects of iron overload can be monitored primarily in liver cells as it is one of the earliest and most severely affected organs.⁸

In transfusion dependent beta thalassemia major patients repeated blood transfusions, increased intestinal iron absorption from ineffective erythropoiesis, and decreased hepcidin levels lead to iron overload.⁹

Accumulation of iron in hepatocytes and Kupffer cells facilitates the generation of reactive oxygen species (ROS) through Fenton reaction.² This oxidative stress triggers necrosis and apoptosis of hepatocytes, which in turn activates hepatic stellate cells and deposition of collagen, leading to fibrosis and, in more severe cases, cirrhosis.¹⁰ All these pathological changes are reflected clinically by deranged liver function test results.¹¹ Additionally, in thalassemia major patients, hepatitis B and C can still be transmitted through repeated blood transfusions and may contribute to the destruction of the liver cells, although blood screening has greatly reduced the incidence of viral infections.¹² Nevertheless, excessive iron deposition alone in liver cells is sufficient to disrupt liver functions and is believed to be a primary contributor to hepatic morbidity in beta thalassemia major patients.¹³

Serum ferritin, an intracellular iron storage protein, is an essential indicator of total body iron stores; clinically it is used as a useful investigation for assessing the degree of iron overload and guiding the course of iron chelation therapy.^{14, 15} The levels of serum ferritin are usually markedly elevated in thalassemia major patients, often exceeding 1000 ng/mL.² Also ferritin is an acute-phase reactant, and in certain conditions, like inflammation, infections, metabolic syndromes and malignancies its level may be elevated.¹⁴ However, in clinical practice, it remains a practical and widely accessible tool to evaluate iron overload.¹⁵

A positive correlation between serum ferritin levels and liver iron concentration has been demonstrated in various studies. Along with the elevated levels of serum ferritin, the possibility and severity of liver cell destruction increase, manifesting as elevated levels of liver enzymes.^{16, 17} Specifically, increased levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) are highly indicative of liver damage.¹⁶ Moreover, the bilirubin levels are also important in thalassemia major patients for the evaluation of hemolysis, iron overload and to assess the status of hepatic functions. Hemolysis, complications of gallstones, and destruction of liver tissues because of

excessive iron deposition can lead to increased bilirubin levels.¹⁸ An elevated level of direct bilirubin is commonly seen in conditions leading to destruction of liver cells, while increased levels of indirect bilirubin usually indicate destruction of red blood cells.¹⁹ Therefore, in transfusion dependent beta thalassemia major patients the regular monitoring of serum ferritin levels and liver function test is essential and helpful for the early detection of irreversible liver cell damage, assessment of liver functions and adjustments in iron chelation therapy.^{20,21,22}

Hence, this research study aims to determine the association of iron overload with hepatic function abnormalities in transfusion dependent beta thalassemia major patients, as serum ferritin is a less invasive, cost effective and more affordable indicator of iron induced liver injury, as compared to liver biopsy or magnetic resonance imaging (MRI T2*) in our country Pakistan. Furthermore, due to the paucity of local research studies, the findings of this study will also help in establishing the local data for the betterment of thalassemia major patients and timely prevention of the complications.

Methodology

After approval from Baqai Medical University Ethics Committee and Board of Advance Studies and Research (BASR), this cross-sectional study was conducted in Baqai Medical University Karachi and Muhammadi Thalassemia Center Karachi. From the city of Karachi the study population of transfusion dependent beta thalassemia major patients was included; duration of study was 6 months from December 2021 to May 2022. The ethical approved was obtained from Baqai Medical University, Karachi Ref no BMU-EC/04-2021. After obtaining written and informed consent from the parents and guardians, 100 transfusion-dependent beta-thalassemia major patients who had received more than 20 units of red blood cell transfusions, of both genders and of age group 2 years to 16 years were selected for this research study.

Blood sample for serum ferritin and LFTs were collected. For serum ferritin Roche Cobas e411 analyzer was used and for liver function test Roche Cobas c111 analyzer was used. Data was analyzed on SPSS version 22. Frequencies and percentages were calculated for the categorical variables. The Shapiro-Wilk test was used to check the normality of data variables. Descriptive

statistics were used to calculate the median and IQR (interquartile range) of quantitative variables. Mann-Whitney U test was used to identify significant difference in ferritin levels between gender and statistical differences in the median ALT, ALP and bilirubin levels between the two study groups (group 1 = ferritin level <2500 and group 2 = ferritin level >2500). A p-value of <0.05 was considered statistically significant. Spearman's correlation coefficient was used to identify a significant correlation of rise in ALT, ALP and bilirubin levels with iron overload (<2500 and >2500), which also determined the strength and direction of the linear relationship between serum ferritin and LFT levels.

Results

Serum Ferritin, Bilirubin, ALT, and ALP levels from blood samples of 100 patients with ages ranging from 2 to 16 years were analysed. Females constituted 51 % of the samples. Ferritin levels (median 1950.50 (IQR: 1259.75–2934.00)) were not significantly different between genders (p= 0.1429). (Figure 1)

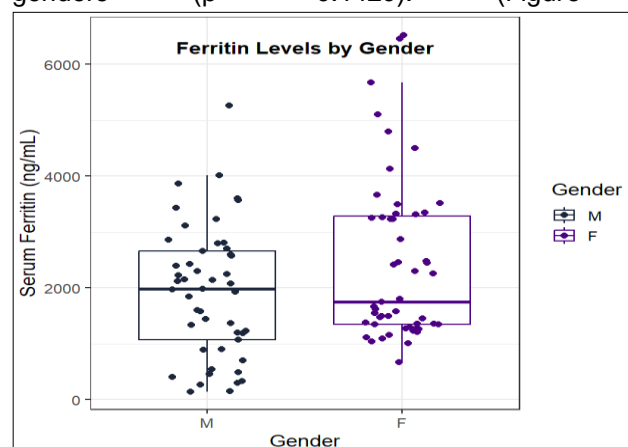


Figure 1: Mann–Whitney U test was performed to check if

there is a significant difference in ferritin levels between genders, $p < 0.05$ (considered significant). * No significant difference in ferritin levels between genders was observed as $p > 0.05$

Patients were grouped according to ferritin levels, with group 1 having ferritin (<2500 ng/mL, median 1365.00 (IQR: 1104.00–1950.50)) and group 2 having (>2500 ng/mL, median 3352.00 (IQR: 3120.00–4010.00)). Median (IQR) for ALT level of group 1 and 2 was 47.0 (34.0–67.0) and 164.0 (89.0–200.0) respectively, whereas median (IQR) ALP level for each group was 125.0 (92.5–161.5) and 200.0 (172.0–224.0) respectively. (Table I)

There was a significant, strong positive correlation of rise in ALT (Spearman $r = 0.675$) as well as ALP (Spearman $r = 0.677$) as ferritin levels rose >2500 ng/mL. (Table II and Figure 2). Bilirubin levels were high (Direct Bilirubin = 89.6%, Total Bilirubin = 49.3% and Indirect Bilirubin = 23.9%) in patients with ferritin levels < 2500 ng/mL as compared to those with ferritin levels > 2500 ng/mL (Direct Bilirubin = 78.8%, Total Bilirubin = 21.2% and Indirect Bilirubin = 3%) shown in (Figure 3). There was a weak and non-significant correlation of rise in bilirubin levels, Total Bilirubin (Spearman $r = -0.049$, $p = 0.787$), Direct Bilirubin (Spearman $r = 0.205$, $p = 0.254$), and Indirect Bilirubin (Spearman $r = -0.342$, $p = 0.052$) with iron overload >2500 ng/mL. (Table 2 and Figure 4-6).

Discussion

In beta thalassemia major patients, life expectancy has been significantly increased, and quality of life has been enhanced with blood transfusions by reducing the risks and complications of anemia.^{5, 6} However, repeated blood transfusions lead to the accumulation of iron in various body organs, particularly in vital organs, and despite of

Table 1: Descriptive statistics of all variables included in the study (n=100) and according to ferritin level groups for each variable

Variables	All patients (n=100) Median (IQR)	Group1: Ferritin <2500 (n=67), (M=34), (F=33), Median (IQR)	Group2: Ferritin >2500 (n=33), (M=15), (F=18), Median (IQR)	P- value
Age	8.0 (6.0–9.0)	8.0 (6.0–10.0)	7.0 (6.0–8.0)	
ALP	152.5 (100.8–199.3)	125.0 (92.5–161.5)	200.0 (172.0–224.0)	6.684e-07 *
ALT	67.0 (40.0–121.3)	47.0 (34.0–67.0)	164.0 (89.0–200.0)	1.945e-12*
Ferritin	1950.50 (1259.8–2934.0)	1365.00 (1104.00–1950.50)	3352.00 (3120.00–4010.00)	
T. Bil	1.09 (0.86–1.46)	1.19 (0.94–1.64)	0.97 (0.78–1.14)	0.0034**
D. Bil	0.47 (0.35–0.61)	0.49 (0.36–0.64)	0.41 (0.33–0.54)	0.1263
In. Bil	0.61 (0.45–0.90)	0.72 (0.48–0.99)	0.49 (0.36–0.69)	0.0015**

Median (IQR: Q1-Q3 = 25th-75th percentile) was calculated for non-normally distributed variables. ALT and ALP levels were further analysed by Mann–Whitney U test, $p < 0.05$ (considered significant). * ALT and ALP levels were significantly higher in group 2 as compared to group 1. **A significant difference in median bilirubin levels between the two groups was observed. M=male, F=female, ALP= alkaline phosphatase and ALT= alanine aminotransferase. T. Bil = total bilirubin, D. Bil= Direct bilirubin and In. Bil= indirect bilirubin

iron chelating therapy in beta thalassemia major patients, iron overload and its associated repercussions still need major focus and attention.⁶

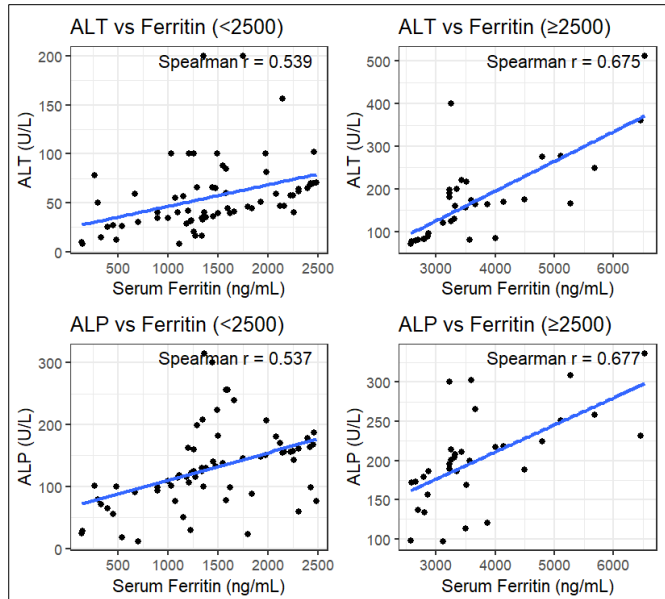


Figure 2: Shows that ALT and ALP levels rise with ferritin overload and there is significant positive correlation. Spearman correlation coefficient r for ALT and ALP shows strong positive correlation with ferritin levels >2500 .

Table II: Spearman correlation coefficient to measure the association between the rise in LFT levels and the increase in ferritin levels in Group 1 vs Group 2 ($n=100$)

Group 1 <2500	Enzyme	Spearman coefficient r	P-value
	ALT	0.5393860*	2.480639e-06*
	ALP	0.5366233*	2.855265e-06*
	Total Bilirubin	0.29 ^d	0.017 ^d
	Direct Bilirubin	0.192	0.12
	Indirect Bilirubin	0.232	0.059
Group 2 ≥2500	Enzyme	Spearman coefficient r	P-value
	ALT	0.6750230**	1.640241e-05**
	ALP	0.6767508**	1.531015e-05**
	Total Bilirubin	-0.049 ^a	0.787 ^a
	Direct Bilirubin	0.205 ^b	0.254 ^b
	Indirect Bilirubin	-0.342 ^a	0.052 ^a

The p-value was considered significant if <0.05 . * Significant moderate positive correlation. **Significant strong positive correlation. ^a Not statistically significant weak/moderate negative correlation. ^b non-significant weak positive correlation. ^d Significant weak positive correlation

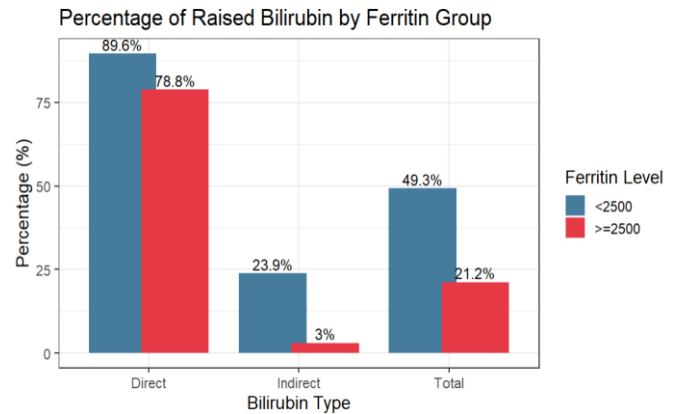


Figure:3a Percentage of patients having raised bilirubin levels between two groups.

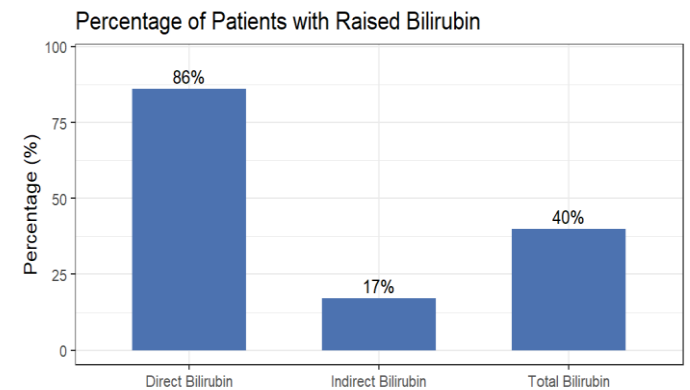


Figure:3b Percentage of patients having raised bilirubin levels.

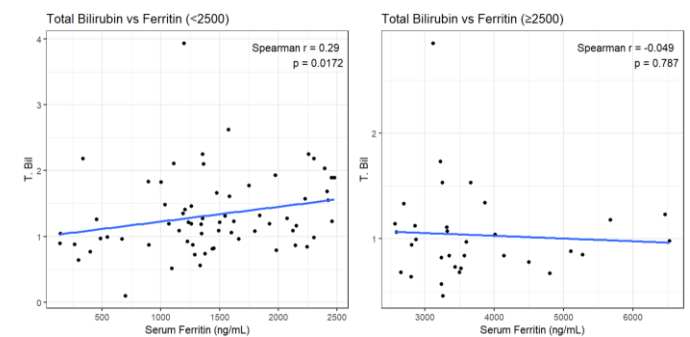


Figure 4: Shows the Spearman correlation coefficient of Total bilirubin vs ferritin levels. Total bilirubin shows a significant weak positive correlation with Ferritin levels <2500 but a weak negative correlation with Ferritin levels >2500 ng/ml, which was not significant. A rise in total bilirubin levels was not associated with iron overload

In our study there was a strong positive correlation between elevated levels of ALT ($r=0.675$) and ALP ($r=0.677$) with increase serum ferritin levels greater than 2500 ng/mL. These results are

supporting the clinical significance of serum ferritin as a reliable marker for assessing the liver damage. The bilirubin levels were raised in the patients with serum ferritin levels less than 2500 ng/mL, reflecting increased hemolysis and relatively preserved liver functions, as compared to the patients with serum ferritin levels more than 2500 ng/mL, indicating significant iron deposition in the liver cells leading to impaired bilirubin metabolism and a decrease in hemolysis due to frequent blood transfusions and chelation therapy.

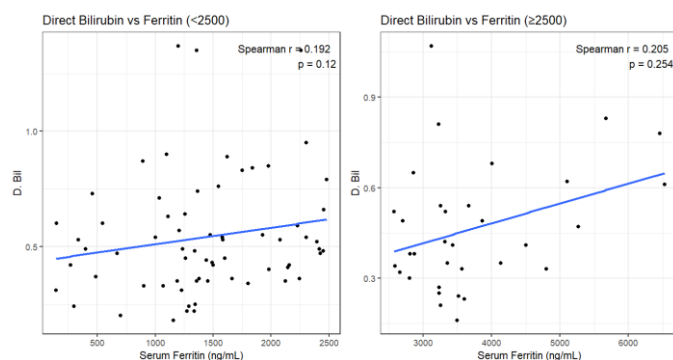


Figure 5: Shows the Spearman correlation coefficient of Direct bilirubin vs ferritin levels. Direct bilirubin shows a non-significant weak positive correlation with Ferritin levels <2500 but a weak negative correlation with Ferritin levels >2500 ng/ml, which was not significant. A rise in direct bilirubin levels in patients was not associated with iron overload.

In contrast to our study, Akbar M. et al (2021) found no association between serum ferritin and liver function tests.²³ This discrepancy may be attributed to difference in sample size; only 30 patients were included in this research. Also the serum bilirubin levels were not analyzed in this study.

Results of various international as well as national research studies are supporting the findings of our present study, highlighting the significance of regular monitoring and a dire need for timely analysis of the serum ferritin levels and liver function tests for early identification of liver dysfunction and to prevent the drastic complications of iron overload in thalassemia major patients.

Faiq AB et al. (2022) determined serum ferritin as a crucial marker in beta thalassemia major patients to

detect derangement of liver enzymes as a result of an adverse effect of iron overload in the liver, as in this research an excellent correlation between serum ferritin and liver enzymes was established. Moreover, they identified serum bilirubin and alkaline phosphatase as beneficial indicators for assessing the hepatic dysfunction and cholestasis.¹⁸ Banafa AM et al. (2022) also reported an evident correlation between serum ferritin levels and liver enzymes ALT and AST.²⁴ Kubran LA et al. (2023) reported serum ferritin as a strong predictor of liver cell injury in transfusion dependent beta thalassemia major patients, and established that serum ferritin is helpful in indicating the different degrees of iron overload in liver. Therefore, to avoid the consequences of iron overload and to start iron chelating therapy at an appropriate time, serum ferritin levels can play an important role.¹⁷ In contrast to our study, serum bilirubin level was not evaluated in this research. Also they compared serum ferritin with T2*-MR, but in our study, because of limited financial resources and budget, we were not able to conduct T2*-MRI in our patients to examine the effects of iron overload in liver tissues. Saravani K et al. (2024) concluded serum ferritin as an effective marker to identify liver damage in beta thalassemia major patients receiving repeated blood transfusions.²⁵

The findings of our study are almost similar to the results of the study conducted by Rabadiya SM et al. (2024) who reported that there is an association of iron overload with liver cell dysfunction and destruction of liver cells in transfusion dependent beta thalassemia major patients can be predicted by evaluating serum ferritin levels and LFTs, and to prevent the liver functions a proper monitoring on iron overload is mandatory.¹⁶ In contrast to our study serum bilirubin levels were not analyzed in this study. Aligning with our present study, by investigating

serum ferritin, liver enzymes, and serum bilirubin, Faruqi A et al. (2024) established that excessive iron deposition in thalassemia major patients leads to impaired liver functions, and there is an association of increased serum ferritin levels with increased AST and ALT levels. Also, an increased serum ferritin level has a weak correlation with serum bilirubin.¹⁹ These findings are similar to our study and emphasizing on regular follow up of beta thalassemia major patients and vigilant evaluation of baseline investigations to prevent serve complications of iron

overload. Similarly, Ayulinda CN et al. (2025) concluded that serum ferritin is a useful tool to measure the liver injury as there is an association of increase level of serum ferritin with liver enzymes in the beta thalassemia major children.²⁰ Ikram AB et al and Akter A et al. (2025) also reported the positive correlation of serum ferritin with liver enzymes and highlighted the importance of monitoring the serum ferritin levels and liver function tests for the early detection of hepatic dysfunction.^{21,22}

Study Limitations: The main restriction was limitation of time and the physical movement from one place to another as this study was conducted during COVID-19 pandemic. Also because of the limited budget and constrain financial resources we were not able to perform T2* MRI to analyze iron deposition in liver tissues.

Conclusion

There is an association of increased levels of serum ferritin with elevated levels of liver enzymes (ALT and ALP). Serum ferritin is a reliable marker for screening and monitoring the complications of iron overload in the liver. Moreover, elevated serum bilirubin levels are indicative of the breakdown of red blood cells and, along with serum ferritin and liver function tests, can predict the status of liver cells. Regular follow-up and timely monitoring of baseline investigations are essential in transfusion dependent beta thalassemia major patients to avoid adverse effects of iron overload.

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