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MONITORING IRON TOXICITY-MEDIATED ORGAN DAMAGE IN β-THALASSEMIA MAJOR PATIENTS: ROLE OF HEPCIDIN AND FERRITIN

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ABSTRACT

Post-transfusion iron toxicity in β-thalassemia major can lead to organ damage, which may include injury to the liver and kidneys. Monitoring the iron levels through hepcidin is crucial for patients due to thalassemia. This study was aimed to assess correlation between serum ferritin and hepcidin, and evaluate their role in tracking iron availability following frequent transfusions in these patients. Thirty subjects (19 males and 11 females) suffering β-thalassemia were chosen at Brainware Diagnostic & Research Center, Kolkata, West Bengal (India) against 15 normal individuals. The medical records of patients were examined by using standard criteria. Hematological parameters were recorded, and biochemical assessment was done for serum ferritin, hepcidin, TIBC, LFT, and RFT. The results showed lower RBC indices and notably higher serum ferritin and hepcidin levels in thalassemic patients than in controls. ALP, AST, and ALT levels in patients were 79.03 ± 10.82, 67.12 ± 11.13, and 253.18 ± 22.01 IU L^{-1}, respectively. Significantly ($p<0.001$) higher levels of creatinine were found in β-thalassemia patients than in control. The study revealed a positive correlation between ferritin and hepcidin as well as LFT parameters. Hepcidin was found to be positively correlated with creatinine. Hence, assessing hepcidin and ferritin concentrations is an important marker for identifying a heightened risk for renal and hepatic toxicity in patients diagnosed with β-thalassemia major.

Keywords: β-Thalassemia, blood transfusion, ferritin, hepcidin, iron toxicity, kidney function, liver function

INTRODUCTION

Thalassemia is one of the most prevalent inherited single-gene disorders in the world. Each year, approximately 1 lakh children suffering from thalassemia major are born globally, with an estimated 66,000 individuals currently affected by β-thalassemia major in India (Bhalodiya *et al.,* 2023). In southern India, the carrier rate for the β-thalassemia gene is observed to be between 1 and 3%, whereas in northern India it ranges from 5 to 15% (Madan *et al.,* 2010). Due to an increase in β-thalassemia patients in West Bengal state, the state government initiated the State Thalassemia Control Programme (STCP) in 2008, which is active in thalassemia prevention (Maji *et al.,* 2020).

In individuals with β-thalassemia major, the marked reduction of β-globin results in an excessive build-up of α-globin chains within erythroid precursors (Bou-Fakhredin *et al.*, 2022). This imbalance creates a condition referred to as 'ineffective erythropoiesis', wherein erythroid cells undergo premature apoptosis, ultimately leading to chronic haemolytic anaemia, which requires persistent blood transfusions (Basu *et al.,* 2023). Reduced hepcidin levels in human cause iron-overload by decreasing hepcidin and simultaneously increasing ferroportin content, thus boosting the iron export from cells (Yadav *et al.,* 2022). Hepcidin, a 25-amino acid peptide produced by hepatocytes, acts as

the key hormone controlling iron balance between erythropoiesis and iron reserves (Kaddah *et al.*, 2017). As hepcidin levels become heightened, iron persists in its intracellular storage form, associated with the ferritin molecule. Hence, ferritin and hepcidin are the crosstalk for iron homeostasis (Nemeth and Ganz, 2021).

The sustained blood transfusion is the primary treatment choice for β-thalassemic patients (Panigrahi and Agarwal, 2007). Regular blood transfusions can cause iron build-up in the body, leading to severe complications such as liver, kidney, heart, and endocrine issues (Soliman *et al.*, 2014). Liver is crucial for the storage of ferritin and hepcidin, however, free iron in the liver can lead to free radical formation, causing lipid peroxidation and liver damage (Zheng *et al.*, 2023). Reports reveal that a relationship exists between serum ferritin levels and hepcidin in numerous thalassemia patients who received multiple blood transfusions (Wahidiyat *et al.*, 2018). However, scanty information is available about the association between iron-overload and impairment of liver and kidney function in thalassemia patients (Alshami and Alzomor, 2025), and the link between hepcidin and iron overload among thalassemia patients is still a matter of controversy. So, targeting hepcidin therapeutically may assist in managing the iron overload in patients with β-thalassemia. Therefore, this study aimed to explore the relationship between liver and kidney function with ferritin and hepcidin levels so as to monitor the iron overload in β-thalassemia major patients who have undergone multiple transfusions.

MATERIALS AND METHODS

Selection of subjects

Patients with β-thalassemia major were selected from Paschim Medinipur, West Bengal (India). The blood samples were collected and analysed in the Brainware Diagnostic & Research Center (BDRC), Barasat, Kolkata, West Bengal (India). Informal consent was obtained from each patient, and a comprehensive clinical history was compiled by a registered pathologist. Thirty subjects were selected, who included 19 males and 11 females, who had received frequent blood transfusions. A control group comprising of 15 healthy individuals (non-β-thalassemic and of the same age and sex) was included in this study.

Research cohort and data origin

A cross-sectional analysis was conducted between March 2024 to February 2025 to assess the effects of iron overload on liver and kidney functions, as well as to explore their relationship with ferritin and hepcidin in patients diagnosed with β-thalassemia major. Patient information, encompassing demographic details, lifestyle choices (such as smoking and alcohol consumption), medical history, medication, duration, history of blood transfusion, and all biochemical test results, was collected from the designated medical records. The medical records were reviewed by two certified pathologists. Blood samples were obtained as per the guidelines of the World Medical Association's Code of Ethics. All procedures adhered to the applicable laws and institutional regulations, and received prior approval from the Institutional Ethics Committee at BDRC, Barasat, Kolkata, India vide Memo No. Inst/IEC/2024/003.

The inclusion criteria considered during selection of subjects were: i) the patients diagnosed with β-thalassemia through high-performance liquid chromatography (HPLC) or electrophoresis, ii) the individuals with β-thalassemia classified as major, minor, or intermedia, regardless of gender; iii) regular blood transfusions, with or without chelating therapy, iv.) paediatric patients, and the patients aged 1-16 years with β-thalassemia major who had no communicable or non-communicable diseases. The individuals with various haemoglobin disorders, including sickle cell anaemia, were excluded from the study. This also applies to those diagnosed with type 1 diabetes mellitus, patients undergoing diuretic treatment, individuals taking anti-epileptic medications, and those suffering from primary

renal disease, as well as liver, renal, endocrine, or cardiac conditions, along with any communicable or non-communicable diseases.

Parameters studied

Blood samples (5 mL) were collected in a clot vial from each subject, and serum was separated following a 20 min clotting period, after which the specified parameters were examined. The haematological parameters such as haemoglobin (Hb; g dL^{-1}), mean corpuscular volume (MCV, Fl), mean corpuscular haemoglobin (MCH, pg $cell^{-1}$), and mean corpuscular haemoglobin count (MCHC, g dL^{-1}) were recorded using a semi-Auto Analyzer (Erba 5 Part Haematology Analyzer-H560). Serum ferritin and hepcidin were measured by the enzyme-linked immunosorbent assay (ELISA) method (Jyothi *et al.*, 2023). Also, biochemical assessments were made for fasting serum iron concentration, total iron-binding capacity (TIBC), liver function parameters (including bilirubin, serum glutamic-oxaloacetic transaminase (SGOT) (Erba SGOT kit, Transasia Bio-Medicals, Cat No. 120204), serum glutamic pyruvate transaminase (SGPT) (Erba SGPT kit, Transasia Bio-Medicals, Cat No. 120207), alkaline phosphatase (ALP) (Erba alkaline phosphatase kit, Transasia Bio-Medicals, Cat No. 120191), and renal function indicators (such as uric acid (Erba uric acid DES kit, Transasia Bio-Medicals, Cat No. 120216), urea (Erba urea BUN kit, Transasia Bio-Medicals, Cat No. 120214), and creatinine (Erba creatinine kit, Transasia Bio-Medicals, Cat No. 120246) were measured.

Statistical analysis

The statistical significance of the data generated and the comparison between control and β-thalassemia groups were performed using an unpaired two-tailed t-test using Origin 8 (OriginLab, Northampton, USA). Correlation analyses were performed using Pearson's correlation coefficient. Data were presented as means ± standard deviation (SD), and statistical significance was considered at $p < 0.05$.

RESULTS AND DISCUSSION

In this study, the mean age for thalassemic patients was (9.63 ± 2.79 years), and the mean age for control subjects was (10 ± 4.72 years).

Haematological analysis

The red blood cell indices of β-thalassemic patients showed a significant decrease as compared to the control (Table 1). The serum ferritin level of β-thalassemic patients significantly ($P < 0.001$) increased to 2149.93 ± 540.8 μg L^{-1} as compared to the control (63.97 ± 12.51 μg L^{-1}). Conversely, in the control group, the concentration of serum hepcidin was 6.6 ng mL^{-1}, which was significantly ($p < 0.001$) higher (18.28 ng mL^{-1}) in β-thalassemic major patients. Further, significantly higher levels of serum iron and lower levels of TIBC were observed in the patients with β-thalassemic major.

Table 1: Hematological analysis of control subjects and β-thalassemic major patients

Haematological parameters	Control (n = 15)	β-thalassemic major patients (n = 30)	p-value
Hb (g dL^{-1})	12.65 ± 0.84	7.68 ± 1.60	0.020
MCH (pg $cell^{-1}$)	28.98 ± 1.40	22.02 ± 1.58	0.029
MCHC (g dL^{-1})	32.34 ± 3.21	24. 13 ± 2.29	0.002
Ferritin (μg L^{-1})	63.97 ± 12.51	2149.93 ± 540.8	0.001
Hepcidin (ng mL^{-1})	6.60 ± 4.28	18.28 ± 2.33	0.001
TIBC (μg dL^{-1})	286.24 ± 36.18	234.50 ± 58.39	0.003
Serum iron (μg dL)	122.48 ± 12.66	272.82 ±158.42	0.001

The values are mean ± SD; the lowest statistical significance was considered at $p < 0.05$.
Hb = Haemoglobin; MCH = Mean corpuscular haemoglobin; MCMC = Mean corpuscular haemoglobin count; TIBS = Total iron- binding capacity.

It is not well understood that individuals with β-thalassemia major are susceptible to metabolic issues that can lead to dysfunction in multiple organs. β-thalassemia is recognized for its association with anaemia and iron overload, with organ failure being the primary contributor to the mortality and morbidity due to iron deposition (Pinto and Forni, 2021). The elevated level of serum ferritin observed in the present study may be associated with transfusion-related iron overload, which may have induced the hepcidin levels, thus resulting in the suppression of erythropoiesis. However, higher hepcidin levels promote intracellular iron sequestration and lead to ferritin accumulation. The concomitant elevation of serum iron and decreased TIBC demonstrates transferrin saturation and iron transport impairment. This observation suggested that patients with β-thalassemia major suffer from profound iron imbalance, which contributes to systemic iron toxicity.

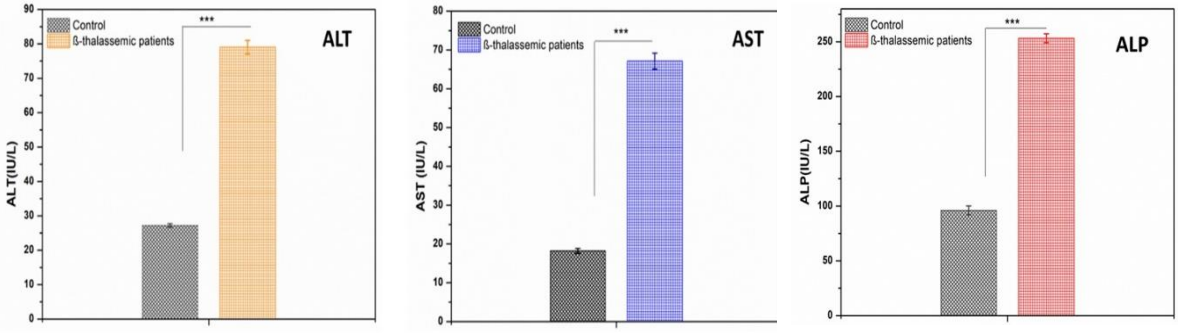

Fig. 1: Serum alanine transaminase (ALT), aspartate transaminase (AST) and serum alkaline phosphatase (ALP) levels of control and β-thalassemic patients. Results are expressed as mean ± SD; ***p <0.001

Liver function test (LFT) analysis

The comparison of LFT of β-thalassemic patients with the control group revealed that the serum activities of liver enzymes like alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were significantly ($p < 0.001$) higher in β-thalassemic patients than in control subjects. The values of AST, ALT, and ALP were 79.03 ± 10.82, 67.12 ± 11.13, and 253.18 ± 22.01 IU L^{-1}, respectively, in β-thalassemic patients (Fig. 1 & 2). The correlation analysis using Pearson's coefficient of ferritin and LFT parameters (ALT, AST, and ALP) depicted a positive correlation between ferritin and LFT parameters (Table 2). This indicated chronic liver dysfunction with hepatocellular injury, iron accumulation, leading to cirrhosis and hepatoma (Nemeth, 2020). Elevated ferritin and hepcidin are linked to cirrhosis in β-thalassemia (Kaddah *et al.*, 2017). Transfusion-related liver issues may arise from infections or hepatic siderosis, contributing to liver disease oxidatively.

Table 2: Correlation between ferritin and RFT and LFT parameters in β-thalassemic major patients

Ferritin with	r value	F-value	p value
Urea	– 0.12872	0.4549	≤ 0.505
Uric acid	– 0.21768	1.34297	≤ 0.256
Creatinine	– 0.07524	0.15372	≤ 0.698
ALT	+ 0.11957	0.39162	≤ 0.546
AST	+ 0.31875	3.05358	≤ 0.091
ALP	+ 0.52648	0.87298	≤ 0.769

ALT = Serum alanine transaminase, AST = aspartate transaminase; ALP = serum alkaline phosphatase

Table 3: Renal function test (RFT) analysis of control subjects and β-thalassemic major patients

Renal function test (RFT) parameters	Control (n = 15)	β-thalassemic patients (n = 30)	p-value
Urea (mg dL^{-1})	14.22 ± 2.28	24.12 ± 3.68	0.002
Uric acid (mg dL^{-1})	2.81 ± 0.65	5.68 ± 1.18	0.028
Creatinine (mg dL^{-1})	0.65 ± 0.15	0.82 ± 0.13	0.002

Results are expressed as mean ± SE; the lowest statistical significance was considered at $p < 0.05$

Renal function test (RFT) analysis

The comparisons of RFT of β-thalassemic patients with the control group showed that urea, uric acid,

and creatinine levels in β-thalassemic patients were significantly higher ($p < 0.001$) than those of the control group. The urea, uric acid, and creatinine levels were 24.12 ± 0.68, 5.68 ± 1.18, and 0.82 ± 0.025 (mg dL^{-1}), respectively, in β-thalassemic patients (Table 2). The degree of anemia, accumulation of iron, and frequency of blood transfusions contribute to the tubular and glomerular dysfunction in individuals suffering from thalassemia (Sadeghi *et al.*, 2021). Bhowad *et al.* (2022) have identified the signs of renal tubular dysfunction in individuals with thalassemia. Thalassemia enhances creatinine clearance and glomerular filtration rate, while anaemia reduces systemic vascular resistance, leading to hyperdynamic circulation, which elevates renal plasma flow and GFR (Darabont *et al.*, 2023). These changes can cause damage to the glomerular capillary wall and impact endothelial and epithelial cells. Further, higher urea and creatinine levels are observed in thalassemia patients as compared to the uric acid levels.

Correlation study

Correlation analysis, using Pearson's coefficient, of ferritin and RFT parameters (urea, uric acid, and creatinine) indicated a negative correlation between ferritin and RFT parameters (Table 2; Fig. 3) in β-thalassemic patients. Conversely, a strong positive correlation of hepcidin was found with ferritin and creatinine, but hepcidin showed a negative correlation with TIBC in β-thalassemic major patients (Table 4, Fig. 3). Several studies have proposed that reduced serum hepcidin levels in patients with β-thalassemia major may result in heightened iron absorption and subsequent iron overload (Jabeen *et al.*, 2025). The disparity in hepcidin levels and iron overload may probably be attributed to the transfusion therapy, which hinders erythropoiesis and raises body iron levels, leading to the increased hepcidin levels. However, high hepcidin has a direct impact on renal function as it induces creatinine levels (Pasricha *et al.*, 2013). Hence, hepcidin is considered a suitable marker for the detection of hepatic and renal impairment due to iron overload in patients with β-thalassemia major.

Table 4: Correlation of hepcidin with ferritin, TIBC and creatinine in β-thalassemic major patients

Hepcidin with	r value	F-value	p value
Ferritin	+ 0.70450	27.59024	≤ 1.390
Creatinine	+ 0.81737	54.35016	≤ 6.254
TIBC	– 0.66180	21.04757	≤ 0.41724

TIBS = Total iron-binding capacity

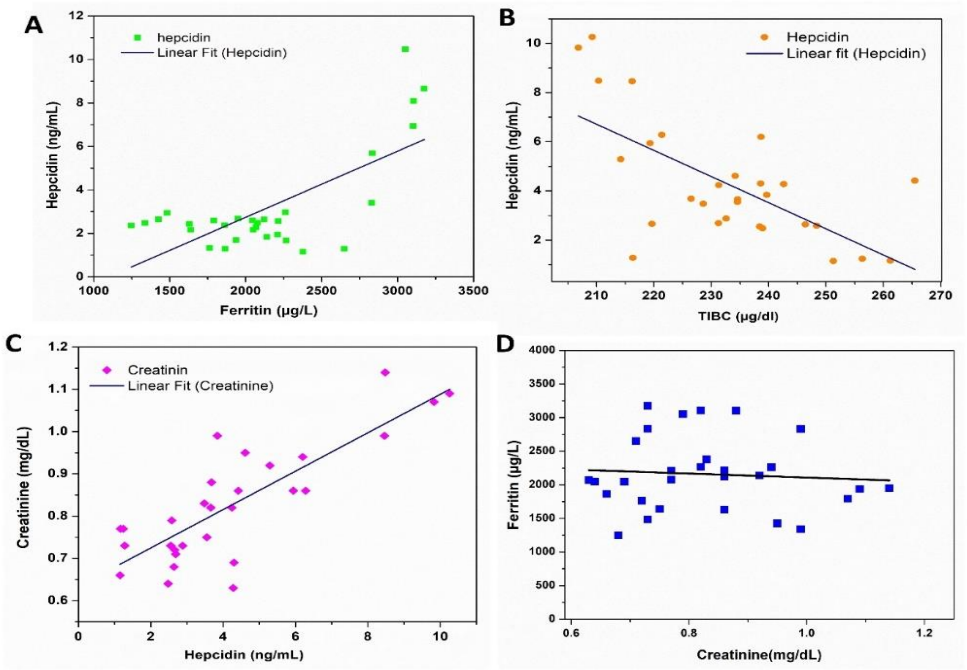

Fig. 3: Linear correlation of hepcidin with ferritin (A), total iron binding capacity (TIBC) (B), and creatinine (C), and linear correlation between ferritin and creatinine (D)

Conclusion: This study reveals compromised renal and liver functions in β-thalassemia patients due to iron overload. Early identification of high-risk patients allows targeted interventions to slow kidney and liver damage, thereby reducing mortality. Hepcidin levels indicate elevated iron toxicity, crucial for assessing iron availability in organs. We found a direct relationship between hepcidin and ferritin with AST, ALT, and creatinine, which suggests increased risks of hepatic and renal complications in β-thalassemia major patients who received frequent blood transfusions. Monitoring hepcidin and ferritin can help in early detection and timely prevention of iron overload. Nevertheless, appropriate and timely monitoring of hepcidin and ferritin can aid in early detection and prevention of iron overload. A more comprehensive diagnostic strategy must assess the evaluation of hepcidin and its fundamental mechanism.

Conflict of interest: The authors declare that they have no conflict of interest in the work presented.

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Ethical statement: Informed consent was obtained from each patient prior to the study, and a comprehensive clinical history was compiled by a registered pathologist. All the procedures were performed in compliance with the relevant laws and institutional guidelines and were approved by the Institutional Ethics Committee, Brainware Diagnostic & Research Center, Barasat, Kolkata, West Bengal (India) vide No. Inst/IEC/2024/003.

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