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HAEMATOLOGICAL (CBC) AND HEPATOLOGICAL (LFT) STATUS OF BETA THALASSEMIA MAJOR PATIENTS IN DHQ HOSPITAL, MIRPUR, AZAD JAMMU AND KASHMIR: COMPARATIVE ANALYSIS

Mobeen Hameed Rana[*1], Aamna Hanif[2], Sobia Nazir[3], Sumen Arouj[4], M. Sharjeel Abid[5], Shamail Hussnain[6], Kashif Ali[7].

[*1,2,3,4,5,6,7]*Department of Allied Health Sciences (Medical Lab Technology), Faculty of Health and Medical Sciences, Mirpur University of Science and Technology (MUST), Mirpur, Azad Jammu and Kashmir, Pakistan*

[*1]mobeenhameedrana@gmail.com

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Mobeen Hameed Rana

Abstract

Background: *β-thalassemia major (β-TM) is an inherited disorder of β-globin synthesis that causes severe anemia and requires lifelong red cell transfusion. Repeated transfusion leads to iron overload that damages the liver and other organs. Pakistan carries one of the highest burdens of β-TM worldwide, yet no study has described the laboratory profile of patients in Mirpur, Azad Jammu and Kashmir (AJK).*

Objective: *To compare the hematological (complete blood count, CBC) and Hepatological (liver function test, LFT) profiles of transfusion-dependent β-TM patients with those of age- and sex-matched healthy individuals.*

Methods: *This comparative cross-sectional study enrolled 50 previously diagnosed β-TM patients (25 male, 25 females; age 1–26 years; hepatitis B and C negative) attending the Thalassemia Department of the District Headquarters (DHQ) Hospital, Mirpur, and 50 healthy controls matched for age and sex. CBC was measured on an automated three-part hematology analyzer (Mindray BC-3000 Plus), and alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total serum bilirubin (TSB) on a semiautomated biochemistry analyzer (Microlab 300). Groups were compared using Welch's two-sample t-test ($p < 0.05$).*

Results: *Most participants (58%) were aged 1–9 years. Compared with controls, patients had significantly lower hemoglobin (7.30 ± 1.64 vs 13.29 ± 1.08 g/dL), red cell count (3.15 ± 0.76 vs 4.91 ± 0.39 ×10⁶/μL), hematocrit (23.94 ± 5.49 vs 39.29 ± 2.93%), MCV (76.33 ± 3.81 vs 80.75 ± 3.42 fL) and MCH (26.89 ± 2.15 vs 28.91 ± 1.50 pg) (all $p < 0.001$), and significantly higher WBC (11.18 ± 7.98 vs 7.45 ± 1.89 ×10³/μL; $p = 0.002$), platelets (392.06 ± 169.57 vs 263.58 ± 78.93 ×10³/μL; $p < 0.001$) and*

MCHC (34.78 ± 0.84 vs 33.77 ± 0.90 g/dL; p < 0.001). All liver parameters were raised in patients: TSB: 1.91 ± 1.22 vs 0.71 ± 0.09 mg/dL, ALT: 72.54 ± 55.52 vs 27.78 ± 8.67 U/L and ALP: 380.18 ± 136.51 vs 150.66 ± 42.38 U/L (all p < 0.001). The same pattern was present in both sexes.

Conclusion: *Transfusion-dependent β-TM patients in Mirpur show marked anemia despite regular transfusion, together with biochemical evidence of hepatic stress. Routine monitoring of CBC and LFTs, alongside iron status assessment, should be an integral part of their care, and community awareness and carrier screening should be strengthened.*

INTRODUCTION

Thalassemia is a group of autosomal recessive disorder caused by mutations or deletions in the globin genes that reduce or abolish the synthesis of the α- or β-globin chains of hemoglobin (Hb) (Al-Abadi, Lahmood, & Abbas, 2023). β-thalassemia was first described clinically by Cooley and Lee in 1925 (Hashim, Abdullah, & Ibadi, 2020), and the name derives from the Greek thalassa, "the sea", reflecting its early recognition in Mediterranean populations (Kumar, 2019). The β-globin gene is located on chromosome 11 and the two α-globin genes on chromosome 16 (Lee, Cho, Park, & Seong, 2021). Both α- and β-thalassemia are subdivided into minor (carrier), intermedia and major forms (Baird, Batten, & Sparks, 2022).

Normal adult Hb is a tetramer of two α- and two β-chains (HbA, $\alpha_2\beta_2$), with smaller amounts of HbA_2 ($\alpha_2\delta_2$) and fetal HbF ($\alpha_2\gamma_2$) (Longo, 2012). In β-thalassemia, deficient β-chain production leaves an excess of unpaired α-chains, which are unstable and precipitate inside developing red cells. These inclusions damage the cell membrane, so that most erythroid precursors die in the bone marrow (ineffective erythropoiesis) and the cells that reach the circulation are removed prematurely by the spleen (chronic extravascular hemolysis) (McKenzie, 2004). The resulting anemia and tissue hypoxia drive erythropoietin production, marrow expansion and extra medullary hematopoiesis, which produce skeletal deformities and hepatosplenomegaly (Ribeil et al., 2013). Expanded erythropoiesis also increases erythroferrone, which suppresses hepcidin and enhances intestinal iron absorption (SanchezVillalobos, Blanquer, Moraleda, Salido, & Perez-Oliva, 2022).

β-thalassemia major (β-TM) usually presents between 6 and 24 months of age with pallor, feeding difficulty, growth retardation and progressive abdominal enlargement from hepatosplenomegaly. Survival depends on regular red cell concentrate transfusion, and iron chelation is required to counter the resulting iron load (Ravanbod et al., 2016). Transfusional iron, together with increased gut absorption, accumulates in the liver, heart, pancreas and endocrine glands, and iron overload is the principal cause of cardiac failure, liver damage and endocrinopathy in these patients (Farmakis, Giakoumis, Polymeropoulos, & Aessopos, 2003; Olivieri, 1999; Shanaki et al., 2016). Hematopoietic stem cell transplantation remains the established curative option, and gene therapy has more recently entered clinical use (Mansilla-Soto, Riviere, Boulad, & Sadelain, 2016).

Worldwide, an estimated 270 million people carry a thalassemia trait, 80 million of them for βthalassemia, and around 23,000 infants are born with β-TM each year, about 90% of them in low- and middle-income countries (Longo, 2012; Mairbäurl & Weber, 2012). The disorder is common across the Mediterranean, the Middle East, the Indian subcontinent and South-East Asia (De Sanctis et al., 2017). In Pakistan, 5–7% of the population carries β-thalassemia trait, amounting to more than 10 million carriers, and approximately 5,000 children are diagnosed with β-TM each year (Bajwa & Basit, 2019). Around 50,000 patients are registered for treatment at centers across the country (Ehsan et al., 2020). High carrier frequency, a large population, high birth rates and frequent consanguineous marriage together explain this burden (Qurat-ul-Ain, Hassan, Rana, & Jabeen, 2011; Khaliq, 2022).

Regular monitoring of hematological and biochemical indices allows timely adjustment of transfusion and chelation and early detection of complications (Khaliq, 2022). Studies from Iraq, Iran, Bangladesh, Egypt and other regions of Pakistan have documented characteristic changes in the CBC and liver

function of β-TM patients (Al-Ghanimi et al., 2019; Hashim et al., 2020; Karim, Ismail, Hasan, & Shekhar, 2016; Khedr, Kotb, Abd El-Kaream, & El-Bayady, 2020; Sharif et al., 2021). However, no such data are available for Mirpur, AJK, where the thalassemia ward of the District Headquarters (DHQ) Hospital registers new patients continually.

The aim of this study was therefore to compare the hematological (CBC: Hb, red cell count and indices, white cell and platelet counts) and hepatic (ALT, ALP and total serum bilirubin) profiles of transfusion dependent β-TM patients at DHQ Hospital, Mirpur, with those of healthy age- and sex-matched individuals, and to identify the principal patterns of abnormality.

Materials and Methods

2.1 Study design and setting

This was an observational, comparative cross-sectional study. Patients were recruited from the Department of Thalassemia, DHQ Hospital, Mirpur, AJK, where they attended as outpatients for scheduled blood transfusion. Hematological and biochemical analyses were performed in the Department of Pathology, Ahsan Medical Complex (AMC), Mirpur.

2.2 Participants

Fifty patients with a previously established diagnosis of β-TM who were receiving regular packed red cell transfusion during the study period were selected by purposive sampling. Inclusion criteria were age 1–26 years, either sex, and negative serology for hepatitis B and C. Patients with α-thalassemia (any form), β-thalassemia minor or intermediate, age below 1 year or above 26 years, or positive hepatitis B or C serology were excluded.

Fifty healthy controls were selected by matched sampling from outpatients attending DHQ Hospital, Mirpur, for minor ailments unrelated to the study. Each control was matched to a patient for sex and age group; none had anemia or related symptoms. Each group therefore comprised 25 males and 25 females with an identical age distribution.

2.3 Sample collection

After informed consent (from parents or guardians for minors), venous blood was drawn under aseptic conditions. Two milliliter's were collected into an EDTA vacutainer for the CBC, and a further sample into a gel/clot-activator tube for LFTs. Serum was separated by centrifugation at 1,500–2,000 rpm for 5 minutes.

2.4 Laboratory analysis

The CBC was analyzed promptly on an automated three-part differential hematology analyzer (Mindray BC-3000 Plus), which counts cells by electrical impedance and measures Hb photometrically. Reported parameters were white blood cell count (WBC), red blood cell count (RBC), Hb, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelet count (PLT).

Liver function tests were measured on a semi-automated spectrophotometric biochemistry analyzer (Microlab 300). Total serum bilirubin (TSB) was determined by the sulfanilic acid (diazo) method using BIOLABO reagents (sulfanilic acid 30 mmol/L, DMSO 7 mol/L and hydrochloric acid 130 mmol/L, with sodium nitrite 0.74 mmol/L). ALT was measured by a kinetic NADH/LDH-coupled method using SPINREACT reagents (TRIS buffer pH 7.8 100 mmol/L, L-alanine 500 mmol/L, NADH 0.18 mmol/L, LDH 1,200 U/L and α-ketoglutarate 15 mmol/L; 100 µL serum per 1 mL working reagent at 37 °C).

ALP was measured by the p-nitrophenyl phosphate method in diethanolamine buffer using SPINREACT reagents (p-NPP 10 mmol/L, magnesium chloride 0.5 mmol/L; 20 µL serum per 1 mL working reagent at 37 °C). The laboratory reference ranges used for interpretation are given in Table 1.

Table 1. Laboratory reference ranges used in the study

Parameter	Male	Female
WBC ($\times 10^3/\mu L$)	4–11	4–11
RBC ($\times 10^6/\mu L$)	4.5–6.0	3.8–5.2
Hb (g/dL)	13–17	12–15
HCT (%)	40–50	36–46
MCV (fL)	75–95	75–95
MCH (pg)	26–32	26–32
MCHC (g/dL)	32–35	32–35
PLT ($\times 10^3/\mu L$)	150–450	150–450
TSB (mg/dL)	0.2–1.0	0.2–1.0
ALT (U/L)	10–45	10–45
ALP (U/L)	30–270	30–270

Adult reference ranges; pediatric ALP values are physiologically higher during growth.

2.5 Statistical analysis

Data were entered into Microsoft Excel. Categorical variables are presented as frequencies and percentages, and continuous variables as mean ± standard deviation (SD) with minimum and maximum. Patients and controls were compared overall and separately by sex using Student's two-sample t-test assuming unequal variances (Welch's test). A p-value below 0.05 was considered statistically significant.

RESULTS

3.1 Demographic characteristics

A total of 100 participants were studied: 50 β-TM patients and 50 matched controls, each group comprising 25 males and 25 females aged 1–26 years. The largest age group in both arms was 1–9 years (58.0%), followed by 10–19 years (36.0%) and 20 years or older (6.0%) (Table 2). Because controls were matched to patients, the age and sex distribution of the two groups was identical.

Table 2. Age and sex distribution of β-TM patients (identical in the matched control group)

Age group (years)	Male, n (%)	Female, n (%)	Total, n (%)
1-9	15 (60.0)	14 (56.0)	29 (58.0)
10-19	8 (32.0)	10 (40.0)	18 (36.0)
≥ 20	2 (8.0)	1 (4.0)	3 (6.0)
Total	25 (100)	25 (100)	50 (100)

n = 50 per group; the control group (n = 50) had exactly the same distribution by design.

3.2 Hematological findings

Patients were markedly anemic. Mean Hb in the patient group was 7.30 ± 1.64 g/dL (range 1.7–9.8 g/dL) compared with 13.29 ± 1.08 g/dL (range 11.6–15.8 g/dL) in controls, and mean RBC count and HCT were likewise substantially lower (Table 3; all $p < 0.001$). MCV and MCH were significantly lower in patients ($p < 0.001$), although the mean values (76.33 fL and 26.89 pg) lay at the lower limit of the reference range rather than well below it. MCHC was slightly but significantly higher in patients (34.78 ± 0.84 vs 33.77 ± 0.90 g/dL; $p < 0.001$).

Mean WBC was higher in patients (11.18 ± 7.98 vs 7.45 ± 1.89 $\times 10^3/\mu L$; $p = 0.002$), with a wide range (2.66–54.13 $\times 10^3/\mu L$). Mean platelet count was also higher (392.06 ± 169.57 vs 263.58 ± 78.93 $\times 10^3/\mu L$; $p < 0.001$), with individual values reaching 787 $\times 10^3/\mu L$.

3.3 Hepatic findings

All three liver parameters were significantly raised in patients (Table 3). Mean TSB was 1.91 ± 1.22 mg/dL (range 0.77–6.25) versus 0.71 ± 0.09 mg/dL in controls; mean ALT was 72.54 ± 55.52 U/L (range 12–315) versus 27.78 ± 8.67 U/L; and mean ALP was 380.18 ± 136.51 U/L (range 199–700) versus 150.66 ± 42.38 U/L (all $p < 0.001$). Mean TSB, ALT and ALP in patients all exceeded the upper reference limit. Figure 1 summarizes the relative size of each difference: bilirubin, ALT and ALP were each about 2.5-fold higher in patients, whereas Hb was about 45% lower.

Table 3. Comparison of hematological and hepatic parameters between β-TM patients and healthy controls

Parameter	β-TM patients (n = 50) Mean ± SD	Controls (n = 50) Mean ± SD	t-test	p-value
Hematological parameters				
WBC ($\times 10^3/\mu L$)	11.18 ± 7.98	7.45 ± 1.89	3.198	= 0.002
RBC ($\times 10^6/\mu L$)	3.15 ± 0.76	4.91 ± 0.39	14.237	< 0.001
Hb (g/dL)	7.30 ± 1.64	13.29 ± 1.08	21.311	< 0.001
HCT (%)	23.94 ± 5.49	39.29 ± 2.93	17.228	< 0.001
MCV (fL)	76.33 ± 3.81	80.75 ± 3.42	6.189	< 0.001
MCH (pg)	26.89 ± 2.15	28.91 ± 1.50	5.570	< 0.001
MCHC (g/dL)	34.78 ± 0.84	33.77 ± 0.90	5.647	< 0.001
PLT ($\times 10^3/\mu L$)	392.06 ± 169.57	263.58 ± 78.93	4.731	< 0.001
Hepatic (liver function) parameters				
TSB (mg/dL)	1.91 ± 1.22	0.71 ± 0.09	6.951	< 0.001
ALT (U/L)	72.54 ± 55.52	27.78 ± 8.67	5.666	< 0.001
ALP (U/L)	380.18 ± 136.51	150.66 ± 42.38	11.100	< 0.001

All differences statistically significant (Welch's t-test, $p < 0.05$). WBC, white blood cells; RBC, red blood cells; Hb, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelets; TSB, total serum bilirubin; ALT, alanine aminotransferase; ALP, alkaline phosphatase.

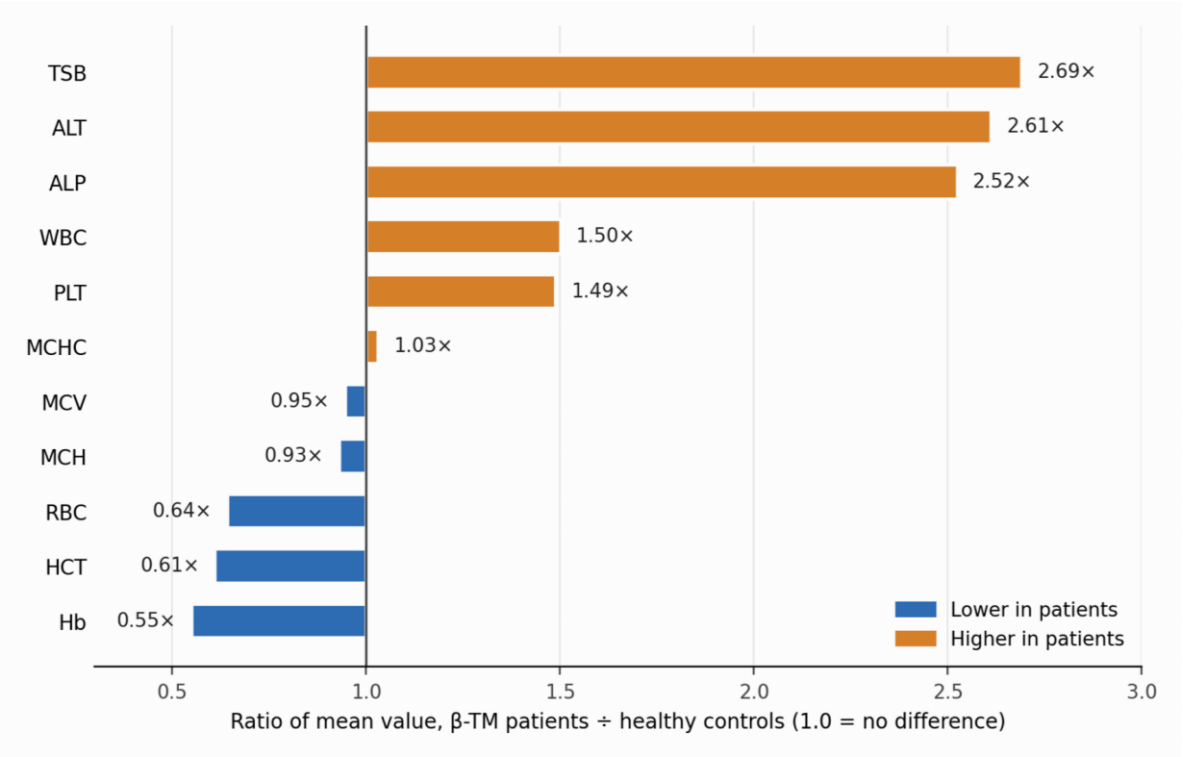


Figure 1. Ratio of the mean value in β-TM patients to that in healthy controls for each parameter. Bars to the left of 1.0 indicate lower values in patients; bars to the right indicate higher values. All differences were statistically significant ($p < 0.05$).

3.4 Findings by sex

The differences between patients and controls were consistent in males and females (Table 4). In both sexes, patients had significantly lower Hb, RBC, HCT, MCV and MCH, and significantly higher WBC, platelet count, MCHC, TSB, ALT and ALP. Among patients, males had numerically lower mean Hb (6.99 vs 7.59 g/dL) and higher mean ALT (84.04 vs 57.04 U/L) and TSB (2.12 vs 1.57 mg/dL) than females, and the widest ranges of WBC (2.66–54.13 $\times 10^3/\mu L$) and ALT (20–315 U/L) were seen in male patients; mean ALP was almost identical in the two sexes (about 380 U/L). These within-patient sex differences were not formally tested.

Table 4. Comparison of hematological and hepatic parameters between patients and controls, stratified by sex

Males (n = 25 per group)				Females (n = 25 per group)		
Parameter	Patients	Controls	p value	Patients	Controls value	p value
Hematological parameters						
WBC ($\times 10^3/\mu L$)	11.76 ± 10.14	7.22 ± 1.92	= 0.038	10.84 ± 5.11	7.15 ± 1.64	< 0.001
RBC ($\times 10^6/\mu L$)	3.05 ± 0.82	4.93 ± 0.47	< 0.001	3.29 ± 0.64	4.90 ± 0.29	< 0.001
Hb (g/dL)	6.99 ± 1.86	13.50 ± 1.31	< 0.001	7.59 ± 1.39	13.07 ± 0.74	< 0.001
HCT (%)	23.26 ± 5.89	39.90 ± 3.53	< 0.001	24.64 ± 5.06	38.67 ± 2.07	< 0.001
MCV (fL)	75.63 ± 4.28	81.01 ± 3.72	< 0.001	76.92 ± 3.22	80.49 ± 3.15	< 0.001
MCH (pg)	26.62 ± 2.15	29.30 ± 1.72	< 0.001	27.25 ± 2.12	28.52 ± 1.15	= 0.010
MCHC (g/dL)	34.58 ± 0.72	33.93 ± 1.09	= 0.029	34.93 ± 0.91	33.61 ± 0.65	< 0.001
PLT ($\times 10^3/\mu L$)	389.92 ± 153.41	269.24 ± 89.82	= 0.002	411.92 ± 183.47	257.92 ± 67.72	< 0.001

Males (n = 25 per group)				Females (n = 25 per group)		
Parameter	Patients	Controls	p value	Patients	Controls	p value
Hepatic (liver function) parameters						
TSB (mg/dL)	2.12 ± 1.35	0.73 ± 0.09	< 0.001	1.57 ± 0.92	0.69 ± 0.09	< 0.001
ALT (U/L)	84.04 ± 67.04	28.60 ± 7.98	< 0.001	57.04 ± 30.15	26.96 ± 9.40	< 0.001
ALP (U/L)	380.00 ± 132.01	154.80 ± 41.11	< 0.001	380.36 ± 143.60	146.52 ± 44.06	< 0.001

Values are mean ± SD; p-values from Welch's t-test. All comparisons statistically significant ($p < 0.05$).

DISCUSSION

To our knowledge, this is the first study to describe the hematological and hepatic profile of transfusion-dependent βTM patients in Mirpur, AJK. Compared with matched healthy individuals, patients showed severe anemia with reduced red cell indices, rai sed leucocyte and platelet counts, and elevation of all three liver parameters measured. These findings are broadly consistent with reports from other regions of Pakistan and from neighboring countries (Table 5).

Table 5. Mean values in β-TM patients in the present study compared with previously published studies

Parameter	Present study Mean ± SD	Previous studies, Mean ± SD (source)
WBC ($\times 10^3/\mu L$)	11.18 ± 7.98	18.77 ± 13.74 (Hashim et al., 2020)
RBC ($\times 10^6/\mu L$)	3.15 ± 0.76	3.43 ± 1.34 (Al-Ghanimi et al., 2019) 3.07 ± 0.77 (Sharif et al., 2021) 3.04 ± 0.68 (Khedr et al., 2020)
Hb (g/dL)	7.30 ± 1.64	8.95 ± 2.88 (Hashim et al., 2020) 8.01 ± 1.02 (Sharif et al., 2021) 7.36 ± 1.50 (Karim et al., 2016)
HCT (%)	23.94 ± 5.49	22.51 ± 4.99 (Kumar, 2019) 21.50 ± 5.30 (Karim et al., 2016)
MCV (fL)	76.33 ± 3.81	74.03 ± 2.73 (Al-Ghanimi et al., 2019) 68.85 ± 16.35 (Shanaki et al., 2016) 70.00 ± 9.50 (Karim et al., 2016)
MCH (pg)	26.89 ± 2.15	22.43 ± 8.07 (Hashim et al., 2020) 24.77 ± 9.80 (Al-Ghanimi et al., 2019) 20.90 ± 4.04 (Shanaki et al., 2016)
MCHC (g/dL)	34.78 ± 0.84	35.2 ± 3.0 (Jameel, Baig, Ahmed, Hussain, & bin Doghaim Alkhamaly, 2017)
PLT ($\times 10^3/\mu L$)	392.06 ± 169.57	388.70 ± 6.57 (Al-Ghanimi et al., 2019) 313.40 ± 39.27 (Sharif et al., 2021)
TSB (mg/dL)	1.91 ± 1.22	2.32 ± 0.91 (Hussain et al., n.d.) 1.56 ± 1.28 (AL-hameedawi & Al-Shawi, 2022)
ALT (U/L)	72.54 ± 55.52	60.52 ± 99.33 (Khedr et al., 2020) 117.12 ± 32.00 (Sharif et al., 2021) 69.42 ± 23.72 (Hussain et al., n.d.)
ALP (U/L)	380.18 ± 136.51	210.51 ± 98.74 (AL-hameedawi & Al-Shawi, 2022) 302.00 ± 26.05 (Khedr et al., 2020)

4.1 Red cell parameters

Mean Hb in our patients (7.30 g/dL) closely matched the value reported from Bangladesh (7.36 g/dL; Karim et al., 2016) and was lower than values from Pakistan (8.01 g/dL; Sharif et al., 2021) and Iraq (8.95 g/dL; Hashim et al., 2020). RBC count and HCT were similarly comparable to previous reports (Al-Ghanimi et al., 2019; Karim et al., 2016; Khedr et al., 2020; Kumar, 2019; Sharif et al., 2021). The anemia reflects the combined effect of ineffective erythropoiesis, in which α-chain precipitates destroy erythroid precursors in the marrow, and accelerated splenic removal of damaged circulating red cells (McKenzie, 2004; Ribeil et al., 2013). Samples were drawn when patients attended for transfusion, so these values probably approximate pre-transfusion Hb. International guidelines recommend maintaining pre-transfusion Hb at 9–10.5 g/dL to suppress ineffective erythropoiesis and limit marrow expansion (Farmakis et al., 2022); a mean of 7.3 g/dL, with some values below 5 g/dL, suggests that many patients in this cohort were under-transfused, which may reflect limited blood availability, irregular attendance or long transfusion intervals.

MCV and MCH were significantly lower than in controls, in agreement with earlier studies (AlGhanimi et al., 2019; Hashim et al., 2020; Karim et al., 2016; Shanaki et al., 2016). The reduction was, however, modest, and mean values lay near the lower limit of normal rather than in the markedly microcytic range typical of untransfused thalassemia. This is expected in transfusion-dependent patients, because a large proportion of circulating red cells are normocytic donor cells that dilute the patient's own microcytic, hypochromic cells. The small increase in MCHC, although statistically significant, stayed close to the reference range; it may reflect the mixed donor and recipient red cell population and the fact that MCHC is a calculated index on three-part analyzers, and it should be interpreted with caution.

4.2 White cell and platelet counts

Raised WBC counts in β-TM have been reported previously (Hashim et al., 2020). Part of this increase may be genuine, reflecting infection, splenectomy or altered immune function associated with iron overload (Farmakis et al., 2003). An important additional explanation is analytical: β-TM patients often have large numbers of circulating nucleated red blood cells, which impedance-based analyzers count as leucocytes unless a correction is applied. The very high individual value of 54.13 $\times 10^3/\mu L$ is compatible with this. Future studies should report WBC corrected for nucleated red cells on a peripheral smear. The raised platelet count agrees with Al-Ghanimi et al. (2019) and Sharif et al. (2021) and is commonly attributed to reactive thrombocytosis in chronic hemolysis and, particularly, to prior splenectomy; splenectomy status was not recorded in this study.

4.3 Liver function

Mean TSB in patients (1.91 mg/dL) was about 2.7 times that of controls and fell between previously reported values of 1.56 and 2.32 mg/dL (AL-hameedawi & Al-Shawi, 2022; Hussain, Abdullah, & Hussein, n.d.). In β-TM, hyperbilirubinaemia is predominantly unconjugated and results from hemolysis and intramedullary destruction of erythroid precursors; because bilirubin was not fractionated, a hepatocellular or cholestatic contribution cannot be separated in our data.

ALT, the most liver-specific of the markers measured, was raised 2.6-fold, in agreement with other studies (Hussain et al., n.d.; Khedr et al., 2020; Sharif et al., 2021). Because patient's positive for hepatitis B and C were excluded, the most likely explanation is hepatocellular injury from transfusional iron overload, in which labile iron generates oxidative stress, inflammation and ultimately fibrosis (Olivieri, 1999; Shanaki et al., 2016). The wide spread of ALT values (12–315 U/L) indicates that liver injury varies considerably between patients, plausibly with transfusion burden and adherence to chelation.

ALP was also markedly raised (380.18 U/L), exceeding values reported from Iraq and Egypt (AL-hameedawi & Al-Shawi, 2022; Khedr et al., 2020). ALP is derived from both liver and bone, and in βTM the expanded marrow and increased bone turnover contribute substantially to serum ALP, in addition to any hepatobiliary injury. Most participants were children, in whom bone-derived ALP is physiologically higher; however, because controls were age-matched, the difference between groups cannot be explained by age alone.

4.4 Clinical implications

Taken together, the findings indicate that patients attending the DHQ Hospital thalassemia ward remain considerably anemic between transfusions and show biochemical evidence of liver stress early in life, since most were under 10 years of age. Regular CBC and LFT monitoring provides an inexpensive means of identifying patients who need more frequent transfusion, closer chelation review or further investigation of liver disease. These results also support wider public awareness of thalassemia and premarital and cascade carrier screening, given the high carrier frequency and rates of consanguineous marriage in the region (Khaliq, 2022; Qurat-ul-Ain et al., 2011).

4.5 Limitations

This study has several limitations. It was conducted at a single center with a modest sample size and purposive sampling, which limits generalizability. Serum ferritin, liver iron concentration and other markers of iron status were not measured, so liver abnormalities could not be linked directly to the degree of iron overload. Information on transfusion frequency, time since last transfusion, chelation regimen and adherence, and splenectomy status was not analyzed. Bilirubin was not fractionated, WBC counts were not corrected for nucleated red cells, and ALP was not separated into bone and liver isoenzymes. Finally, adult reference ranges were applied to a largely pediatric population.

CONCLUSION

Transfusion-dependent β-thalassemia major patients in Mirpur, AJK, differ significantly from healthy age- and sex matched individuals in both hematological and hepatic parameters. Patients had severe anemia with reduced red cell count, hematocrit, MCV and MCH, raised WBC and platelet counts, and elevated bilirubin, ALT and ALP. Regular monitoring of the CBC and liver function, combined with assessment of iron status and optimization of transfusion and chelation, is essential for improving outcomes. Larger, multicenter studies incorporating serum ferritin and treatment history are recommended, alongside community education and carrier screening to reduce the number of new cases.

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ETHICAL APPROVAL AND CONSENT

Written informed consent was obtained from all participants, or from parents or guardians for participants under 18 years of age.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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