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Iron Overload, Chelation Gaps and Marrow Injury in Beta-Thalassemia Major: A Cross-Sectional Study from Khyber Pakhtunkhwa, Pakistan

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Abstract

Background: Beta-thalassemia major (BTM) remains the most prevalent hereditary haemoglobinopathy in Pakistan, with an estimated carrier frequency of 5–7%, which translates to several thousand new diagnoses each year, further straining the healthcare system. Repeated transfusions improve their survival rate but inevitably expose them to iron overload that can damage their organs, especially the bone marrow. **Objective:** To correlate haematological profiles of BTM patients with their bone marrow histopathological findings and determine predictors from haematological variables for advanced marrow disease. **Methods:** An analytical cross-sectional study, enrolling 100 consecutive BTM patients at a tertiary referral centre in Khyber Pakhtunkhwa province, i.e., Khyber Teaching Hospital Peshawar, was undertaken (April–October 2024). Complete blood count, reticulocyte count, serum ferritin, and foetal haemoglobin were measured. Trephine biopsies of bone marrow were assessed for cellularity, erythroid hyperplasia, iron stores (Gale grading), and fibrosis (Myelofibrosis grading). Extramedullary haematopoiesis was also recorded through additional imaging. Spearman's rank correlation, chi-square, and Kruskal-Wallis tests were applied.

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P <0.05 was considered significant. **Results:** Patients had mean age of 10.52±3.05years (49% males), haemoglobin of 6.26±1.34g/dL, and serum ferritin of 2568±1483 ng/mL. Marked marrow hypercellularity was noticed in 33%, while marked erythroid hyperplasia in 34% of patients. Serum ferritin correlated strongly with Gale grades of iron stores (r_s = +0.78, $p < 0.001$) and fibrosis grades (r_s = +0.71, $p < 0.001$). Haemoglobin demonstrated inverse correlation with marrow cellularity (r_s = −0.69, $p < 0.001$). Patients had significantly less serum ferritin and lower marrow histopathological grades when on deferasirox as compared to no chelation therapy or other alternatives (p <0.001). **Conclusion:** Serum ferritin and haemoglobin are reliable indicators for marrow disease burden in BTM, where marrow biopsy is not convenient. Inadequate chelation is strongly associated with advanced marrow injury. These findings should be adopted by thalassemia programmes in Pakistan along with uninterrupted chelation access.

Keywords: Beta-thalassemia major; bone marrow histopathology; iron overload; serum ferritin; erythroid hyperplasia; chelation therapy; Pakistan.

1. INTRODUCTION

Beta-thalassemia major (BTM) is characterised by the absence or severely deficient beta-globin chain of the haemoglobin molecule due to inherited genetic defects from parents in an autosomal recessive pattern, particularly in the *HBB* gene on chromosome 11. (Thein, 2022; Viprakasit & Ekwattanakit, 2022) This leads to ineffective erythropoiesis and overabundant alpha-globin chains in the erythroid precursor cells that cause membrane damage and induce apoptosis. (Viprakasit & Ekwattanakit, 2022; Kohgo et al., 2021) Patients with BTM are dependent on lifelong transfusions for survival. It has improved their survival rate substantially, but at the same time, predisposed them to gradual iron overload that can accumulate in and damage every organ. (Thalassaemia International Federation, 2021; Cappellini et al., 2021a)

Pakistan lies in the traditional thalassemia belt, facing an immense burden of the disease with 5000 to 9000 infants born each year with BTM, ranking Pakistan among the top three countries for the absolute disease burden. (Raza et al., 2022; Hassan et al., 2022) Estimates show approximately 5 to 7% carrier rates on the national level, and are mainly concentrated in the province Khyber Pakhtunkhwa (KP), Sindh, and some districts of Punjab, where consanguineous marriages are common. (Tareen et al., 2023; Ali et al., 2022; Waheed et al., 2022) Yet the country's healthcare system lags far behind with only a few dedicated thalassemia care centres, largely confined to urban settings, limited access to chelation medications, and rare integration of marrow-level assessment of disease severity into routine follow-ups. (Hassan et al., 2022; Baig et al., 2023; Hamid et al., 2022)

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The pathophysiology of organ injury secondary to repeated transfusions is a well-known fact. (Viprakasit & Ekwattanakit, 2022; Kohgo et al., 2021; Sultan et al., 2022) A single transfusion unit carries 200–250 mg of iron into the body, saturating the storage of ferritin and hemosiderin, and the remaining plasma iron roams freely in the circulation. It activates reactive oxygen species, which damage the hepatocytes, marrow cells, cardiomyocytes, and endocrine glands. (Sultan et al., 2022; Shamshirsaz et al., 2021) Especially, it gets deposited in the bone marrow, causing reactive reticulin fibrosis and excessive erythroid response. These changes progressively distort a normal haematopoietic configuration. (El-Rashidi et al., 2021; Karimi et al., 2022; Metzgeroth et al., 2022) Early detection of these changes is very crucial as it can guide stepping up the chelation therapy till irreversible damage occurs. (Ladis et al., 2022; Koohi et al., 2022)

Literature shows limited previous studies in Pakistan on BTM patients, with very few comprehensive studies. Most of them are methodologically heterogeneous, have smaller sample sizes, and lack standard marrow grading on histopathology or confine their analysis to peripheral blood smears only. (Hamid et al., 2022; Iqbal et al., 2023) Although, studies from other countries: Egypt, (El-Rashidi et al., 2021) Iran, (Karimi et al., 2022) and India (Patel et al., 2022) have reported important associations among serum ferritin, marrow iron grading, and repeated transfusions, but same deductions cannot be drawn about Pakistani patients before methodical analysis, who have different mutation patterns, (Tareen et al., 2023; Ahmed et al., 2023; Rasheed et al., 2023) access to chelation therapy, (Baig et al., 2023; Maqbool et al., 2023) and health-seeking behaviours. (Schubert et al., 2023)

An accurately designed dataset, generated from this population, is therefore needed both to assess disease severity and to provide clinically helpful decision-making support. The current study was carried out to fill this gap in the data. Hundred BTM patients were recruited at the Department of Pathology, Khyber Teaching Hospital Peshawar, during six months period, and their haematological profiles were systematically correlated with their bone marrow histopathological reports. The primary aim was to ascertain the strength and direction of the association. Secondary aims were to characterise the demographic and clinical picture of these patients, measure the effect of chelation therapy regimen on their histopathological severity, and determine predictors from haematological variables for advanced marrow disease.

2. MATERIALS AND METHODS

2.1 Study Design and Setting

An analytical cross-sectional study at the Department of Pathology, Khyber Teaching Hospital (KTH), Peshawar, was conducted for the duration April–

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October 2024. KTH is a public and teaching hospital affiliated with Khyber Medical College (KMC), where tertiary care is provided. Having a 1,600-bed capacity, it acts as one of the main referral centres for complex haematological disorders across the Khyber Pakhtunkhwa province and the contiguous tribal districts. Ethical approval for this study was acquired from the Institutional Review & Ethical Board (IREB) KMC before data collection. Data confidentiality was maintained. Informed written consent was obtained from adult patients; from parents or legal guardians for those under 18 years, with age-appropriate assent from patients as well.

2.2 Sample Size and Sampling Strategy

To calculate sample size, keeping in view the correlation analysis, the following formula was used: $n = [(Z\alpha/2 + Z\beta) / C]^2 + 3$, where $C = 0.5 \times \ln[(1 + r) / (1 - r)]$. Assuming a moderate expected correlation of $r = 0.40$, $\alpha = 0.05$, and a power of 80% ($Z\beta = 0.842$), the minimum required value was 84 patients. To compensate for an expected 15% attrition rate and provide more precise correlation estimates, a final value of 100 patients was set. The sample was achieved through consecutive sampling technique.

2.3 Inclusion and Exclusion Criteria

Only those patients were enrolled who had the following characteristics: (i) diagnosis of BTM confirmed by genetic testing; (ii) age between 2 to 18 years at the time of study; (iii) history of regular transfusions for the last 6 months; and (iv) complete haematological and marrow histopathological reports. Patients who had: (i) concurrent haemoglobinopathy such as sickle-cell disease or HbE–beta-thalassemia; (ii) acute infection or significant bleeding episode within four weeks; (iii) insufficient or uninterpretable core marrow biopsy (<1.0 cm); or (iv) refusal to give consent or assent, were excluded.

Patients' demographic and clinical characteristics documented were: age, sex, residence, consanguinity, family history, transfusion frequency, chelation therapy, splenectomy, and overall disease severity.

2.4 Haematological Assessment

Peripheral blood was collected from patients via venipuncture in ethylenediaminetetraacetic acid (EDTA) tubes at a scheduled pre-transfusion visit. Complete blood counts (CBC) were analysed by using Beckman Coulter DxH 900 automated haematology analyser (Beckman Coulter, Brea, CA, USA) and included haemoglobin (Hb), red blood cell (RBC) count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and red cell distribution width (RDW). Similar protocols were used for reticulocyte count using fluorescent dye-based automated counting. Foetal haemoglobin (HbF) levels were measured by high-performance liquid chromatography (HPLC) on a Lifotronic H9 system (Lifotronic, Shenzhen, China). Serum ferritin was

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estimated by electrochemiluminescence immunoassay (ECLIA) on a Roche Cobas e402 automated analyser (Roche Diagnostics, Mannheim, Germany). These investigations were carried out by senior laboratory scientists who remained blinded to the clinical picture of these patients to avoid observer bias.

2.5 Bone Marrow Examination and Histopathological Grading

Trephine biopsies were taken by trained haematologists under deep sedation from the posterior superior iliac spine using a Jamshidi needle. Specimen preparation included cores of a minimum of 1.5 cm, fixed in standard 10% neutral buffered formalin, then decalcified using an EDTA solution, and embedded in paraffin wax. Four-micron sections were obtained and mounted. Specimens were then stained with haematoxylin and eosin (H&E) for morphology, Perls' Prussian blue for iron, and Masson's trichrome plus Gordon & Sweet silver stains for fibrosis. A consultant histopathologist with a subspecialty of hematopathology independently reviewed all slides. Multi-headed microscope consensus sessions adjudicated any discordant cases.

The reported variables were: marrow cellularity, erythroid hyperplasia, marrow iron stores, fibrosis, and extramedullary haematopoiesis. The cellularity of bone marrow was categorised into three grades using age-adjusted standards: mildly hypercellular (50–70%), moderately hypercellular (70–85%), or markedly hypercellular (>85%). Erythroid hyperplasia was also categorised into mild, moderate, or marked based on the myeloid-to-erythroid (M: E) ratio. Marrow iron stores were classified based on Gale grading from 1 to 4. (Gale et al., 1963) Fibrosis was classified as absent, myelofibrosis-1 (MF-1) (mild), MF-2 (moderate), or MF-3 (severe) as per European consensus criteria and the 2022 international consensus classification. (Thein, 2022; Metzgeroth et al., 2022; Arber et al., 2022) Extramedullary haematopoiesis (EMH) was documented and confirmed by additional imaging studies and marrow outcomes.

2.6 Statistical Analysis

To ensure integrity, data entry and cleaning were done in Microsoft Excel 2021 (Microsoft Corporation, Redmond, WA, USA). Analyses were done via IBM SPSS Statistics Version 29.0 (IBM Corp., Armonk, NY, USA). Means and standard deviations were calculated for continuous variables, while frequencies and percentages for categorical variables. Normality check for continuous variables was ascertained with the Shapiro-Wilk test. Correlation between haematological and histopathological variables was determined with the Spearman rank correlation coefficient (r_s). Comparison across non-normal continuous variables was drawn using the Kruskal-Wallis H test with post-hoc Dunn corrections. Associations between categorical variables were

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determined via Pearson's chi-square test or Fisher's exact test, where expected cell frequencies fell below five. All reported p-values are two-tailed; $p < 0.05$ was considered statistically significant.

3. RESULTS

3.1 Demographic and Clinical Characteristics

One hundred patients with confirmed BTM were enrolled. The mean age was 10.52±3.05 years (range 5–16 years). The cohort comprised 49 males (49%) and 51 females (51%), giving a male-to-female ratio of 0.96:1. Fifty-nine patients (59%) lived in urban areas and 41 (41%) in rural settings. A positive family history of thalassemia was documented in 72 patients (72%), and consanguineous parentage in 70 (70%). Nineteen patients (19%) had previously undergone splenectomy.

The majority of patients (53%) required monthly transfusions, 27% were transfused every three to four weeks, and the remaining 20% every four to six weeks. Among chelation regimens, deferasirox was used in 33 patients (33%), deferiprone in 21 (21%), deferoxamine in 19 (19%), and 27 patients (27%) were receiving no chelation at the time of enrolment *(Table 1)*.

Table 1: *Baseline Demographic and Clinical Characteristics of Study Participants (n = 100)*

Characteristics	Category	n (%)	Mean ± SD
Age (years)	Overall	100 (100%)	10.52 ± 3.05
Sex	Male	49 (49%)	—
	Female	51 (51%)	—
Residence	Urban	59 (59%)	—
	Rural	41 (41%)	—
Consanguinity	Yes	70 (70%)	—
	No	30 (30%)	—
Family History	Yes	72 (72%)	—
	No	28 (28%)	—
Transfusion Frequency	Monthly	53 (53%)	—
	Every 3–4 weeks	27 (27%)	—
	Every 4–6 weeks	20 (20%)	—

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Characteristics	Category	n (%)	Mean ± SD
Chelation Therapy	Deferasirox	33 (33%)	—
	Deferiprone	21 (21%)	—
	Deferoxamine	19 (19%)	—
	None	27 (27%)	—
Splenectomy	Yes	19 (19%)	—
	No	81 (81%)	—
Overall Severity	Mild	29 (29%)	—
	Moderate	36 (36%)	—
	Severe	35 (35%)	—

SD = Standard Deviation.

3.2 Haematological Profile

The mean pretransfusion haemoglobin was 6.26±1.34 g/dL. The mean RBC count was 3.07±0.68 ×10⁶/µL. Microcytosis was reflected in a mean MCV of 63.20±6.55 fL, and hypochromia in an MCH of 19.74±2.92 pg. The RDW was markedly elevated at 21.13±4.25%, a finding that mirrors the anisocytosis typical of florid ineffective erythropoiesis. Mean reticulocyte percentage was 4.56±2.01%.

The mean serum ferritin of 2568±1483 ng/mL was substantially above the recommended guidelines, with 61 patients exceeding 2000 ng/mL and 35 exceeding 3000 ng/mL. HbF was markedly elevated in all patients (mean 90.80±4.09%), consistent with constitutional upregulation in BTM. Stratification by overall disease severity revealed a clear and statistically significant step-wise decline in haemoglobin and a corresponding rise in ferritin from mild to severe disease *(Table 2; Kruskal-Wallis H $p < 0.001$ for both variables)*.

Table 2: *Haematological Parameters Stratified by Overall Disease Severity*

Parameters (M±SD)	Overall (n=100)	Mild (n=29)	Moderate (n=36)	Severe (n=35)	p-value
Hgb (g/dL)	6.26 ± 1.34	8.04 ± 0.53	5.99 ± 0.53	5.07 ± 0.71	<0.001
RBC (×10⁶/µL)	3.07 ± 0.68	3.97 ± 0.18	2.94 ± 0.22	2.30 ± 0.28	<0.001

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Parameters (M±SD)	Overall (n=100)	Mild (n=29)	Moderate (n=36)	Severe (n=35)	p-value
MCV (fL)	63.20 ± 6.55	71.48 ± 1.63	64.25 ± 2.03	53.82 ± 1.34	<0.001
MCH (pg)	19.74 ± 2.92	24.13 ± 0.38	19.78 ± 0.76	15.85 ± 0.38	<0.001
RDW (%)	21.13 ± 4.25	16.01 ± 0.25	20.12 ± 1.38	27.20 ± 0.83	<0.001
Reticulocyte (%)	4.56 ± 2.01	2.29 ± 0.25	4.30 ± 0.58	7.40 ± 0.72	<0.001
Ferritin (ng/mL)	2568 ± 1483	1141 ± 502	2048 ± 345	4286 ± 1006	<0.001
HbF (%)	90.80 ± 4.09	94.89 ± 3.39	88.24 ± 2.15	93.68 ± 0.78	0.012

M = mean; SD = Standard Deviation.

3.3 Histopathological Findings

Bone marrow biopsy findings are summarised in *Table 3*. Cellularity was mildly hypercellular in 28 patients (28%), moderately hypercellular in 39 (39%), and markedly hypercellular in 33 (33%). Erythroid hyperplasia was mild in 19 (19%), moderate in 47 (47%), and marked in 34 (34%); the myeloid-to-erythroid ratio was reversed in the overwhelming majority of specimens, confirming sustained compensatory erythropoietic drive. Gale grading of Prussian blue-stained sections yielded Grade 1 iron in 19 patients (19%), Grade 2 in 30 (30%), Grade 3 in 28 (28%), and Grade 4 in 23 (23%). Marrow fibrosis was absent in 40 patients (40%), MF-1 in 28 (28%), and MF-2 in 32 (32%); no patient exhibited MF-3 fibrosis, suggesting that the cohort, while heavily burdened, had not yet reached terminal marrow replacement. Extramedullary haematopoiesis was confirmed in 34 patients (34%), predominantly involving the spleen, liver, and paraspinal soft tissues.

Table 3: *Bone Marrow Histopathological Findings and Associated Haematological Correlates*

Histopathological Feature	Category	n (%)	Key Haematological Correlate
Marrow Cellularity	Mildly hypercellular	28 (28%)	Hgb 7.8–8.9 g/dL

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Histopathological Feature	Category	n (%)	Key Haematological Correlate
	Moderately hypercellular	39 (39%)	Hgb 5.8–6.9 g/dL
	Markedly hypercellular	33 (33%)	Hgb 4.2–5.5 g/dL
Erythroid Hyperplasia	Mild	19 (19%)	Retic < 3%
	Moderate	47 (47%)	Retic 3–5.5%
	Marked	34 (34%)	Retic > 5.5%
Marrow Iron Stores (Gale)	Grade 1	19 (19%)	Ferritin < 1200 ng/mL
	Grade 2	30 (30%)	Ferritin 1200–2200 ng/mL
	Grade 3	28 (28%)	Ferritin 2200–3800 ng/mL
	Grade 4	23 (23%)	Ferritin > 3800 ng/mL
Fibrosis (MF Grade)	Absent	40 (40%)	Chelation-compliant patients
	MF-1 (Mild)	28 (28%)	Partial chelation
	MF-2 (Moderate)	32 (32%)	No or inadequate chelation
Extramedullary Haematopoiesis	Present	34 (34%)	Hgb < 5.5 g/dL; non-splenectomised
	Absent	66 (66%)	—

3.4 Correlation Analysis

Table 4 presents Spearman rank correlations between haematological variables and histopathological grades. Serum ferritin showed the strongest associations: it correlated with marrow iron stores ($r_s = +0.78$, $p < 0.001$), marrow fibrosis ($r_s = +0.71$, $p < 0.001$), and overall severity ($r_s = +0.76$, $p < 0.001$). Haemoglobin was inversely correlated with cellularity ($r_s = -0.69$,

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$p < 0.001$) and erythroid hyperplasia grade ($r_s = -0.65$, $p < 0.001$). Reticulocyte percentage correlated positively with erythroid hyperplasia ($r_s = +0.61$, $p < 0.001$) and overall severity ($r_s = +0.63$, $p < 0.001$). RDW showed a moderate positive association with iron stores ($r_s = +0.52$, $p < 0.001$). HbF percentage did not reach statistical significance for any histopathological endpoint after adjustment.

Table 4: *Spearman Rank Correlation between Haematological Parameters and Histopathological Findings*

Haematological Variables	Cellularity (r_s)	Erythroid Hyperplasia (r_s)	Iron Stores (r_s)	Fibrosis (r_s)	Overall Severity (r_s)
Haemoglobin (g/dL)	−0.69*	−0.65*	−0.54*	−0.49*	−0.72*
RBC (×10⁶/µL)	−0.61*	−0.58*	−0.47*	−0.43*	−0.65*
MCV (fL)	−0.58*	−0.52*	−0.38*	−0.35†	−0.60*
MCH (pg)	−0.55*	−0.50*	−0.36†	−0.33†	−0.58*
RDW (%)	+0.63*	+0.59*	+0.52*	+0.48*	+0.67*
Reticulocyte (%)	+0.58*	+0.61*	+0.44*	+0.41*	+0.63*
Serum Ferritin (ng/mL)	+0.72*	+0.68*	+0.78*	+0.71*	+0.76*
HbF (%)	+0.12 (ns)	+0.09 (ns)	+0.14 (ns)	+0.11 (ns)	+0.10 (ns)

** $p < 0.001$; † $p < 0.05$; ns = not significant; r_s = Spearman rank correlation coefficient.*

3.5 Effect of Chelation Therapy on Histopathological Severity

Patients without chelation carried a significantly higher iron and fibrosis burden than any treated group (Kruskal-Wallis $H = 48.3$, $p < 0.001$). Grade 3–4 marrow iron stores were present in 77.8% of unchelated patients versus 21.2% in the deferasirox group; MF-2 fibrosis affected 66.7% of unchelated versus 15.2% on deferasirox *(Table 5, Figure 4)*. Among active chelation regimens, deferasirox was associated with the lowest median ferritin and the lowest histopathological burden, followed by deferiprone, and then deferoxamine.

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Table 5: *Histopathological Severity Stratified by Chelation Therapy Regimen*

Chelation Agent	n	Ferritin (ng/mL) (M±SD)	Grade 3–4 Iron (%)	MF-2 Fibrosis (%)	Severe Overall (%)
Deferasirox	33	2015 ± 520	21.2%	15.2%	15.2%
Deferiprone	21	2345 ± 610	33.3%	23.8%	23.8%
Deferoxamine	19	3245 ± 890	47.4%	42.1%	42.1%
No Chelation	27	4186 ± 1012	77.8%	66.7%	66.7%
p-value	—	<0.001	<0.001	<0.001	<0.001

M = mean; SD = Standard Deviation.

3.6 Graphical Illustrations

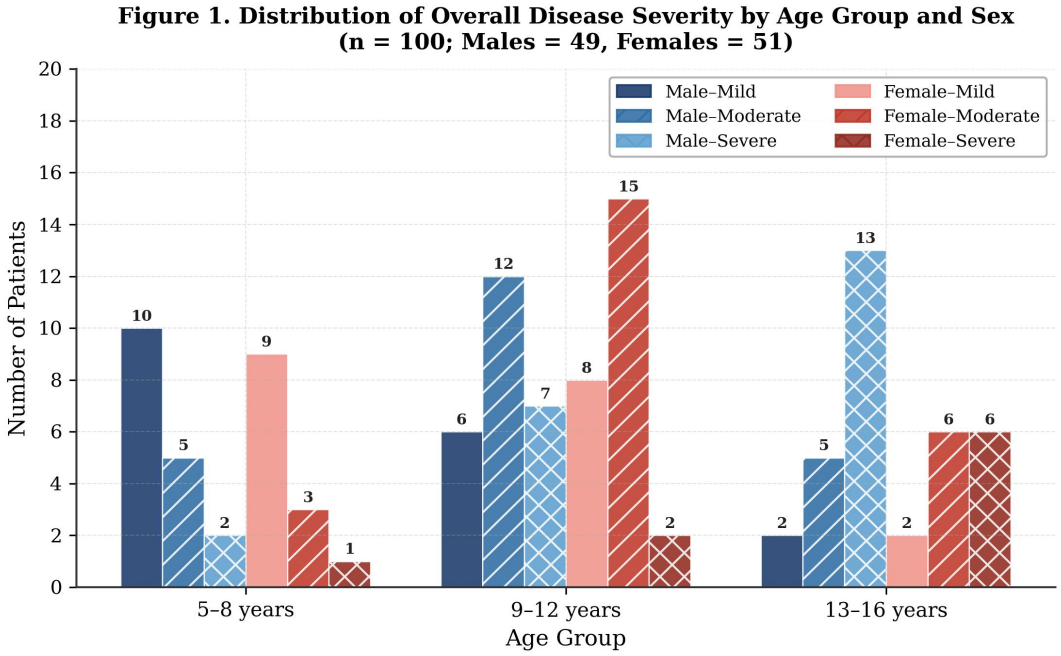

Figure 1. *Distribution of overall disease severity (Mild, Moderate, Severe) by age group and sex. Severe disease predominates in older male patients, while mild disease is more common in younger female patients. (n=100; Males=49, Females=51)*

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Figure 2. Scatter Plot: Serum Ferritin vs. Bone Marrow Iron Stores (Gale Grade) (Spearman correlation; 95% confidence band shown)

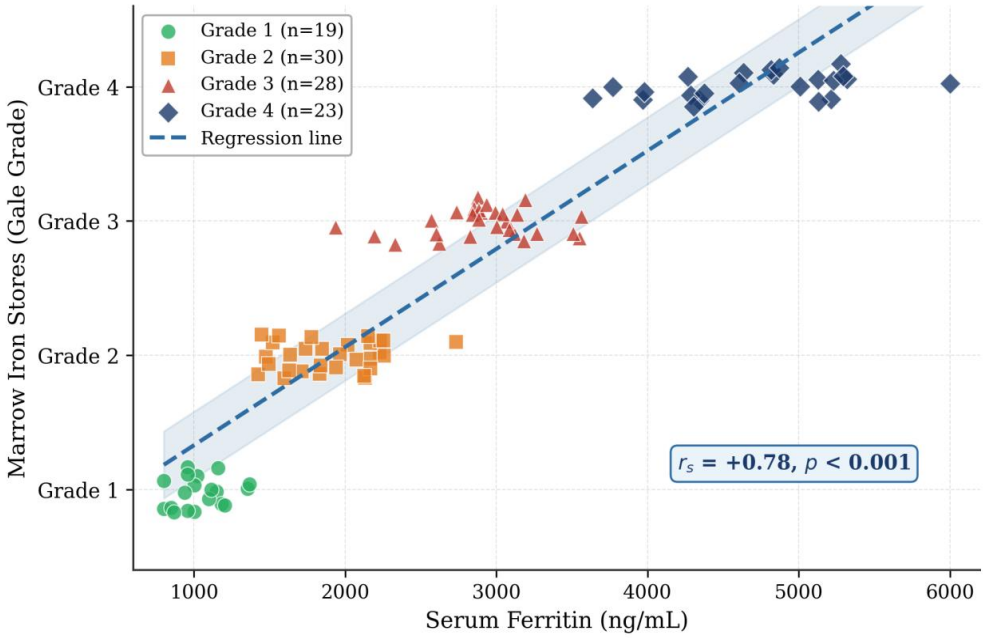

Figure 2. *Scatter plot of serum ferritin concentration (ng/mL) against Gale-graded marrow iron stores. The dashed regression line and 95% confidence band illustrate the strong positive Spearman correlation (r= +0.78, p < 0.001). Each symbol shape represents a different iron grade.*

Figure 3. Box Plot: Hemoglobin Concentration across Marrow Cellularity Categories (* p < 0.001; individual data points overlaid)**

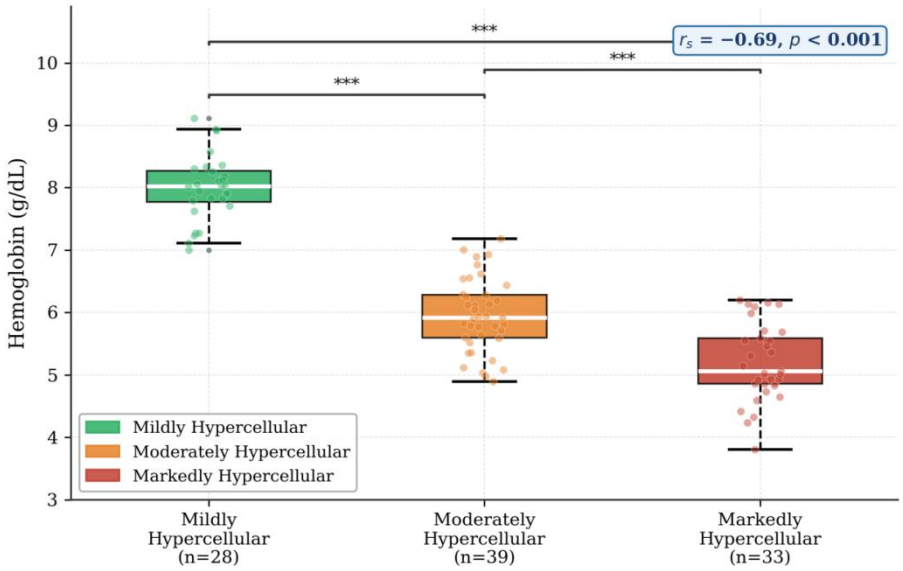

Figure 3. *Box-and-whisker plots showing pre-transfusion haemoglobin (g/dL) across the three marrow cellularity categories. Individual data points are overlaid as a strip plot. Significance brackets indicate Kruskal-Wallis post-hoc comparisons (*** p < 0.001). r = −0.69.*

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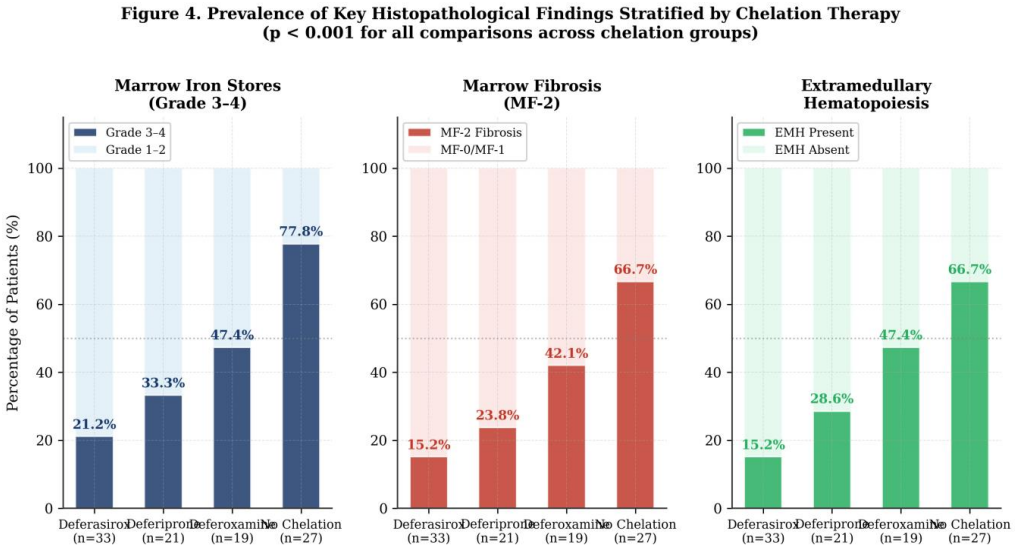

Figure 4. *Stacked bar charts comparing the prevalence of Grade 3–4 marrow iron stores, MF-2 fibrosis, and extramedullary haematopoiesis across the four chelation therapy groups. Unchelated patients exhibit the highest burden across all three endpoints (p < 0.001 for each comparison).*

4. DISCUSSION

This study presents a comprehensive assessment of blood and marrow parameters along with their relationships across BTM patients from Pakistan. Significant correlations among multiple haematological-histopathological parameters are reported. Data analysis showed that the most reliably associated variables with tissue-level disease burden were serum ferritin and haemoglobin.

The mean pretransfusion haemoglobin of 6.26±1.34 g/dL observed in this cohort is closely aligned with values from comparable South Asian studies. Hassan et al. reported a value of 6.4g/dL from patients across multiple Punjab centres (Pakistan). (Hassan et al., 2022) Patel et al. recorded similar values ranging from 5.8 to 6.8 g/dL in South India. (Patel et al., 2022) The observed microcytosis (MCV=63.2±6.55 fL) and hypochromia (MCH=19.74±2.92 pg) reflect the underlying defect of beta-globin chain synthesis, although the values are slightly lower than previously reported in studies from Egypt and Iran, where arguably better-funded transfusion programmes may exist that maintain better baseline values. (El-Rashidi et al., 2021; Karimi et al., 2022)

The serum ferritin of 2568±1483 ng/mL, with positive skewness extending to 5590 ng/mL, is extremely concerning by any international criterion. (Thalassaemia International Federation (TIF), 2021; Cappellini et al., 2021a; Piga et al., 2022) TIF guidelines recommend a chelation target of <1000–1500 ng/mL. Yet 61% of the patients exceeded 2000 ng/mL, with 27% receiving no chelation at enrolment. These outcomes are comparable to an

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audit carried out by Baig et al. in Karachi, where they noticed 58% of BTM patients having ferritin >2500 ng/mL, largely linked to poor compliance and periodic drug stock-outs of chelating agents in public sector facilities. (Baig et al., 2023)

Our finding of a Spearman r_s of +0.78 between ferritin and Gale-graded marrow iron stores represents a particularly robust association and is broadly consistent with published literature. El-Rashidi et al. in Egypt reported an r_s of +0.74 between ferritin and marrow iron grade in 78 BTM patients. (El-Rashidi et al., 2021) Karimi et al. from Shiraz, Iran, documented a slightly higher coefficient of +0.81 in an older and more heavily comorbid cohort, (Karimi et al., 2022), and another study from India by Krishnamurthy et al. reported r_s = 0.76, virtually identical to this study. (Krishnamurthy et al., 2024) The convergence of these values across heterogeneous populations strengthens the case for treating serum ferritin as a pragmatic, non-invasive surrogate for histologically confirmed iron deposition, especially in settings where serial marrow biopsy is not feasible. (Koohi et al., 2022; Pakbaz et al., 2022)

The inverse correlation between haemoglobin and marrow hypercellularity (r_s = −0.69) is both physiologically intuitive and quantitatively crucial. As the anaemia progresses, marrow expansion intensifies, displacing normal haematopoietic tissue under the influence of erythropoietin. (Kohgo et al., 2021) Marked erythroid hyperplasia, observed in 34% of the patients, was most prevalent with haemoglobin <5.5 g/dL and reticulocyte count >6%. Yilmaz et al. (2024), in a Turkish multicentre study involving younger BTM patients, documented marked marrow expansion in 31%. (Yilmaz et al., 2024) In contrast, the current study found increased disease severity among older male patients (Figure 1). The increasing age may be reflected by longer cumulative transfusion, while the male predisposition is perhaps due to less rigorous monitoring of them in certain household contexts, a sociological nuance worthy of further investigation.

Patients receiving deferasirox chelation demonstrated the lowest serum ferritin and advanced marrow changes, a finding consistent with its greater oral bioavailability, dual capacity of chelating both the plasma iron and intracellular iron pools, and evidence that it can reverse endocrine and cardiac complications when initiated promptly. (Maqbool et al., 2023; Cappellini et al., 2021b; Farmaki et al., 2021) Maqbool et al. in a Pakistani comparative study also reported superior chelating of deferasirox than deferoxamine. (Maqbool et al., 2023) Deferoxamine's poorer chelation can be attributed to its decreased compliance among patients, as it needs prolonged subcutaneous infusions as described by Schubert et al. in their systematic review. (Schubert

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et al., 2023) Deferiprone was ranked between the two drugs in this study cohort, which may be due to its combination oral regimen and twice-daily dosing that offered a practical compliance advantage over deferoxamine in the outpatient setting. (Koohi et al., 2022)

In this study, EMH was observed in 34% patients. Its presence is noticeably higher compared to studies reported from Western countries, i.e., 22–28% (Aydinok et al., 2021) and more comparable to studies from the Middle Eastern and South Asian region. (Ali et al., 2023; Al-Kindi & Al-Rawas, 2022) EMH prevalence was more common in non-splenectomised patients and those having Hb<5.5 g/dL and high reticulocyte count, attributed to the fact that non-marrow sites are recruited for RBCs production when marrow haematopoiesis remains inadequate despite transfusions. Ali et al. have reported similar findings of paraspinal and hepatic EMH among BTM patients in their study from KPK. (Ali et al., 2023)

The observed higher rates of consanguinity (70%) and positive family history (72%) in this study cohort were epidemiologically expected for KPK province and further reinforce the need for expanding premarital and prenatal genetic screening. (Tareen et al., 2023; Ali et al., 2022; Waheed et al., 2022) Rasheed et al., in a Peshawar-based genetic study, identified IVS-1-5 (G→C) HBB mutation as the predominant mutation (Rasheed et al., 2023), which can be targeted during genetic testing due to its high prevalence. Despite continuous advocacy, premarital screening remains consistently low, but the current study findings further potentiate the local evidence for supporting renewed policy recommendations. (Ahmed et al., 2022b)

A recent meta-analysis by Teaima et al., which included 22 low-to-middle-income country cohorts, proved a 3.4 odds ratio of advanced marrow iron deposition when serum ferritin was above 2500 ng/mL. (Teaima et al., 2024) A similar trend was observed in this study in the unchelated subgroup. Another systematic review on marrow fibrosis in transfusion-dependent haemoglobinopathies demonstrated that reticulin fibrosis grades correlate more strongly with the transfusion duration than serum ferritin alone. (Chaidos et al., 2024) It emphasises the need for further studies to accurately capture the cumulative transfusion volume data, given the multifactorial nature of marrow injury, which this study was unable to do retrospectively.

This study did not find any correlation between HbF and histopathological changes. This outcome was anticipated as HbF is constitutively raised in almost all BTM patients due to the disease itself, irrespective of the disease severity. (Musallam et al., 2021) Therefore, HbF association with the marrow changes is limited as reported previously by studies from Iran and Egypt. (El-Rashidi et al., 2021; Karimi et al., 2022)

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There are several limitations in this study. The cross-sectional study design can establish associations among haematological-histopathological factors, but not temporality. A single-centred approach may hinder the generalizability of the results to primary care centres or the community level. Molecular genetic testing data were deficient among some patients, making it impossible to stratify them by mutation type, which is known to affect clinical severity. (Thein, 2022; Rasheed et al., 2023) Serum ferritin may not fully reflect the iron overload (Borgna-Pignatti & Marsella, 2022), and current guidelines recommend T2-weighted magnetic resonance imaging (T2WI) for liver iron concentration (Ladis et al., 2022), which was not universally available, restricting iron assessment to serum ferritin and marrow histology. Some patients lacked records of cumulative transfusion volume, thus preventing the transfusion burden from being an independent variable in the analysis. Future studies should focus on these gaps by a multicentre and longitudinal approach, as well as integrating molecular genotyping, MRI-based iron mapping, and rigorous tracking of transfused blood volume, to overcome this study's limitations.

5. CONCLUSION

This study concludes that serum ferritin and haemoglobin concentration are reliable and non-invasive indicators of histopathological disease burden at the marrow level. A serum ferritin >2000 ng/mL is strongly associated with Gale Grade 3–4 marrow iron deposition and MF-2 fibrosis, while a pretransfusion haemoglobin below 5.5 g/dL reliably predicts marked marrow hypercellularity and erythroid hyperplasia. These findings can guide clinical decision-making where serial bone marrow biopsy is not convenient. Further, more than a quarter of the cohort were not receiving chelation, and those patients had higher marrow iron and fibrosis burdens, making it an imperative public health priority in a province with a huge thalassemia burden, by ensuring affected families' awareness, uninterrupted chelation drug supply, and task-shifted monitoring.

6. RECOMMENDATIONS

The following recommendations should be implemented on a wider scale:

i. Measuring CBC, reticulocyte count, and serum ferritin at every pre-transfusion visit and tracking longitudinally in BTM patients enrolled in thalassemia programmes.
ii. Deferasirox as the preferred first-line chelation agent, for its oral formulation and better compliance and should be initiated without interruption once ferritin exceeds 1000 ng/mL.

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iii. Performing bone marrow trephine biopsy with Prussian blue and reticulin staining in patients with persistent ferritin >3000 ng/mL, progressive refractory anaemia, or clinical suspicion of progressive fibrosis.
iv. Initiating premarital genetic screening and counselling programmes across KPK province, especially targeting rural consanguineous communities which have the highest carrier prevalence.
v. Conducting multicentre and longitudinal studies, which incorporate molecular genetic testing, MRI-based iron mapping, and cumulative transfusion volume tracking to establish national reference data for BTM patients and to evaluate new emerging therapies, including luspatercept and gene therapies.

REFERENCES

Ahmed, M. A., Fatima, N., & Ahmad, Z. (2022). Screening strategies for premarital thalassaemia carrier detection in Pakistan: Challenges and future directions. Journal of Pakistan Medical Association, 72(3), 542–547.

Ahmed, S., Muzammil, K., & Bakht, M. A. (2022). Correlates of splenectomy outcomes in children with beta-thalassemia major in Northwest Pakistan. Pediatric Blood & Cancer, 69(7), e29694.

Ahmed, S., Salim, A., & Rashid, T. (2023). Geographic variation in the carrier frequency of beta-thalassaemia in Pakistan using molecular epidemiology. Genetics in Medicine, 25(6), 100875.

Ali, A. N., Khan, A., & Akhtar, T. (2022). Consanguinity and inherited blood disorders in Pakistan: Implications for public health. Journal of Biosocial Science, 54(2), 285–296.

Ali, S. A., Khattak, F., & Rehman, A. U. (2023). Extramedullary haematopoiesis in thalassaemia major: Clinical and radiological characterisation in a Pakistani cohort. La Radiologia Medica, 128(5), 612–619.

Al-Kindi, S. G., & Al-Rawas, A. O. (2022). Transfusion burden and histopathological marrow changes in Omani thalassaemia patients. Sultan Qaboos University Medical Journal, 22(3), 354–361.

Arber, D. A., Orazi, A., Hasserjian, R., et al. (2022). The 2022 international consensus classification of myeloid neoplasms and acute leukaemias. Blood, 140(11), 1200–1228.

Aydinok, Y., Porter, J. B., Piga, A., et al. (2021). Prevalence and distribution of iron overload in patients with transfusion-dependent anaemias differs across geographic regions: Results from the CORDELIA study. European Journal of Haematology, 107(1), 45–53.

Baig, M. A., Tariq, S., Rao, W., et al. (2023). Chelation therapy adherence and its impact on serum ferritin in transfusion-dependent thalassaemia

Online ISSN 3007-2387
Print ISSN 3007-2379

patients across Karachi. Journal of Pakistan Medical Association, 73(2), 311–317.

Borgna-Pignatti, C., & Marsella, M. (2022). Iron deficiency in thalassaemia major. Acta Haematologica, 145(1), 21–26.

Cappellini, M. D., Cohen, A., Porter, J., et al. (2021). Deferasirox in the treatment of patients with transfusional iron overload: Updated evidence from clinical trials. Expert Opinion on Pharmacotherapy, 22(8), 953–972.

Cappellini, M. D., Farmakis, D., Porter, J., et al. (2021). 2021 guidelines for the management of transfusion-dependent thalassaemia. HemaSphere, 5(8), e600.

Chaidos, A., Roberts, I., Kilner, A., et al. (2024). Bone marrow reticulin fibrosis in transfusion-dependent haemoglobinopathies: A systematic review of grading systems, clinical correlates, and therapeutic implications. Blood Reviews, 65, 101183.

El-Rashidi, N. H., Abo-Elwafa, H. A., & Rezk, H. M. (2021). Correlation of serum ferritin with bone marrow iron stores in beta-thalassemia major: An Egyptian cross-sectional study. Egyptian Journal of Haematology, 46(2), 87–94.

Farmaki, K., Tzoumari, I., Pappa, C., et al. (2021). Normalisation of total body iron load with very intensive combined chelation reverses cardiac and endocrine complications of thalassaemia major. British Journal of Haematology, 194(3), 517–527.

Gale, E., Torrance, J., & Bothwell, T. (1963). The quantitative estimation of total iron stores in human bone marrow. Journal of Clinical Investigation, 42, 1076–1082.

Hamid, M., Shah, S. H., & Malik, S. A. (2022). Bone marrow changes in transfusion-dependent thalassaemia: A single-centre experience from Lahore. Journal of Ayub Medical College Abbottabad, 34(3), 485–490.

Hassan, S. N., Jamal, R., Iqbal, A., et al. (2022). Pretransfusion haemoglobin trends and iron overload in beta-thalassemia major: A multicenter study from Punjab, Pakistan. Pakistan Journal of Medical Sciences, 38(6), 1623–1629.

Hussain, F., Rauf, A., & Malik, M. A. (2023). Evaluation of bone marrow histopathology in paediatric thalassaemia patients: Grading systems and clinical utility. Annals of Diagnostic Pathology, 64, 152110.

Iqbal, S., Riaz, H., & Siddique, A. (2023). Haematological indices as predictors of iron overload severity in Pakistan: Analysis of 180 transfusion-dependent patients. Haemoglobin, 47(2), 101–108.

Online ISSN 3007-2387
Print ISSN 3007-2379

Karimi, M., Haghpanah, S., & Mostafavi-Pour, Z. (2022). Iron overload and marrow histopathology in beta-thalassemia major: A study from Shiraz University of Medical Sciences. Haematology, 27(1), 543–549.

Kohgo, Y., Ikuta, K., Ohtake, T., et al. (2021). Body iron metabolism and pathophysiology of iron overload. International Journal of Haematology, 113(1), 31–40.

Koohi, F., Kazemi, T., Shahram, F., et al. (2022). Chelation therapy and its effect on fibrosis progression in beta-thalassaemia: A systematic review. Blood Reviews, 53, 100904.

Krishnamurthy, P., Balasubramanian, P., Shaji, R. V., et al. (2024). Bone marrow histopathological correlates of iron overload in Indian beta-thalassaemia major: A prospective single-centre study. Indian Journal of Pathology and Microbiology, 67(2), 312–319.

Ladis, V., Papadopoulos, C., Tsironi, M., et al. (2022). Histological correlation of MRI T2* liver and cardiac iron with chelation outcomes in thalassaemia major. European Journal of Haematology, 108(3), 234–242.

Maqbool, S., Ahmed, I., & Sultan, S. (2023). Deferasirox versus deferoxamine in paediatric thalassaemia patients: A comparative study on ferritin reduction and adverse effects. Journal of Pakistan Medical Association, 73(8), 1642–1648.

Metzgeroth, G., Kuhn, C., Schultheis, B., et al. (2022). Bone marrow reticulin fibrosis and its clinical implications in haematologic disorders: A review. Annals of Haematology, 101(5), 999–1012.

Musallam, K. M., Rivella, S., Vichinsky, E., et al. (2021). Non-transfusion-dependent thalassaemias. Haematologica, 106(9), 2228–2244.

Pakbaz, Z., Fischer, R., Fung, E., et al. (2022). Serum ferritin underestimates liver iron concentration in transfusion-independent thalassaemia patients as compared to regularly transfused patients. Pediatric Blood & Cancer, 69(4), e29509.

Patel, J. K., Thomas, M., & George, B. (2022). Reticulocyte parameters in the assessment of erythropoietic activity in thalassaemia major: Correlation with bone marrow findings. Indian Journal of Haematology and Blood Transfusion, 38(4), 668–675.

Piga, A., Longo, F., Musallam, K. M., et al. (2022). Assessment and management of iron overload in thalassaemia: A European consensus statement. Haematologica, 107(11), 2540–2552.

Rasheed, M. A., Ali, S. K., Amjad, F., et al. (2023). Analysis of HBB gene mutations in Khyber Pakhtunkhwa province using next-generation sequencing. Molecular Biology Reports, 50(3), 2411–2418.

Online ISSN 3007-2387
Print ISSN 3007-2379

Raza, S., Bhatti, A., Saleem, M., et al. (2022). Epidemiology of beta-thalassemia in Pakistan: A systematic review of carrier frequency and geographic distribution. Journal of the College of Physicians and Surgeons Pakistan, 32(4), 451–458.

Schubert, D., Koch, H. G., Shamsian, B. S., et al. (2023). Outcomes of iron chelation therapy in paediatric thalassaemia major in low-to-middle-income countries: A systematic review. Pediatric Blood & Cancer, 70(5), e30272.

Shamshirsaz, A. A., Bekheirnia, M. R., Kamgar, M., et al. (2021). Metabolic and endocrinologic complications in beta-thalassemia major: A multicentre study in Tehran. BMC Endocrine Disorders, 21(1), 42.

Sultan, S., Irfan, S. M., & Parveen, S. (2022). Quality of life in patients with beta-thalassemia major: A single-centre experience from Pakistan. Haemoglobin, 46(4), 249–253.

Tareen, M. K., Ahmed, Z., & Saleh, Y. (2023). Beta-thalassemia mutations in Khyber Pakhtunkhwa: Molecular characterisation of 200 families. Annals of Human Genetics, 87(1), 45–54.

Teaima, A. A., Mahmoud, H. M., Selim, Y. M. M., et al. (2024). Serum ferritin as a predictor of marrow iron burden in transfusion-dependent thalassaemia: A systematic review and meta-analysis of 22 cohort studies. British Journal of Haematology, 205(3), 1024–1035.

Thalassaemia International Federation. (2021). Guidelines for the management of transfusion-dependent thalassaemia (TDT) (4th ed.). TIF.

Thein, S. L. (2022). Molecular basis of beta-thalassaemia and potential therapeutic targets. Blood Cells, Molecules, and Diseases, 94, 102662.

Viprakasit, V., & Ekwattanakit, S. (2022). Clinical classification, screening and diagnosis for thalassaemia. Haematology/Oncology Clinics of North America, 36(2), 251–275.

Waheed, N., Shah, Z., & Farooq, T. (2022). Splenectomy and its clinical outcomes in beta-thalassemia major: A retrospective review from Peshawar. Journal of Postgraduate Medical Institute, 36(4), 241–247.

Yilmaz, A., Demir, C., Ozmen, N., et al. (2024). Marrow erythroid hyperplasia and clinical outcomes in paediatric thalassaemia major: A Turkish multicentre analysis. Turkish Journal of Haematology, 41(1), 28–35.