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Prevalence and Patterns of Iron Deficiency in Pediatric Beta Thalassemia Trait: Diagnostic Challenges in Overlapping Conditions

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Abstract

Background: *Iron deficiency (ID) and beta thalassemia trait (BTT) are common causes of microcytic hypochromic anemia in children, but their coexistence poses diagnostic challenges. Overlapping hematological features may lead to misclassification, delaying targeted management. This study aimed to determine the prevalence and patterns of iron deficiency in pediatric patients with BTT.*

Methods: *This cross-sectional study was conducted at Thalassemia and DNA laboratory in the Department of Biochemistry and Molecular Biology of Bangladesh Shishu Hospital and Institute, Dhaka, Bangladesh, from January 2024 to July 2025. A total of 155 children aged 6 months to 12 years with microcytic anemia were enrolled. Patients with chronic illness, recent blood transfusion, or acute infection were excluded. Hematological parameters were measured using an automated hematology analyzer, while iron status was assessed by serum iron, total iron-binding capacity (TIBC) and ferritin. Hemoglobin electrophoresis was performed to detect BTT (HbA_2 ≥3.5% and HbF<10%). Data were analyzed using SPSS version 25.*

Results: *The mean age was 2.1 ± 1.8 years, with male predominance (65.8%). The mean hemoglobin was 7.2 ± 1.3 g/dL and red cell indices showed microcytic hypochromic anemia. Ferritin levels indicated iron deficiency in 50.3% (n = 78), while 49.7% had normal iron stores. BTT was present in 4.5% (n = 7) of cases. Both ID and BTT contributed significantly to microcytic hypochromic anemia in this cohort.*

Conclusion: *Iron deficiency is highly prevalent among children with microcytic hypochromic anemia and a small proportion has beta thalassemia trait. Accurate differentiation between these conditions is crucial for appropriate management and genetic counseling.*

Keywords: *Iron deficiency, Beta thalassemia trait, Microcytic hypochromic anemia, Ferritin, Pediatric anemia*

1. Introduction

Beta thalassemia is one of the most common inherited hemoglobin disorders worldwide, resulting from mutations in the β-globin gene that lead to reduced or absent β-globin chain synthesis [1]. The heterozygous form, known as beta thalassemia trait (BTT), is usually asymptomatic but is characterized by microcytosis, hypochromia and a mild anemia [2]. While BTT itself does not cause severe clinical problems, its accurate diagnosis is essential for genetic counseling and prevention of birth of severe forms such as beta thalassemia major or hemoglobin E beta thalassemia. In populations with a high prevalence of BTT, screening programs are crucial for early identification and carrier detection [3].

Iron deficiency (ID) is the most prevalent nutritional deficiency globally, particularly affecting children in developing countries due to inadequate dietary intake, poor absorption and chronic blood loss from infections or parasitic infestations [4]. Like BTT, iron deficiency anemia (IDA) presents with microcytosis and hypochromia, making it difficult to distinguish between the two solely on the basis of red cell indices [5]. The coexistence of ID and BTT poses an additional diagnostic challenge: iron deficiency can lower HbA_2 levels, potentially masking the presence of BTT, while failure to recognize iron deficiency in a child with BTT can lead to untreated anemia and its sequelae [6]. Differentiating between IDA and BTT has significant clinical and public health implications. In BTT, unnecessary iron therapy offers no benefit and may cause iron overload, while in IDA, delayed diagnosis and treatment can impair growth, cognitive development and immunity in children [7]. Therefore, a careful evaluation using complete blood count (CBC), red cell indices, iron profile and hemoglobin electrophoresis or high-performance liquid chromatography (HPLC) is required to correctly classify the etiology of microcytic anemia [8].

Previous studies have shown variable prevalence rates of iron deficiency among BT carriers, ranging from 20% to over 50%, depending on population characteristics, nutritional status and screening methods [9]. In many developing countries, particularly in South Asia, the burden of both ID and BTT is high due to genetic predisposition, nutritional insufficiency and limited access to preventive programs [10]. Bangladesh, situated in the "thalassemia belt," faces a dual challenge of high carrier frequency and widespread iron deficiency among children [11]. Despite this, there is a paucity of data on the co-occurrence of these two conditions in pediatric populations, which can lead to misclassification and missed opportunities for targeted interventions.

In pediatric practice, accurate identification of BTT is particularly important not only for immediate management but also for future reproductive planning, as carriers may unknowingly marry other carriers, leading to offspring with beta thalassemia major, hemoglobin E beta thalassemia or other hemoglobinopathies [3]. At the same time, timely detection and correction of iron deficiency is essential to prevent irreversible neurodevelopmental consequences. Overlapping hematological features and biochemical variations, especially in the presence of dual pathology, underscore the need for comprehensive diagnostic protocols [12].

Given the limited data from Bangladesh on the prevalence and pattern of iron deficiency in children with BTT, there is a clear need for research that addresses this gap. This study aimed to determine the prevalence of iron deficiency among pediatric patients diagnosed with BTT, to describe the hematological and biochemical patterns in such cases and to highlight the diagnostic pitfalls when these two conditions coexist. Understanding these patterns will aid clinicians in formulating accurate diagnoses, avoiding unnecessary treatments and implementing effective public health strategies for both iron deficiency and thalassemia prevention.

2. Methodology & Materials

This cross-sectional study was conducted at the Thalassemia and DNA Laboratory of Department of Biochemistry and Molecular Biology of Bangladesh Shishu Hospital and Institute, Dhaka, Bangladesh, from January 2024 to July 2025. A total of 155 pediatric patients, aged between 6 months and 12 years, presenting with microcytic hypochromic anemia were included. Microcytic hypochromic anemia was defined as hemoglobin concentration below the specific reference values with low mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) according to age and sex of the individuals. Patients with chronic illnesses, recent blood transfusions, or acute infections at the time of sampling were excluded to avoid confounding factors.

Venous blood samples were collected under aseptic conditions. Complete blood count (CBC) parameters including red blood cell indices-RBC count, hemoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC)] and red cell distribution width (RDW) were determined using an automated hematology analyzer. Serum iron and total iron-binding capacity (TIBC) were measured by standard spectrophotometric methods and serum ferritin concentration was determined using enzyme-linked immunosorbent assay (ELISA). Iron deficiency was defined as serum ferritin less than 15 ng/mL. Beta thalassemia trait (BTT) screening was performed using high-performance liquid chromatography (HPLC) for hemoglobin fractionations, with $HbA_2 \geq 3.5\%$ and HbF<10% considered diagnostic.

All instruments were calibrated daily and internal quality control measures were strictly followed to ensure accuracy and reliability of results. Data were recorded in a structured case record form and entered into IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp., Armonk, NY, USA) for analysis. Descriptive statistics were calculated for all variables and results were expressed as mean ± standard deviation (SD) for continuous data and as frequencies and percentages for categorical data.

3. RESULTS

Table 1. *Age Distribution (n = 155)*

Age Group	Frequency (n)	Percentage (%)
<1 year	45	29.00%
1–2 years	72	46.50%
2–5 years	23	14.80%
>5 years	15	9.70%

Table 1 shows the age distribution of the 155 pediatric patients with beta thalassemia trait included in the study. The majority of patients were between 1–2 years of age (72 cases, 46.5%), followed by those <1 year (45 cases, 29.0%). Children aged 2–5 years accounted for 23 cases (14.8%), while the smallest group was those >5 years old (15 cases, 9.7%).

Table 2. *Sex Distribution*

Sex	Frequency (n)	Percentage (%)
Male	102	65.80%
Female	53	34.20%

Table 2 presents the sex distribution of the study patients. Out of the 155 participants, 102 (65.8%) were male and 53 (34.2%) were female, indicating a male predominance among the study population.

Table 3. *Hematological Parameters of the Study Patients*

Parameter	Mean ± SD	Range
RBC ($\times 10^6/\mu L$)	4.48 ± 0.72	1.78–6.40
Hb (g/dL)*	7.2 ± 1.3	3.1–10.6
HCT (%)	27.6 ± 4.5	11.9–39.6
MCV (fL)	62.1 ± 5.8	48.1–91.6
MCH (pg)	16.3 ± 2.4	11.6–24.8
MCHC (g/dL)	26.3 ± 2.6	19.7–39.2
RDW (%)	22.1 ± 2.7	14.5–28.2
Platelet ($\times 10^3/\mu L$)	388 ± 158	109–878

Table 3 summarizes the hematological parameters of the study population. The mean red blood cell (RBC) count was 4.48 ± 0.72 × 10^6/µL (range: 1.78–6.40), with a mean hemoglobin (Hb) level of 7.2 ± 1.3 g/dL (range: 3.1–10.6), indicating overall anemia among participants. The hematocrit (HCT) averaged 27.6 ± 4.5%, while mean corpuscular volume (MCV) was 62.1 ± 5.8 fL, reflecting microcytosis. Mean corpuscular hemoglobin (MCH) was 16.3 ± 2.4 pg and mean corpuscular hemoglobin concentration (MCHC) averaged 26.3 ± 2.6 g/dL, both below normal limits indicating hypochromia. The red cell distribution width (RDW) was elevated (22.1 ± 2.7%), consistent with anisocytosis. Platelet counts varied widely, with a mean of 388 ± 158 × 10^3/µL (range: 109–878).

Table 4. *Iron Profile Parameters*

Parameter	Mean ± SD	Range
Serum Iron (µg/dL)	27.4 ± 38.1	8–347
TIBC (µg/dL)	404 ± 123	134–732
Ferritin (ng/mL)	46.2 ± 142.6	1–637

Table 4 presents the iron profile parameters of the study patients. The mean serum iron level was 27.4 ± 38.1 µg/dL (range: 8–347), indicating that many participants had markedly reduced iron stores, although a few outliers with high values increased the mean. Total iron-binding capacity (TIBC) averaged 404 ± 123 µg/dL (range: 134–732), reflecting increased binding capacity in iron-deficient states. The mean ferritin level was 46.2 ± 142.6 ng/mL (range: 1–637), with a wide distribution suggesting that while most patients had depleted iron stores, some had normal or elevated ferritin, possibly due to inflammation or coexisting conditions.

Table 5. *Iron Deficiency Status (Ferritin <15 ng/mL)*

Iron Deficiency	Frequency (n)	Percentage (%)
Deficient	78	50.30%
Normal	77	49.70%

Table 5 shows the iron deficiency status of the study population, classified based on serum ferritin levels (<15 ng/mL indicating deficiency). Out of 155 participants, 78 patients (50.3%) were iron deficient, while 77 patients (49.7%) had normal ferritin levels.

Table 6. *Beta Thalassemia Trait (HbA2 ≥3.5%, HbF <10%)*

BTT Diagnosis	Frequency (n)	Percentage (%)
Present	7	4.50%
Absent	148	95.50%

Table 6 presents the distribution of beta thalassemia trait (BTT) among the study participants, diagnosed based on HbA_2 levels ≥3.5% and HbF <10% Out of 155 children, 7 patients (4.5%) were identified as having BTT, while the vast majority, 148 patients (95.5%), did not exhibit the trait.

Table 7. *Hematological and Iron Profile Parameters in Children with Iron Deficiency Alone and Those with Iron Deficiency plus Beta Thalassemia Trait*

Parameter	Iron Deficiency Only (n = 71)	Iron Deficiency + BTT (n = 7)	p-value
RBC count (×10⁶/µL)	4.21 ± 0.65	5.12 ± 0.58	0.002 **
Hemoglobin (g/dL)	7.1 ± 1.2	7.3 ± 1.1	0.62
Serum Iron (µg/dL)	25.2 ± 14.5	22.8 ± 12.1	0.58
TIBC (µg/dL)	410 ± 105	428 ± 98	0.47

Table 7 summarizes the hematological and iron profile parameters in children with iron deficiency alone and those with iron deficiency coexisting with beta thalassemia trait (BTT). The mean red blood cell (RBC) count was significantly higher in the ID + BTT group compared to children with iron deficiency only (5.12 ± 0.58 vs. 4.21 ± 0.65 ×10⁶/µL; p = 0.002), reflecting the compensatory erythropoiesis typically seen in thalassemia carriers. In contrast, hemoglobin levels were comparable between the two groups (7.3 ± 1.1 vs. 7.1 ± 1.2 g/dL; p = 0.62). Similarly, serum iron (22.8 ± 12.1 vs. 25.2 ± 14.5 µg/dL; p = 0.58) and total iron-binding capacity (TIBC) (428 ± 98 vs. 410 ± 105 µg/dL; p = 0.47) did not differ significantly, indicating that the severity of iron deficiency was similar in both groups.

4. Discussion

The present study investigated the prevalence and patterns of iron deficiency among pediatric patients with or without beta thalassemia trait (BTT) in a tertiary care setting in Bangladesh, highlighting the diagnostic challenges posed by overlapping hematological features. Among the 155 children studied, we observed a high prevalence of iron deficiency (50.3%) and a smaller but clinically important proportion with BTT (4.5%). These findings are consistent with the dual burden of nutritional deficiencies and inherited hemoglobin disorders reported in previous Bangladeshi and regional studies [13, 14].

The predominance of male participants (65.8%) aligns with earlier hospital-based studies in Bangladesh, where male children often present more frequently to healthcare facilities, possibly reflecting gender-based health-seeking patterns rather than a true biological difference [15, 16]. The highest proportion of cases occurred in the 1–2 years age group, which may be related to the weaning period, during which children are at increased risk of iron deficiency due to dietary inadequacy and high growth demands [17].

Our hematological findings revealed microcytic, hypochromic anemia with elevated RDW, features characteristic of both IDA and BTT. The overlap in red cell indices underscores the diagnostic challenge emphasized by Jahan et al., who reported that coexisting iron deficiency can lower HbA_2 levels, potentially masking the diagnosis of BTT [18]. Similar diagnostic limitations have been discussed by Laengsri et al., who proposed algorithmic approaches and discriminant indices to improve differentiation [19]. However, these indices often lose sensitivity in the presence of concurrent iron deficiency, highlighting the need for combined hematological and biochemical assessment.

The prevalence of BTT in our cohort (4.5%) is lower than rates reported in community-based screenings in Bangladesh, which range from 8–10% in certain regions [13, 16]. This difference may be due to our

hospital-based sampling, focused on children already suspected of anemia rather than population-level carrier screening. Nonetheless, the presence of BTT in this pediatric group underscores the importance of early detection for genetic counseling, as emphasized by Angastiniotis et al. and Farmakis et al [20, 21].

Iron deficiency was present in half of the children studied, consistent with the high burden of nutritional anemia in Bangladeshi children reported in national surveys and pediatric studies [17, 22]. Our finding of low mean serum iron and elevated TIBC in deficient children aligns with classical biochemical profiles of IDA [23]. The coexistence of ID and BTT in pediatric patients has significant implications: while iron therapy is essential in IDA to prevent neurodevelopmental deficits, inappropriate supplementation in isolated BTT offers no benefit and carries a risk of iron overload [24].

Internationally, similar coexistence rates have been reported. Jahan et al., found that 39% of BTT carriers also had iron deficiency, while McGann et al., observed a dual burden in African pediatric populations, linking nutritional anemia with inherited hemoglobinopathies [18, 25]. These findings reinforce the global nature of this diagnostic dilemma, particularly in low- and middle-income countries. The wide range of ferritin values in our study reflects the heterogeneous iron status in the cohort. As Garcia-Casal et al., note, ferritin remains the most practical biomarker for iron stores but may be influenced by infection or inflammation, common in pediatric populations [26]. This highlights the importance of interpreting ferritin alongside other iron indices and clinical context.

From a diagnostic perspective, the overlap between IDA and BTT continues to challenge for pediatricians. Several discriminant formulas have been proposed to improve differentiation, but their accuracy diminishes when the two conditions coexist [27, 28]. Advanced diagnostic approaches, such as those discussed by Singh et al. and Laengsri et al., may improve screening efficiency, particularly in resource-limited settings [19, 29].

Our study also underscores the need for public health interventions that integrate nutritional programs with hemoglobinopathy screening. As recommended by the Thalassemia International Federation and the SPOG Pediatric Hematology Working Group, targeted strategies should include early childhood screening for both iron deficiency and thalassemia carriers, coupled with parental education and genetic counseling [21, 23]. In Bangladesh, such integrated programs could reduce the burden of undiagnosed carriers and untreated anemia.

The clinical implications of misclassification are significant. Unrecognized BTT may lead to missed opportunities for genetic prevention, while untreated iron deficiency can impair growth and cognitive development [17]. Conversely, unnecessary iron therapy in BTT risks iron overload, which may cause long-term complications, especially in individuals requiring transfusions later in life [24, 30].

5. Limitations of the Study

Limitations of our study include its hospital-based design, which may limit generalizability to the broader pediatric population. Additionally, inflammatory markers were not routinely assessed, which may have influenced ferritin interpretation in children with concurrent illness. Nonetheless, the strength of our study lies in its combined hematological and biochemical approach, providing a comprehensive picture of the co-occurrence of IDA and BTT in Bangladeshi children.

6. Conclusion

Our findings confirm that iron deficiency remains highly prevalent among pediatric patients with microcytic hypochromic anemia and a small but important proportion have BTT. The significant overlap in hematological features demands a careful, multi-parameter diagnostic approach to avoid misclassification. Integrating nutritional interventions with systematic hemoglobinopathy screening in childhood could address both conditions effectively, reducing the clinical and public health burden in Bangladesh.

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Conflicts of Interest

There are no conflicts of interest.

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