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Original Research Article

Role of Estimation of Serum Ferritin, Vitamin B12 and Folic Acid in Management of Beta Thalassemic Children

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Abstract

Background: Beta thalassemia major (β-TM) is a transfusion-dependent hemolytic anemia in which repeated blood transfusions lead to progressive iron overload, while chronic hemolysis and increased erythropoietic turnover raise the demand for hematinic micronutrients such as vitamin B12 and folic acid. Periodic biochemical monitoring of these parameters is central to rational management.

Objective: To estimate serum ferritin, vitamin B12, and folic acid levels in children with beta thalassemia major and to evaluate their relationship with transfusion burden and chelation therapy, thereby defining their role in routine management.

Methods: A hospital-based cross-sectional study was conducted on 60 children (2–14 years) with transfusion-dependent β-TM and 30 age- and sex-matched healthy controls. Serum ferritin was measured by chemiluminescent immunoassay; vitamin B12 and folate were measured by electrochemiluminescence. Data were analyzed using Student's t-test, chi-square test, and Pearson correlation; $p < 0.05$ was considered significant.

Results: Mean serum ferritin was markedly higher in cases than controls (2,864 ± 1,212 ng/mL vs 58 ± 24 ng/mL; $p < 0.001$), and 71.7% of cases had ferritin > 2,000 ng/mL. Mean vitamin B12 (312 ± 138 pg/mL vs 486 ± 152 pg/mL; $p < 0.001$) and serum folate (5.8 ± 2.9 ng/mL vs 9.6 ± 3.1 ng/mL; $p < 0.001$) were significantly lower in cases. Vitamin B12 deficiency was found in 23.3% and folate deficiency in 31.7% of thalassemic children. Ferritin correlated positively with the number of transfusions ($r = 0.68$, $p < 0.001$) and duration of transfusion therapy ($r = 0.61$, $p < 0.001$), and was lower in children with good chelation compliance.

Conclusion: Children with β-TM show significant iron overload alongside frequent subclinical deficiencies of vitamin B12 and folic acid. Serial estimation of serum ferritin should guide chelation therapy, while routine screening and supplementation of vitamin B12 and folic acid should be incorporated into standard thalassemia care protocols.

Keywords: Beta Thalassemia Major; Serum Ferritin; Vitamin B12; Folic Acid; Iron Overload; Chelation Therapy.

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Introduction

Beta thalassemia is one of the most common monogenic disorders worldwide, arising from mutations in the beta-globin gene that reduce or abolish synthesis of beta-globin chains, resulting in ineffective erythropoiesis and chronic hemolytic anemia [1]. It is estimated that about 1.5% of the global population carries a beta thalassemia trait, and approximately 60,000 symptomatic infants are born annually, with the highest burden concentrated in the Mediterranean basin, the Middle East, and South and Southeast Asia [2]

In India, the carrier frequency ranges from 3% to 4%, translating into an estimated 10,000–12,000 affected births each year, making thalassemia a substantial public health problem and a major consumer of pediatric transfusion services [3]. Children with beta thalassemia major (β-TM) typically present within the first two years of life with severe anemia, failure to thrive, and hepatosplenomegaly, and become dependent on lifelong regular blood transfusions to sustain growth and suppress extramedullary hematopoiesis [1,4]. While hypertransfusion regimens have transformed survival, each unit of packed red cells delivers approximately 200–250 mg of elemental iron into a body that has no physiological mechanism for iron excretion [4].

Transfusional hemosiderosis, compounded by increased gastrointestinal iron absorption driven by suppressed hepcidin, leads to progressive iron deposition in the liver, heart, and endocrine glands. Cardiac siderosis remains the leading cause of death, while hepatic fibrosis, diabetes mellitus, hypogonadism, hypothyroidism, and growth failure constitute the major morbidities of iron overload [5,6]. Serum ferritin, although an acute-phase reactant, remains the most widely used, inexpensive, and accessible surrogate marker of total body iron burden; serial ferritin values above 1,000–2,500 ng/mL are used to initiate and intensify iron chelation therapy and are correlated with hepatic iron concentration and adverse outcomes [6,7].

A second, frequently under-recognized dimension of thalassemia care concerns hematinic micronutrients. The markedly expanded and ineffective erythropoiesis of β-TM, with erythroid activity up to 10–15 times normal, dramatically increases the consumption of folic acid, a co-factor essential for DNA synthesis in proliferating erythroblasts [8]. Chronic hemolysis further accelerates folate turnover, and dietary intake in developing countries is often marginal, so subclinical or overt folate deficiency may supervene and aggravate anemia, increase transfusion requirement, and elevate homocysteine levels [8,9]. Vitamin B12, intimately linked with folate metabolism, has similarly been reported to be reduced in a subset of multitransfused thalassemic children, possibly related to increased utilization, poor nutritional intake, and altered gut function [9,10]. Deficiency of either vitamin can produce superimposed megaloblastic changes, neurological symptoms, and a blunted response to transfusion therapy that may be misattributed to the underlying hemoglobinopathy [10].

Despite international guidelines recommending regular monitoring of iron status and nutritional supplementation in thalassemia [4], data from resource-limited settings on the combined assessment of serum ferritin, vitamin B12, and folic acid in the same cohort of children remain sparse. The present study was therefore undertaken to estimate these three parameters in children with β-TM, compare them with healthy controls, correlate them with transfusion and chelation variables, and define their practical role in the routine management of beta thalassemic children.

Materials and Methods

Study Design and Setting: This hospital-based, observational, cross-sectional case–control study was conducted in the Department Of Pathology in collaboration with the Department of Biochemistry of a tertiary care teaching hospital over a period of 18 months, after obtaining approval from the Institutional Ethics Committee. Written informed consent was obtained from the parents or legal guardians of all participants, and consent was taken from children older than 7 years.

Study Population: Sixty children aged 2–14 years with an established diagnosis of beta thalassemia major, confirmed by high-performance liquid chromatography (HPLC) of hemoglobin, who were receiving regular packed red cell transfusions at the thalassemia day-care unit, were enrolled consecutively as cases. Thirty apparently healthy, age- and sex-matched children attending the outpatient department for minor non-hematological complaints served as controls.

Inclusion Criteria: (i) HPLC-confirmed beta thalassemia major; (ii) age 2–14 years; (iii) minimum of 10 lifetime transfusions; (iv) parental consent.

Exclusion Criteria: Children with thalassemia intermedia or other hemoglobinopathies, acute febrile illness or documented infection within the preceding 2 weeks, chronic liver or renal disease unrelated to thalassemia, malignancy, chronic inflammatory disorders, current vitamin B12 or folic acid supplementation within the previous 3 months, and post-splenectomy status less than 6 months old were excluded, since these conditions independently alter ferritin, B12, or folate levels.

Data Collection: A structured proforma was used to record demographic details, age at diagnosis, age at first transfusion, total number and frequency of transfusions, duration of transfusion therapy, type of chelation (deferasirox, deferiprone, or combination), compliance with chelation (good compliance defined as intake ≥ 6 days per week by parental report and pill count), splenectomy status, and anthropometry. Clinical examination documented pallor, icterus, hepatosplenomegaly, and growth parameters plotted on WHO/IAP growth charts.

Laboratory Methods: Under aseptic precautions, 5 mL of venous blood was collected from each participant immediately before a scheduled transfusion (in cases) after an overnight fast.

Complete blood counts were performed on an automated hematology analyzer. Serum ferritin was estimated by chemiluminescent microparticle immunoassay (normal reference: 12–150 ng/mL for children).

Serum vitamin B12 and folate were measured by electrochemiluminescence immunoassay. Vitamin B12 deficiency was defined as < 200 pg/mL, borderline as 200–300 pg/mL; folate deficiency was defined as serum folate < 3 ng/mL and borderline as 3–5 ng/mL. Iron overload was graded

by ferritin as mild (< 1,000 ng/mL), moderate (1,000–2,000 ng/mL), and severe (> 2,000 ng/mL).

Statistical Analysis: Data were compiled in Microsoft Excel and analyzed using SPSS version 26.0. Continuous variables were expressed as mean ± standard deviation and compared using the unpaired Student's t-test; categorical variables were expressed as proportions and compared using the chi-square test. Pearson's correlation coefficient was used to assess the relationship of serum ferritin with transfusion variables and of ferritin with vitamin levels. A p-value < 0.05 was considered statistically significant.

Results

A total of 60 children with beta thalassemia major and 30 healthy controls were studied. The two groups were comparable in age and sex distribution (Table 1).

Table 1: Baseline characteristics of cases and controls

Parameter	Cases (n = 60)	Controls (n = 30)	p-value
Mean age (years)	8.4 ± 3.2	8.1 ± 3.0	0.67
Male : Female	34 : 26	17 : 13	0.98
Weight-for-age < −2 SD, n (%)	26 (43.3%)	3 (10.0%)	0.001
Height-for-age < −2 SD, n (%)	22 (36.7%)	2 (6.7%)	0.002
Hemoglobin (g/dL, pre-transfusion)	7.2 ± 0.9	12.4 ± 1.1	<0.001

Cases and controls were well matched for age and sex, validating the comparison. A significantly higher proportion of thalassemic children were underweight and stunted, reflecting the combined impact of chronic anemia, iron toxicity to endocrine organs, and nutritional deficits.

Table 2: Comparison of serum ferritin, vitamin B12 and folic acid between cases and controls

Parameter	Cases (n = 60)	Controls (n = 30)	p-value
Serum ferritin (ng/mL)	2,864 ± 1,212	58 ± 24	<0.001
Vitamin B12 (pg/mL)	312 ± 138	486 ± 152	<0.001
Serum folate (ng/mL)	5.8 ± 2.9	9.6 ± 3.1	<0.001

Serum ferritin in thalassemic children was nearly fifty times that of controls, confirming severe transfusional iron overload. Conversely, mean vitamin B12 and folate levels were significantly lower in cases, indicating increased consumption of hematinic vitamins by the hyperactive erythron and suboptimal nutritional reserves.

Table 3: Grading of iron overload by serum ferritin among cases (n = 60)

Ferritin category	n (%)
< 1,000 ng/mL (mild)	6 (10.0%)
1,000–2,000 ng/mL (moderate)	11 (18.3%)
> 2,000 ng/mL (severe)	43 (71.7%)

Nearly three-quarters of the children had severe iron overload despite being on chelation, underscoring gaps in chelation adequacy and compliance in this cohort.

Table 4: Vitamin B12 and folate status among cases (n = 60)

Status	Vitamin B12, n (%)	Folate, n (%)
Deficient	14 (23.3%)	19 (31.7%)
Borderline	17 (28.3%)	15 (25.0%)
Normal	29 (48.4%)	26 (43.3%)

Approximately one in four children was frankly B12-deficient and nearly one in three was folate-deficient; more than half had either deficient or borderline levels of at least one vitamin, supporting a policy of routine screening and supplementation.

Table 5: Correlation of serum ferritin with transfusion and chelation variables

Variable	Correlation coefficient (r) / comparison	p-value
Total number of transfusions	r = 0.68	<0.001
Duration of transfusion therapy (years)	r = 0.61	<0.001
Serum folate	r = −0.34	0.008
Serum vitamin B12	r = −0.26	0.045
Ferritin: good vs poor chelation compliance	2,214 ± 986 vs 3,412 ± 1,187 ng/mL	<0.001

Serum ferritin rose significantly with increasing transfusion number and duration, confirming its utility as a cumulative marker of transfusional iron load. Children with good chelation compliance had significantly lower ferritin, demonstrating that ferritin monitoring directly reflects chelation

effectiveness. The modest inverse correlation between ferritin and both vitamins suggests that children with the highest disease and transfusion burden are also at greatest risk of hematinic depletion.

Discussion

The present study demonstrates the triad of severe iron overload, subclinical vitamin B12 deficiency, and folate depletion in multitransfused beta thalassemic children, and highlights the practical value of monitoring all three analytes in routine management.

The mean serum ferritin of 2,864 ng/mL, with 71.7% of children above 2,000 ng/mL, is consistent with reports from other Indian and regional cohorts where the majority of transfusion-dependent children exceed the 2,500 ng/mL threshold associated with cardiac disease and mortality [6,7,11]. Olivieri et al. showed that sustained ferritin below 2,500 ng/mL confers markedly better cardiac disease-free survival, establishing ferritin-guided chelation as a cornerstone of therapy [7]. The strong positive correlation of ferritin with transfusion number and duration observed here mirrors earlier studies and reaffirms that, in resource-limited settings where MRI T2* and liver iron quantification are not routinely available, serial serum ferritin remains a pragmatic and reasonably reliable surveillance tool [6,11]. The significantly lower ferritin in compliant chelation users provides an actionable message: ferritin trends can identify poor compliance early and prompt counselling or a change in chelator [5,12].

The finding of vitamin B12 deficiency in 23.3% and borderline levels in a further 28.3% of cases accords with studies by Mojtahedzadeh et al. and others who documented reduced cobalamin levels in thalassemic patients compared with controls, attributed to accelerated erythropoietic consumption and marginal dietary intake (9,10). Folate deficiency in 31.7% of our cases is similarly in line with the classical observation that chronic hemolytic anemias impose a folate demand several-fold above normal [8]. Even in transfusion-dependent patients, in whom endogenous erythropoiesis is partly suppressed, residual ineffective erythropoiesis and hemolysis continue to consume folate, and international guidelines therefore recommend routine folic acid supplementation in thalassemia syndromes [4,8]. The inverse correlation between ferritin and both vitamins in our cohort suggests that the most heavily transfused children — those with the longest disease duration and the greatest marrow drive — are precisely the subgroup most likely to harbor hematinic deficiency; unrecognized deficiency in these children may manifest as a falling pre-transfusion hemoglobin, shortened inter-transfusion interval, cytopenias, or neurodevelopmental concerns wrongly ascribed to the primary disease [9,10,13].

The clinical implications are threefold. First, serum ferritin should be estimated every three months in all transfusion-dependent children, with chelation initiated or intensified according to established thresholds [4,6]. Second, annual (or symptom-triggered) estimation of vitamin B12 and folate should be integrated into thalassemia day-care protocols, with supplementation of deficient and borderline cases — a low-cost intervention with meaningful hematological benefit [8,9]. Third, growth monitoring must accompany biochemical surveillance, since growth failure in our cohort tracked with severe iron overload, as reported previously [5,14].

The limitations of this study include its cross-sectional design, single-center setting, modest sample size, reliance on ferritin rather than MRI-based iron quantification, and measurement of total serum B12 rather than holotranscobalamin or methylmalonic acid, which are more specific markers of functional deficiency (13). Longitudinal multicentric studies incorporating these markers are warranted.

Conclusion

Children with beta thalassemia major exhibit severe transfusional iron overload, with more than two-thirds showing serum ferritin above 2,000 ng/mL, alongside a high prevalence of vitamin B12 (23.3%) and folic acid (31.7%) deficiency. Serum ferritin rises predictably with transfusion burden and falls with good chelation compliance, validating its role as the principal bedside tool for guiding and monitoring chelation therapy in resource-limited settings. Routine periodic estimation of serum ferritin, together with screening for and supplementation of vitamin B12 and folic acid, should be incorporated as standard components of the comprehensive management of beta thalassemic children to reduce iron-related organ damage, optimize erythropoiesis, and improve growth and quality of life.

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