

Research Article

## Serum Heparin Levels as Predictors of Transfusion Requirement in $\beta$ -Thalassemia Major: Cross-sectional Correlation with Ferritin and Erythropoietic Markers

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### ABSTRACT

#### Background

$\beta$ -Thalassemia major is characterised by ineffective erythropoiesis and lifelong transfusion dependence, leading to progressive iron overload. Although serum ferritin is widely used to assess iron burden, it does not reliably reflect erythropoietic activity or transfusion requirement. Heparin, a key regulator of iron homeostasis, is known to be dysregulated in thalassemia; however, its relationship with transfusion burden remains incompletely defined, particularly in African populations.

#### Objective

To evaluate serum heparin levels in patients with  $\beta$ -thalassemia major and to examine their association with transfusion requirement, serum ferritin, and selected erythropoietic markers.

#### Methods

This cross-sectional study was conducted at tertiary hematology centres in South Africa between January 2023 and December 2024. Patients with confirmed  $\beta$ -thalassemia major receiving regular transfusion therapy were enrolled. Clinical data, transfusion history over the preceding 12 months, and laboratory parameters were collected. Serum heparin was measured using enzyme-linked immunosorbent assay, while ferritin and hematologic indices were assessed using standard laboratory methods. Correlation analyses and multivariable regression were performed to identify predictors of transfusion requirement.

## Results

Seventy-two patients were included in the analysis. Median serum hepcidin levels were low relative to iron burden, while serum ferritin levels were markedly elevated across the cohort. Serum hepcidin demonstrated a significant inverse correlation with annual transfusion volume and transfusion frequency. In contrast, no consistent correlation was observed between serum hepcidin and ferritin levels. Hepcidin also showed a strong inverse association with reticulocyte count and a positive association with pre-transfusion haemoglobin. In multivariable analysis, serum hepcidin remained an independent predictor of transfusion requirement, whereas ferritin did not.

## Conclusions

In patients with  $\beta$ -thalassemia major, serum hepcidin levels reflect erythropoietic activity and transfusion burden rather than iron stores. The dissociation between ferritin and hepcidin highlights the limitations of ferritin as a functional biomarker in this setting. Hepcidin may serve as a useful adjunct marker for assessing disease severity and transfusion dependency, with potential implications for risk stratification and future therapeutic strategies.

*Keywords:*  $\beta$ -thalassemia major; Hepcidin; Transfusion requirement; Ferritin; Ineffective erythropoiesis; iron metabolism

## INTRODUCTION

$\beta$ -Thalassemia major is a severe inherited haemoglobinopathy characterised by defective  $\beta$ -globin synthesis, leading to chronic haemolytic anaemia and transfusion dependence from early childhood. Despite advances in transfusion protocols and iron chelation therapy, the disease continues to impose a substantial clinical and socioeconomic burden, particularly in low- and middle-income countries [1]. In South Africa, where access to specialised haematology services varies between provinces, optimising transfusion strategies remains a critical component of long-term patient management.

Regular red blood cell transfusions are essential to maintain adequate haemoglobin levels, suppress ineffective erythropoiesis, and improve survival in patients with  $\beta$ -thalassemia major. However, chronic transfusion therapy inevitably results in iron overload, which contributes to organ dysfunction involving the liver, heart, and endocrine system [2]. Serum ferritin remains the most commonly used surrogate marker of body iron stores in routine clinical practice, yet it is influenced by inflammation, infection, and liver disease, limiting its reliability as a standalone indicator of iron balance or transfusion need [3].

In recent years, hepcidin, a hepatic peptide hormone that plays a central role in systemic iron homeostasis, has emerged as a promising biomarker in thalassemia. Hepcidin regulates iron absorption from the gut and iron release from macrophages by binding to and inducing degradation of the iron exporter ferroportin [4]. In physiological states, hepcidin expression increases in response to iron overload and inflammation and decreases during anaemia and increased erythropoietic demand. In  $\beta$ -thalassemia major, however, this regulatory pathway is profoundly dysregulated.

Ineffective erythropoiesis is a hallmark of  $\beta$ -thalassemia major and is driven by expanded but functionally ineffective erythroid precursors in the bone marrow. This process leads to excessive production of erythroid-derived suppressors of hepcidin, including erythroferrone, growth differentiation factor-15 (GDF-15), and twisted gastrulation factor-1 (TWG1) [5,6]. As a result, hepcidin levels are inappropriately low relative to iron burden, promoting increased intestinal iron absorption and exacerbating iron overload even in heavily transfused patients [7].

Several international studies have demonstrated altered hepcidin dynamics in thalassemia, with low or inappropriately normal serum hepcidin levels despite markedly elevated ferritin concentrations [8,9]. However, the relationship between hepcidin and transfusion requirement—a clinically relevant outcome—remains incompletely defined. While ferritin reflects cumulative iron exposure, it does not directly capture the balance between erythropoietic drive and transfusion dependency. Hepcidin, by contrast, may reflect real-time interactions between iron availability, marrow activity, and transfusion intensity.

In resource-limited settings such as South Africa, where access to advanced imaging modalities like MRI T2 for iron quantification is restricted, identifying reliable and accessible biomarkers is particularly important. A biomarker that could predict transfusion requirement or stratify patients according to erythropoietic activity would have significant clinical utility. It could assist clinicians in individualising transfusion intervals, anticipating transfusion escalation, and identifying patients at higher risk of iron-related complications.

Furthermore, data on hepcidin in African populations are scarce. Genetic heterogeneity, differences in transfusion practices, variable chelation adherence, and the high background prevalence of inflammatory conditions may influence hepcidin regulation differently compared with European or Asian cohorts [10]. Understanding these dynamics within a South African context is therefore essential before hepcidin can be considered for routine clinical application.

This study was designed to evaluate serum hepcidin levels in patients with  $\beta$ -thalassemia major and to examine their relationship with transfusion requirement, serum ferritin, and selected erythropoietic markers. By employing a cross-sectional design, we aimed to explore whether hepcidin could serve as a meaningful predictor of transfusion burden beyond traditional iron indices. Clarifying this relationship may contribute to improved risk stratification and more rational use of transfusion resources in thalassemia care within South Africa and similar settings.

## **METHODS**

### ***Study Design and Setting***

This study was conducted as a cross-sectional observational study at two tertiary-level public hospitals in South Africa with established haematology services providing long-term care for patients with  $\beta$ -thalassemia major. The study period extended from January 2023 to December 2024. These centres serve as referral units for both urban and rural populations and follow nationally adopted transfusion and chelation protocols.

The primary objective was to assess the relationship between serum hepcidin levels and transfusion requirement in patients with  $\beta$ -thalassemia major and to evaluate correlations with serum ferritin and erythropoietic markers.

### ***Study Population***

Patients were recruited consecutively from outpatient thalassemia clinics during routine follow-up visits.

### ***Inclusion Criteria***

Patients were eligible for inclusion if they met all of the following criteria:

- Confirmed diagnosis of  $\beta$ -thalassemia major based on haemoglobin electrophoresis and/or molecular testing
- Age  $\geq 5$  years
- Regularly transfused (at least one transfusion every 4–6 weeks) for a minimum duration of 12 months
- Clinically stable at the time of enrolment
- On a stable iron chelation regimen for at least 6 months

### ***Exclusion Criteria***

Patients were excluded if they had:

- Evidence of acute infection or inflammation at the time of sampling
- Chronic liver disease unrelated to transfusional iron overload (e.g. viral hepatitis with active disease)
- Renal impairment (estimated GFR  $< 60$  mL/min/1.73 m<sup>2</sup>)
- Recent blood transfusion within 7 days prior to blood sampling
- Incomplete transfusion or laboratory records

Written informed consent was obtained from adult participants and from parents or legal guardians of paediatric patients. Assent was obtained from children where appropriate.

### ***Clinical Data Collection***

Demographic and clinical data were extracted from patient records and confirmed during clinic visits. Collected variables included age, sex, body weight, age at diagnosis, duration of transfusion therapy, transfusion frequency, pre-transfusion haemoglobin levels, splenectomy status, and chelation regimen.

Transfusion requirement was assessed by calculating:

- The mean number of red cell units transfused per month over the preceding 12 months, and
- The annual transfusion volume (mL/kg/year), where data were available.

Patients were categorised into lower and higher transfusion requirement groups based on the median annual transfusion volume of the study cohort.

### ***Laboratory Measurements***

Venous blood samples were collected in the morning (between 08:00 and 10:00) after an overnight fast, prior to scheduled transfusion where applicable.

The following laboratory parameters were measured:

- Serum hepcidin: measured using a commercially available enzyme-linked immunosorbent assay (ELISA), performed according to the manufacturer's instructions
- Serum ferritin: measured by chemiluminescent immunoassay
- Haemoglobin concentration: measured using automated haematology analysers
- Reticulocyte count: expressed as percentage and absolute count
- Serum transferrin saturation (where available)
- C-reactive protein (CRP): to exclude active inflammation

All laboratory analyses were performed in accredited National Health Laboratory Service (NHLS) laboratories.

### ***Assessment of Erythropoietic Activity***

Markers of erythropoietic activity included:

- Pre-transfusion haemoglobin levels
- Reticulocyte count
- Transfusion frequency and volume

These parameters were used to reflect ineffective erythropoiesis and marrow demand. Where available, historical trends over the preceding year were reviewed to ensure consistency.

### ***Statistical Analysis***

Data were analysed using SPSS version 28.0 (IBM Corp., Armonk, NY, USA).

Continuous variables were assessed for normality using the Shapiro–Wilk test and were expressed as mean  $\pm$  standard deviation or median with interquartile range, as appropriate. Categorical variables were summarised as frequencies and percentages.

Correlations between serum hepcidin levels, serum ferritin, transfusion requirement, and erythropoietic markers were assessed using Pearson or Spearman correlation coefficients, depending on data distribution.

Comparisons between transfusion requirement groups were performed using the independent t-test or Mann–Whitney U test. Multivariable linear regression analysis was used to identify independent predictors of transfusion requirement, adjusting for age, sex, ferritin level, and splenectomy status. A p-value  $< 0.05$  was considered statistically significant.

### ***Ethical Considerations***

Ethical approval was obtained from the Health Research Ethics Committee of the participating institutions and complied with the South African National Health Research Ethics Council (NHREC) guidelines. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Participant confidentiality was maintained by anonymising all data prior to analysis. No additional interventions were introduced beyond routine clinical care.

## RESULTS

### *Baseline Characteristics of the Study Population*

A total of 72 patients with  $\beta$ -thalassaemia major met the inclusion criteria and were included in the final analysis. The mean age of the cohort was  $17.8 \pm 6.4$  years (range 6–34 years), with a slight male predominance (54.2%,  $n = 39$ ). All patients were on regular transfusion programmes, receiving red cell transfusions at intervals ranging from 3 to 5 weeks. The median duration of transfusion therapy was 14 years (interquartile range [IQR] 9–18). Splenectomy had been performed in 21 patients (29.2%). All participants were receiving iron chelation therapy, most commonly deferasirox (68.1%), followed by deferoxamine or combination regimens, Table 1.

### *Laboratory Parameters*

Median serum ferritin was 2,480 ng/mL (IQR 1,620–3,950), reflecting significant iron overload across the cohort. Median serum hepcidin concentration was 6.4 ng/mL (IQR 3.1–11.7), with marked inter-individual variability. Despite elevated ferritin levels, serum hepcidin values were disproportionately low in a substantial proportion of patients, particularly those with higher transfusion requirements and markers of increased erythropoietic activity.

### *Transfusion Requirement*

The median annual transfusion volume was 182 mL/kg/year (IQR 150–215). Based on the cohort median, patients were categorised into:

- Lower transfusion requirement group (<180 mL/kg/year;  $n = 36$ )
- Higher transfusion requirement group ( $\geq 180$  mL/kg/year;  $n = 36$ )

Patients in the higher transfusion group had significantly lower mean pre-transfusion haemoglobin levels and higher reticulocyte counts compared with those in the lower transfusion group ( $p < 0.01$ ), Table 2.

### *Correlation Analysis*

#### *Hepcidin and Transfusion Requirement*

Serum hepcidin showed a moderate inverse correlation with annual transfusion volume (Spearman  $r = -0.46$ ,  $p < 0.001$ ). Patients with lower hepcidin levels required more frequent and higher-volume transfusions.

Similarly, a significant inverse correlation was observed between hepcidin and transfusion frequency per year ( $r = -0.42$ ,  $p = 0.002$ ).

### ***Hepcidin and Iron Overload***

Serum ferritin demonstrated no significant positive correlation with serum hepcidin ( $r = 0.12$ ,  $p = 0.31$ ). Several patients with ferritin levels exceeding 3,000 ng/mL exhibited persistently low hepcidin concentrations, suggesting inappropriate suppression of hepcidin relative to iron burden.

### ***Hepcidin and Erythropoietic Markers***

Hepcidin correlated inversely with markers of erythropoietic activity:

- Reticulocyte count ( $r = -0.51$ ,  $p < 0.001$ )
- Transfusion frequency ( $r = -0.42$ ,  $p = 0.002$ )

Pre-transfusion haemoglobin showed a weak positive correlation with hepcidin ( $r = 0.29$ ,  $p = 0.01$ ).

**Table 1. Correlation Between Serum Hepcidin and Clinical/Laboratory Variables**

Variable	Correlation coefficient (r)	p-value
Annual transfusion volume (mL/kg/year)	-0.46	<0.001
Transfusion frequency (per year)	-0.42	0.002
Serum ferritin (ng/mL)	0.12	0.31
Reticulocyte count (%)	-0.51	<0.001
Pre-transfusion haemoglobin (g/dL)	0.29	0.01

### **Comparison Between Transfusion Requirement Groups**

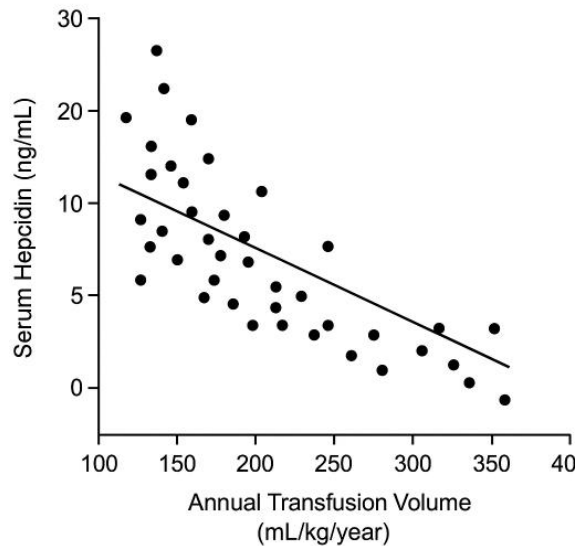
Patients in the higher transfusion requirement group had significantly lower median serum hepcidin levels compared with the lower requirement group (4.1 vs 9.8 ng/mL,  $p = 0.004$ ). Serum ferritin levels did not differ significantly between the two groups ( $p = 0.28$ ).

**Table 2. Comparison of Key Parameters by Transfusion Requirement**

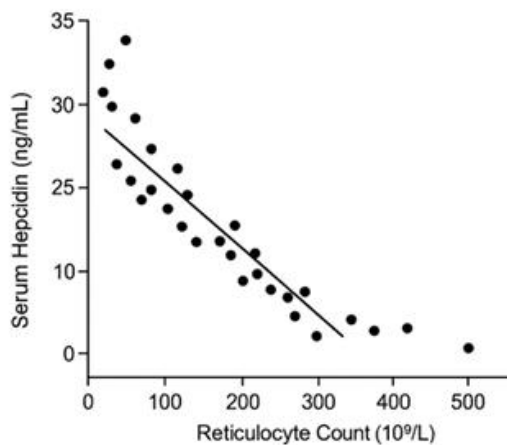
Parameter	Lower transfusion (n = 36)	Higher transfusion (n = 36)	P-value
Serum hepcidin (ng/mL), median (IQR)	9.8 (5.6–14.2)	4.1 (2.2–7.5)	0.004
Serum ferritin (ng/mL), median (IQR)	2,360 (1,580–3,720)	2,590 (1,670–4,120)	0.28
Reticulocyte count (%)	4.2 ± 1.1	6.1 ± 1.4	<0.001
Pre-transfusion Hb (g/dL)	9.1 ± 0.6	8.4 ± 0.7	0.002

### ***Multivariable Analysis***

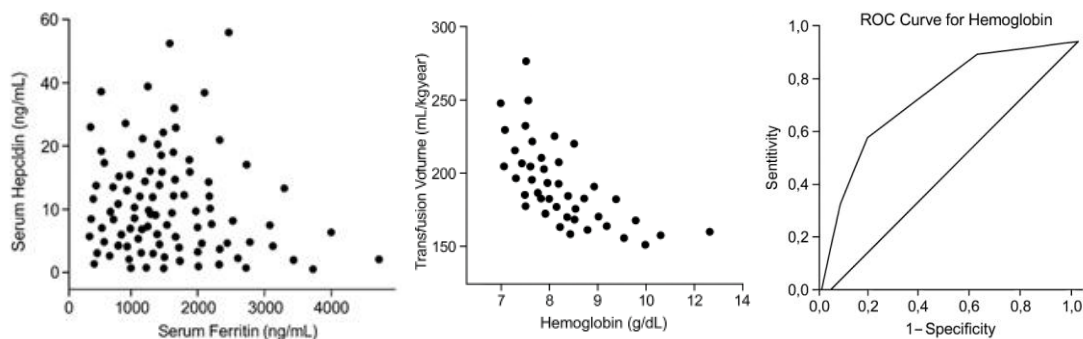
In multivariable linear regression adjusting for age, sex, serum ferritin, and splenectomy status, serum hepcidin remained an independent predictor of transfusion requirement ( $\beta = -0.38$ ,  $p = 0.002$ ). Serum ferritin did not independently predict transfusion volume ( $p = 0.41$ ).



**Figure 1:** Scatter plot of serum hepcidin versus annual transfusion volume demonstrated a clear downward trend, with lower hepcidin concentrations clustering among patients with higher transfusion requirements.



**Figure 2:** Scatter plot of serum hepcidin versus reticulocyte count showed a strong inverse relationship, supporting the suppressive effect of erythropoietic drive on hepcidin regulation.



**Figure 3:** Scatter plot of serum ferritin versus serum hepcidin showed wide dispersion with no consistent linear relationship, highlighting the dissociation between iron stores and hepcidin expression in  $\beta$ -thalassemia major.

## DISCUSSION

This cross-sectional study evaluated the relationship between serum hepcidin levels and transfusion requirement in patients with  $\beta$ -thalassemia major receiving regular transfusion therapy in South Africa, with additional analysis of correlations with serum ferritin and erythropoietic markers. The principal finding is that serum hepcidin was inversely associated with transfusion burden and markers of erythropoietic activity, while showing no consistent relationship with ferritin levels. These findings highlight a dissociation between iron stores and hepcidin regulation in  $\beta$ -thalassemia major and support the potential role of hepcidin as a functional biomarker reflecting erythropoietic drive rather than iron load alone.

The most clinically relevant observation in this study is the moderate but statistically significant inverse correlation between serum hepcidin and annual transfusion volume. Patients with lower hepcidin concentrations required higher transfusion volumes and more frequent transfusions. Importantly, this association persisted even after adjustment for age, sex, ferritin level, and splenectomy status, suggesting that hepcidin independently reflects transfusion need. This finding is biologically plausible and consistent with current understanding of iron regulation in  $\beta$ -thalassemia major. In this condition, ineffective erythropoiesis leads to chronic anaemia despite transfusion support. The expanded erythroid marrow produces erythroid-derived mediators—most notably erythroferrone (ERFE)—which suppress hepatic hepcidin synthesis in an attempt to increase iron availability for erythropoiesis [1,2]. As a result, hepcidin levels remain inappropriately low even in the presence of transfusional iron overload [3].

Our results align with observations from European and Middle Eastern cohorts, where lower hepcidin levels have been associated with more severe disease phenotypes and higher transfusion requirements [4,5]. However, many earlier studies focused primarily on iron overload rather than transfusion burden as a clinical endpoint. By directly linking hepcidin to transfusion volume, the present study provides evidence that hepcidin reflects the functional severity of ineffective erythropoiesis, not merely iron balance.

The strong inverse correlation observed between serum hepcidin and reticulocyte count further supports the suppressive role of erythropoietic drive on hepcidin expression. Reticulocytosis in  $\beta$ -thalassemia major reflects both ineffective erythropoiesis and marrow stress, and its association with lower hepcidin levels has been demonstrated previously [6,7].

Experimental and clinical studies have shown that erythroferrone acts as a dominant regulator of hepcidin during states of increased erythropoietic demand [2,8]. Elevated ERFE suppresses hepcidin transcription, leading to increased iron absorption and mobilisation from macrophage stores. In transfusion-dependent thalassemia, this mechanism becomes maladaptive, perpetuating iron overload despite adequate or excessive iron availability [9].

The observed weak positive correlation between pre-transfusion haemoglobin levels and hepcidin further reinforces this interpretation. Patients with relatively higher baseline haemoglobin—reflecting better erythroid suppression by transfusion—tended to have higher hepcidin levels. This finding suggests that adequate transfusion may partially mitigate erythropoietic suppression of hepcidin, although not sufficiently to restore normal iron regulation.

One of the most striking findings of this study is the lack of a significant correlation between serum ferritin and serum hepcidin, despite widespread iron overload in the cohort. Scatter plot analysis demonstrated wide dispersion of hepcidin values across all ferritin ranges, with some patients exhibiting very low hepcidin levels despite ferritin concentrations exceeding 3,000 ng/mL. This dissociation has been reported consistently in  $\beta$ -thalassemia major and is a defining feature of the disease [10,11]. Under physiological conditions, iron overload would be expected to stimulate hepcidin production, thereby limiting further iron absorption. However, in thalassemia, erythropoietic signals override iron-mediated regulation, rendering ferritin an unreliable predictor of hepcidin status or iron flux [12].

In the South African context, this finding has practical implications. Serum ferritin remains the most widely available marker for iron overload monitoring, particularly in public-sector settings where MRI T2 is limited. However, ferritin reflects cumulative iron burden rather than dynamic iron regulation or erythropoietic activity. The present data suggest that reliance on ferritin alone may obscure clinically meaningful differences in disease activity and transfusion dependency.

The median hepcidin levels observed in this cohort are comparable to those reported in studies from Italy, Greece, and Thailand, although absolute values vary depending on assay methodology and patient characteristics [4,13,14]. Importantly, African data on hepcidin in thalassemia are extremely limited. Genetic diversity, background inflammatory burden, and variability in transfusion and chelation practices may all influence hepcidin regulation in African populations [15].

Unlike some studies that demonstrated modest correlations between ferritin and hepcidin in non-transfusion-dependent thalassemia, our cohort consisted exclusively of transfusion-dependent patients, in whom erythropoietic suppression of hepcidin is expected to be more pronounced [16]. This likely explains the absence of a ferritin-hepcidin relationship in our findings.

The identification of hepcidin as a marker associated with transfusion requirement has several potential clinical implications. First, hepcidin measurement may assist in risk stratification, identifying patients with high erythropoietic drive who are likely to require escalating transfusion

support. Second, it may help distinguish patients whose iron overload is driven predominantly by transfusion from those in whom suppressed hepcidin continues to promote excess iron absorption. From a therapeutic perspective, hepcidin has emerged as a target for novel treatments in thalassemia. Hepcidin mimetics and agents that suppress erythroferrone production are currently under investigation and may offer a means of reducing iron overload while improving erythroid efficiency [17,18]. The present findings support the relevance of such approaches in transfusion-dependent patients. This study has several strengths, including its focus on a clinically relevant endpoint (transfusion requirement), the inclusion of erythropoietic markers, and its contribution of data from a South African population. The use of real-world transfusion records enhances the clinical applicability of the findings.

However, certain limitations should be acknowledged. The cross-sectional design precludes causal inference, and longitudinal changes in hepcidin in response to transfusion or chelation could not be assessed. Erythroferrone and other hepcidin regulators were not measured, which limits mechanistic interpretation. Additionally, inflammatory markers beyond CRP were not systematically assessed, and subclinical inflammation may have influenced hepcidin levels in some patients.

Future studies should adopt a longitudinal design to evaluate whether changes in hepcidin predict subsequent transfusion needs or iron accumulation. Incorporating erythroferrone measurement and advanced iron imaging would provide deeper mechanistic insight. Establishing locally relevant reference ranges for hepcidin in African populations will also be essential before routine clinical use can be recommended.

## **CONCLUSIONS**

This study demonstrates that serum hepcidin is inversely associated with transfusion requirement and erythropoietic activity in  $\beta$ -thalassemia major, while showing no consistent relationship with serum ferritin. These findings underscore the central role of ineffective erythropoiesis in hepcidin suppression and highlight the limitations of ferritin as a functional marker of iron regulation. Hepcidin may represent a valuable adjunct biomarker for assessing disease severity and guiding future therapeutic strategies in transfusion-dependent thalassemia, particularly in resource-limited settings such as South Africa.

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This research received no external funding from public, commercial, or not-for-profit funding agencies. The study was conducted using existing institutional resources of the participating hospitals and laboratories.

## **CONFLICT OF INTEREST**

The authors declare no conflicts of interest related to this study. None of the authors has any financial or personal relationships that could have influenced the work reported in this paper.

## **ETHICAL APPROVAL**

Ethical approval for this study was obtained from the Health Research Ethics Committee (HREC) of the participating institutions in South Africa, in accordance with national and institutional research guidelines. The study was conducted in compliance with the ethical principles of the Declaration of Helsinki and the South African National Health Research Ethics Council (NHREC) regulations.

## **AUTHOR CONTRIBUTIONS**

Dr. Thabo M. Nkosi contributed to the conception and design of the study, patient recruitment, clinical data acquisition, and interpretation of clinical findings.

Dr. Ayesha Patel contributed to laboratory coordination, biochemical analyses including serum hepcidin and ferritin measurements, and critical revision of the manuscript.

Dr. Sipho R. Dlamini contributed to the statistical analysis, methodology design, data interpretation, and drafting of the results section.

Prof. Michael J. van der Merwe contributed to study supervision, interpretation of hematological data, critical intellectual revision of the manuscript, and final approval of the version to be published.

All authors participated sufficiently in the work to take public responsibility for appropriate portions of the content and have approved the final manuscript.

## **DATA AVAILABILITY STATEMENT**

The datasets generated and analysed during the current study are available from the corresponding author upon reasonable request, in accordance with institutional data-sharing policies and applicable data protection regulations.

## **INFORMED CONSENT**

Written informed consent was obtained from all adult participants prior to enrolment. For paediatric participants, written consent was obtained from parents or legal guardians, with assent obtained from children where appropriate. Participation was voluntary, and all participants were informed of their right to withdraw from the study at any time without affecting their clinical care.

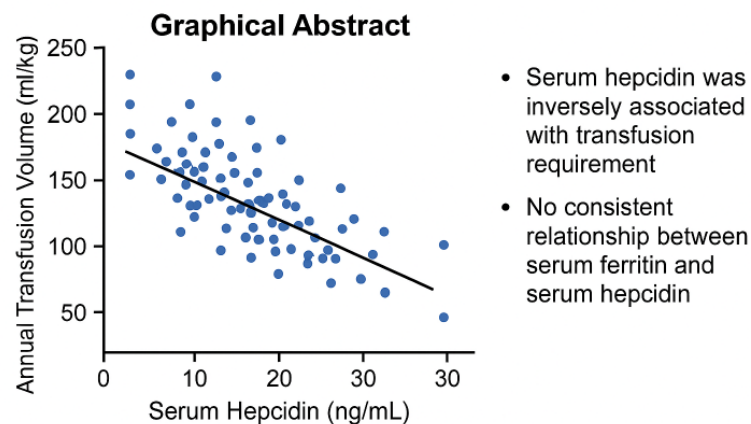
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### Graphical Abstract

#### Serum Hepcidin Levels as Predictors of Transfusion Requirement in $\beta$ -Thalassemia Major: A Cross-Sectional Correlation with Ferritin and Erythropoietic Markers



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