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The effect of blood transfusion on serum hepcidin levels in chronically transfused patients of β-thalassemia major: An observational study in a tertiary care centre in Western Maharashtra

Sujay Bhowmik, Amit Kumar Biswas, Ajay Kumar Baranwal, Amit Ajay Pawar, Ujjwal Dimri

Abstract:

INTRODUCTION: Hepcidin is the key regulator of systemic iron homeostasis. In iron-loading anemias, hepcidin levels are regulated by opposite forces of erythropoiesis and iron overload. In β-thalassemia major patients, transfusions are the predominant cause of iron overload; in such chronically transfused patients, hepcidin concentrations are significantly higher than nontransfused patients, due to both increased iron load of transfusions and the suppression of ineffective erythropoiesis.

AIM: This study aims to evaluate the effect of blood transfusions on serum hepcidin levels in chronically transfused patients of β-thalassemia major and correlate with hemoglobin and serum ferritin levels of pre- and posttransfusion.

MATERIALS AND METHODS: Thirty-three β-thalassemia major patients requiring monthly transfusions were included in the study. Blood samples, collected pretransfusion and 7 days posttransfusion, were evaluated for hemoglobin, serum ferritin, and serum hepcidin using enzyme immunoassay.

STATISTICAL ANALYSIS: Data were statistically analyzed through SPSS software and $P < 0.05$ is considered statically significant.

RESULTS: Posttransfusion levels of hemoglobin, serum ferritin, and serum hepcidin increased. Posttransfusion levels of hepcidin were near normal levels. Pre- and posttransfusion hepcidin concentrations were significantly associated with hemoglobin levels.

CONCLUSION: Serum hepcidin concentrations vary depending on the degree of erythropoiesis drive and level of anemia. We found that the serum hepcidin levels decrease over the inter-transfusion interval and transfusions cause suppression of ineffective erythropoiesis by the increase in hemoglobin. Posttransfusion values of hepcidin in our study were closer to normal levels which may be due to lower erythropoietic drive posttransfusion. We suggest that the measurement of serum hepcidin in chronically transfused β-thalassemia patients can be used as a follow-up investigation for better management of these patients.

Keywords:

β-thalassemia major, hepcidin, iron overload, transfusion-dependent thalassemia

Department of Immunohaematology and Blood Transfusion, Armed Forces Medical College, Pune, Maharashtra, India

Address for correspondence:
Dr. Sujay Bhowmik, Department of Immunohaematology and Blood Transfusion, Armed Forces Medical College, Sholapur Road, Wanowarie, Pune - 411 040, Maharashtra, India.
E-mail: sujay.bhowmik@gmail.com

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Introduction

Hepatocytes are the producers of the iron-regulatory hormone hepcidin; it is a 25-amino acid peptide hormone that inhibits iron entry into the plasma compartment from the three main sources of iron dietary absorption in the duodenum, the release of recycled iron from macrophages, and the release of stored iron from hepatocytes.[1] Iron-loading anemias are disorders in which hepcidin is controlled by opposite forces of ineffective erythropoiesis and iron overload.[2] Hepcidin not only controls dietary iron absorption and plasma iron concentrations but also tissue iron distribution.[3] In β-thalassemia major, transfusions are the most significant cause of iron overload. In chronically transfused patients, hepcidin concentrations are significantly higher than in nontransfused patients, due to both increased iron load and the reduction of ineffective erythropoiesis.[3,4]

However, hepcidin concentrations fall between transfusions, due to an increase in erythropoiesis. During these periods, decreased hepcidin results in increased intestinal iron absorption and contributes to iron load in these patients.[3]

Transfusions would be expected to affect hepcidin production by:

a. Relieving anemia and suppressing the erythropoietic drive which should lessen the suppression of hepcidin, thus increasing hepcidin levels posttransfusion
b. Increasing body iron load which should upregulate hepcidin synthesis.

Hence, we propose to undertake the study to determine pre- and posthepcidin levels in patients with β-thalassemia major. Measurement of these levels may assist in better clinical monitoring and management of transfusions in these patients.

Materials and Methods

The study was an observational comparative study conducted between October 2019 and October 2021 in the department of immunohematology and blood transfusion of a tertiary health-care educational setup in Western India. A total of 33 β-thalassemia major patients registered with the blood bank of a tertiary care center and requiring regular monthly blood transfusions were assessed for serum hepcidin, serum ferritin, and hemoglobin (Hb) levels. Patients with any β-thalassemia type <05 years of age and patients who were seropositive for HIV, HCV, or HBV were excluded from the study. Ethical clearance was granted by the institutional ethical committee and consent was taken before sample collection.

Blood samples were collected (for both pre and 7 days posttransfusion) in three vacutainers, one in sterile and two in K_2 Ethylenediamine tetra acetic acid (EDTA) vacutainers. They were tested for Hb by Sysmex XT 2000-I automated hematology analyzer (Sysmex Corporation, Japan), serum ferritin by siemens dimension EXL 200 (Siemens Healthcare GmbH, Germany), and serum hepcidin by enzyme immunoassay using human hepc enzyme-linked immunosorbent assay (ELISA) kit (Real-Gene Laboratories, Lake Forest, CA, USA), Erba Lisa wash II automated ELISA microplate washer, and Erba Lisa scan EM automated ELISA microplate reader (Transasia Bio-Medicals Limited, Mumbai, India).

The obtained results were subjected to Student's *t*-test paired that were used to compare the means between the two groups (pre- and posttransfusion). Pearson's correlation was performed to assess the associations between red cell indices. For all tests, a probability <0.05 was considered statistically significant. Data were statistically evaluated with International Business Machines Corporation (IBM) SPSS version 20.0 IBM Corp, Chicago, IL, USA.

Results

The patient's demographic and clinical details were obtained, and the mean age standard deviation (SD) (years) was 19.64 ± 4.59 and ranged from 11 to 30 years. The mean weight (Kg) of patients was 46.06 ± 11.01, the weight ranged from 26 to 75 kgs. The frequency of males was predominant at 72.7% (24) than females at 27.3% (9). The mean SD volume of transfusion (mL) was 800.91 ± 130.49 with a range from 600 to 1020 [Table 1].

The pretransfusion parameters with mean serum ferritin (ng/mL) were 3520.30 ± 1335.82 and ranged from 1974 to 6754, followed by mean Hb (g/dL) 8.68 ± 1.10,

Table 1: Demographic details

Demographic details	Mean±SD/frequency (%)
Age (years)	19.64±4.59 (11.00-30.00)
Gender	
Male	24 (72.7)
Female	9 (27.3)
ABO blood group	
A	9 (27.3)
B	10 (30.3)
O	14 (42.4)
Rh antigen	
Negative	4 (12.1)
Positive	29 (87.9)
Weight (kg)	46.06±11.01 (26.00-75.00)
Volume of transfusion (mL)	800.91±130.49 (600.00-1020.00)

SD=Standard deviation

with a range from 6.2 to 10.3. Mean serum hepcidin was 8.62 ± 3.00 (ng/ml) and ranged from 2.1 to 14 [Tables 2-4].

Posttransfusion, the mean serum ferritin (ng/mL) was higher with 5481.21 ± 1799.50 and ranged from 3029 to 9786, mean Hb (g/dL) 10.99 ± 1.07 with a range from 9.2 to 12.9, followed by mean serum hepcidin with 34.61 ± 7.34 (ng/ml) and ranged from 18.6 to 46.9 [Tables 2-4].

The percentage changes posttransfusion in Hb were from 9% to 48%, whereas in serum ferritin were 15%–123% and in serum hepcidin were 144%–903%, which were all statistically significant [Tables 2-4].

Serum hepcidin (Pretransfusion)

The demographic variables (age, gender, and weight) and clinical variables (volume of transfusion, Hb, hepcidin/ferritin) were compared with Pretransfusion hepcidin levels [Table 5].

There was a moderate negative correlation between age (years) and serum hepcidin (pretransfusion), and this correlation was statistically significant (r = −0.35, P = 0.044). There was no significant difference between the gender groups in terms of serum hepcidin (pretransfusion) (W = 128.000, P = 0.430). There was a moderate negative correlation between weight (Kg) and serum hepcidin (pretransfusion), and this correlation was statistically significant (r = −0.36, P = 0.041).

There was no significant difference between the blood groups ($\chi 2$ = 3.620, P = 0.605) and serum ferritin (ng/mL) (pretransfusion) (rho = −0.01, P = 0.937) in terms of serum hepcidin (pretransfusion)

There was a moderate negative correlation between the volume of transfusion (mL) and serum hepcidin (pretransfusion), and this correlation was statistically significant (r = −0.41, P = 0.018).

There was a moderate positive correlation between Hb (g/dL) (pretransfusion) and serum hepcidin (pretransfusion), and this correlation was statistically significant (r = 0.35, P = 0.043).

There was a strong positive correlation between hepcidin/ferritin (pretransfusion) and serum hepcidin (pretransfusion), and this correlation was statistically significant (r = 0.75, $P \leq$ 0.001).

Serum hepcidin (posttransfusion)

The demographic variables (age, gender, and weight) and clinical variables (volume of transfusion, Hb, hepcidin/ferritin) were further compared with Post transfusion hepcidin level [Table 6].

There was no statistically significant correlation between age (years) and serum hepcidin (posttransfusion) (r = −0.31, P = 0.078). There was no significant difference between the gender groups in terms of serum hepcidin

Table 2: Assessment of change in hemoglobin (g/dL) (*n*=33)

Timepoint	Hemoglobin (g/dL)			Paired *t*-test	
	Mean±SD	Median (IQR)	Range	*t*	*P*
Pretransfusion	8.68±1.10	8.70 (1.40)	6.20-10.30	−19.7	<0.001
Posttransfusion	10.99±1.07	11.00 (1.70)	9.20-12.90		
Absolute change	2.31±0.67	2.50 (1.00)	0.80-3.60		
Percent change	27.4±10.2	27.2 (12.6)	9-48		

SD=Standard deviation, IQR=Interquartile range

Table 3: Assessment of change in serum ferritin (ng/mL) (*n*=33)

Timepoint	Serum ferritin (ng/mL)			Wilcoxon test	
	Mean±SD	Median (IQR)	Range	*V*	*P*
Pretransfusion	3520.30±1335.82	3220.00 (1290.00)	1974.00-6754.00	0.0	<0.001
Posttransfusion	5481.21±1799.50	4907.00 (2519.00)	3029.00-9786.00		
Absolute change	1960.91±815.46	1994.00 (887.00)	544.00-3504.00		
Percent change	60.0±27.0	55.0 (26.0)	15-123		

SD=Standard deviation, IQR=Interquartile range

Table 4: Assessment of change in serum hepcidin (*n*=33)

Timepoint	Serum hepcidin			Paired *t*-test	
	Mean±SD	Median (IQR)	Range	*t*	*P*
Pretransfusion	8.62±3.00	8.90 (3.80)	2.10-14.00	−24.6	<0.001
Posttransfusion	34.61±7.34	35.60 (6.80)	18.60-46.90		
Absolute change	25.99±6.08	26.30 (8.00)	14.10-37.30		
Percent change	354.4±197.4	264.1 (160.4)	144-903		

SD=Standard deviation, IQR=Interquartile range

Table 5: Association between serum hepcidin (pretransfusion) and parameters

Parameters	Serum hepcidin (pretransfusion)	*P*
Age (years)***	Correlation coefficient (*r*)=−0.35	0.044[a]
Gender		
Male	8.86±2.88	0.430[b]
Female	8.00±3.42	
ABO blood group		
A	8.70±3.59	0.264[c]
B	9.89±2.15	
O	7.67±2.97	
Rh antigen		0.804[b]
Negative	9.07±2.25	
Positive	8.56±3.12	
Weight (kg)***	Correlation coefficient (*r*)=−0.36	0.041[a]
Volume of transfusion (mL)***	Correlation coefficient (*r*)=−0.41	0.018[a]
Hemoglobin (g/dL) (pretransfusion)***	Correlation coefficient (*r*)=0.35	0.043[a]
Serum ferritin (ng/mL) (pretransfusion)	Correlation coefficient (rho)=−0.01	0.937[d]
Hepcidin/ferritin (pretransfusion)***	Correlation coefficient (*r*)=0.75	<0.001[a]

***Significant at *P*<0.05, [a]Pearson's correlation, [b]Wilcoxon-Mann-Whitney *U*-test, [c]Kruskal-Wallis test, [c]Spearman's correlation

Table 6: Association between serum hepcidin (posttransfusion) and parameters

Parameters	Serum hepcidin (posttransfusion)	*P*
Age (years)	Correlation coefficient (*r*)=−0.31	0.078[a]
Gender		
Male	34.62±7.69	0.856[b]
Female	34.58±6.75	
ABO blood group***		
A	31.73±8.24	0.023[c]
B	39.95±4.56	
O	32.65±6.69	
Rh Antigen		
Negative	34.23±7.09	0.847[b]
Positive	34.67±7.50	
Weight (kg)	Correlation coefficient (*r*)=−0.19	0.298[a]
Volume of transfusion (mL)***	Correlation coefficient (*r*)=−0.39	0.027[a]
Hemoglobin (g/dL) (posttransfusion)	Correlation coefficient (*r*)=0.25	0.160[a]
Serum ferritin (ng/mL) (posttransfusion)	Correlation coefficient (rho)=0.15	0.413[d]
Serum hepcidin (pretransfusion)***	Correlation coefficient (*r*)=0.59	<0.001[a]
Hepcidin/ferritin (posttransfusion)***	Correlation coefficient (rho)=0.52	0.002[d]

***Significant at *P*<0.05, [a]Pearson's correlation, [b]Wilcoxon-Mann-Whitney *U*-test, [c]Kruskal-Wallis test, [d]Spearman's correlation

(posttransfusion) ($W = 113.000$, $P = 0.856$). There was no statistically significant correlation between weight (Kg) and serum hepcidin (posttransfusion) ($r = -0.19$, $P = 0.298$). There was no significant difference between blood groups in terms of serum hepcidin (posttransfusion) ($\chi 2 = 9.440$, $P = 0.093$).

There was a moderate negative correlation between the volume of transfusion (mL) and serum hepcidin (posttransfusion), and this correlation was statistically significant ($r = -0.39$, $P = 0.027$).

There was no statistically significant correlation between Hb (g/dL) (posttransfusion) ($r = 0.25$, $P = 0.160$), serum ferritin (ng/mL) (posttransfusion) (rho = 0.15, $P = 0.413$), and serum hepcidin (posttransfusion).

There was a moderate positive correlation between serum hepcidin (pretransfusion) and serum hepcidin (posttransfusion), and this correlation was statistically significant ($r = 0.59$, $P \leq 0.001$).

There was a moderate positive correlation between hepcidin/ferritin (posttransfusion) and serum hepcidin (posttransfusion), and this correlation was statistically significant (rho = 0.52, $P = 0.002$).

Discussion

Hepcidin is a 25-amino acid peptide that regulates iron metabolism by binding to ferroportin, thereby inducing its internalization and degradation.[1-3] Hepcidin can be detected in both serum and urine and

is being increasingly utilized as a marker for detecting iron-refractory iron deficiency anemia. However, before the same can be utilized in the clinical context, it is important to establish normal ranges in different age groups and populations. Median (ranges) for reference human serum hepcidin levels in adults based on immunoassay analysis is 112 (29–254) ng/mL for men and 65 (17–286) ng/mL for women.[5]

Ineffective erythropoiesis causes iron overload due to suppression of the liver-derived hormone hepcidin that regulates iron absorption and recycling through its effects on ferroportin, the cellular iron export protein. Low hepcidin preserves ferroportin and permits increased intestinal iron absorption and enhanced macrophage iron release (and hence iron recycling), whereas elevated hepcidin causes degradation of ferroportin, thus decreasing iron absorption and recycling. Hepcidin is suppressed by hypoxia and iron deficiency and is elevated by iron loading and inflammation. Erythropoiesis is perhaps the most potent suppressor of hepcidin, although the mechanism remains uncertain.

The total mean Hb during pretransfusion of the study participants was 8.68 ± 1.10, with a range from 6.2 to 10.3. The total mean Hb during posttransfusion of the study participants was 10.99 ± 1.07 with a range from 9.2 to 12.9. Our study results were comparable to other study findings.[6-8]

The total mean serum ferritin pretransfusion among study participants was higher with 3520.30 ± 1335.82 and ranged from 1974 to 6754 ng/mL. The total mean serum ferritin posttransfusion among study participants was higher with 5481.21 ± 1799.50 and ranged from 3029 to 9786 ng/mL. Our study results were comparable to other study findings.[7-9]

In this study, the posttransfusion levels of all three parameters, i.e. Hb, serum ferritin, and serum hepcidin increased. Posttransfusion levels of hepcidin were near normal levels. In all patients, the hepcidin–ferritin ratio was markedly reduced, both pre- and post-transfusion. Although ferritin rose posttransfusion, it is unlikely that increasing iron stores caused the rise in hepcidin, because percentage changes in hepcidin and ferritin were not correlated.

We found that there was a statistically significant association between serum hepcidin (pretransfusion) and age, weight, volume of transfusion, Hb, and hepcidin and ferritin ratio (pretransfusion) and no significant association with the serum ferritin, blood groups, and gender.

Similarly, there was a statistically significant association between serum hepcidin (posttransfusion) and volume of transfusion, Hb, serum hepcidin (pretransfusion), and hepcidin and ferritin ratio (posttransfusion) and no significant association with the serum ferritin, blood groups age, weight, and gender.

In chronically transfused patients with thalassemia, hepcidin levels resemble those in nonthalassemia individuals, potentially implying relatively normal dietary iron absorption, especially posttransfusion. As hepcidin appears to integrate erythropoietin and iron-loading signals, clinical measurement of hepcidin (together with the hepcidin–ferritin ratio) may become a useful indicator of erythropoiesis and iron kinetics in complex patients.

Conclusion

In this study, we wanted to evaluate the effect of blood transfusion on serum hepcidin levels in chronically transfused patients of β-thalassemia major and correlate with Hb and serum ferritin levels pre- and posttransfusion.

We found that the serum hepcidin levels of these patients decrease over the inter-transfusion interval and increase posttransfusion due to reduced erythropoiesis by a transfusion-related increase in Hb. Posttransfusion values for all three parameters Hb, serum ferritin, and hepcidin increased; we also found that hepcidin levels were closer to normal which may lead to suppression of ineffective erythropoiesis. Pre- and posttransfusion hepcidin concentrations were both associated positively with Hb but not with serum ferritin levels.

This study found that hepcidin levels in patients with β-thalassemia major are significantly associated with anemia. Maintaining normal levels of serum hepcidin levels will help reduce ineffective erythropoiesis both medullary and extramedullary. At present, Hb levels are used to monitor the need for transfusions; we suggest that the measurement of serum hepcidin in chronically transfused β-thalassemia patients can also be used as a follow-up investigation in the near future.

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Conflicts of interest
There are no conflicts of interest.

References

1. Nemeth E, Ganz T. The role of hepcidin in iron metabolism. Acta Haematol 2009;122:78-86.
2. Nemeth E, Ganz T. Regulation of iron metabolism by hepcidin. Annu Rev Nutr 2006;26:323-42.

3. Nemeth E. Hepcidin in β-thalassemia. Ann N Y Acad Sci 2010;1202:31-5.
4. Kemna E, Tjalsma H, Willems H, Swinkels D. Hepcidin: From discovery to differential diagnosis. Haematologica 2008; 93:90-7.
5. Pasricha SR, Frazer DM, Bowden DK, Anderson GJ. Transfusion suppresses erythropoiesis and increases hepcidin in adult patients with β-thalassemia major: A longitudinal study. Blood J Am Soc Hematol 2013;122:124-33.
6. Sawicki KT, Chang HC, Ardehali H. Role of heme in cardiovascular physiology and disease. Journal of the American Heart Association 2015;4:e001138. doi: 10.1161/JAHA.114.001138.
7. Tantiworawit A, Khemakapasiddhi S, Rattanathammethee T, Hantrakool S, Chai-Adisaksopha C, Rattarittamrong E, *et al*. Correlation of hepcidin and serum ferritin levels in thalassemia patients at Chiang Mai University Hospital. Biosci Rep 2021;41:BSR20203352. doi: 10.1042/BSR20203352. PMID: 33565577; PMCID: PMC7886874.
8. Channanayaka C, Manovihari V, Prajwala V, George P, Tirin B. Original Article A prospective cross-sectional study of thyroid dysfunction in transfusion-dependent beta-thalassemia patients. Indian Journal of Child Health 2021;8:10.32677/IJCH.2021.v08.i05.002.
9. Jones E, Pasricha SR, Allen A, Evans P, Fisher CA, Wray K, *et al.* Hepcidin is suppressed by erythropoiesis in hemoglobin E β-thalassemia and β-thalassemia trait. Blood 2015;125:873-80.