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Serum Hepcidin in Multi-Transfused Patients with Beta-Thalassemia Major: A Cross-Sectional Study

Dr. Neha Niharika[1], Dr. Meera Sikka[2*], Dr. Mrinalini Kotru[3], Dr. Sunil Gomber[4], Dr. Satendra Sharma[3]
[1]Resident, Department of Pathology, University College of Medical Sciences and GTB Hospital, Delhi, India
[2]Professor and Head, Department of Pathology, University College of Medical Sciences and GTB Hospital, Delhi, India
[3]Professor, Department of Pathology**,** University College of Medical Sciences and GTB Hospital, Delhi, India
[4]Professor, Department of Pediatrics, University College of Medical Sciences and GTB Hospital, Delhi, India

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***Corresponding author**
Dr. Meera Sikka

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Abstract: Beta-thalassemia major (BTM) is associated with significant morbidity and mortality due to iron overload resulting from ineffective erythropoiesis and repeated blood transfusions. The level of hepcidin, a regulator of iron homeostasis, is influenced by anemia and iron overload, both of which are present in children with BTM and have an opposing effect on hepcidin expression. This study aimed to assess the influence of iron overload and enhanced erythropoiesis on the levels of serum hepcidin in multitransfused patients with BTM. Complete blood counts, serum iron (SI), total iron binding capacity (TIBC), percent transferrin saturation (%TS), serum ferritin (SF), serum transferrin receptors (sTfR) and serum hepcidin were measured in 52 patients with BTM and 35 controls. SF and sTfR were significantly ($p<0.001$) elevated in patients with BTM as compared to controls. Serum hepcidin was significantly ($p<0.001$) higher in patients (28.3±3.2 ng/ml) as compared to controls (8.3±4.6 ng/ml). A negative correlation was seen between serum hepcidin and SI, TIBC and %TS. No correlation was observed between serum hepcidin and SF as also sTfR. The mean serum hepcidin/ferritin ratio was significantly ($p<0.001$) lower in patients as compared to controls. The lack of correlation between hepcidin and SF as also sTfR, suggests that in BTM iron stores and erythropoietic activity do not play a role in hepcidin expression. The hepcidin/ferritin ratio was <1 in patients indicating a suppression of hepcidin relative to the degree of iron overload.
Keywords: Thalassemia, Hepcidin, Hemoglobinopathy, Iron Overload, Transfusion Medicine, Anemia.

INTRODUCTION

Thalassemias are inherited hemolytic anemias characterized by deficient synthesis of one or more globin chains of hemoglobin (Hb) [1]. Beta-thalassemia (deficient synthesis of beta chains), is one of the most common inherited hemoglobinopathy in the world. While the heterozygous state is asymptomatic, the homozygous form; β thalassemia major (BTM) is associated with transfusion dependent anemia [2].

The severe anemia in these patients stimulates erythropoietin (EPO) production resulting in an uncontrolled expansion of erythroid progenitors and extramedullary haematopoiesis (EMH) which contributes to skeletal abnormalities and hepatosplenomagaly [2].

These patients are on regular blood transfusion which maintains an adequate level of Hb and suppresses erythropoiesis. Repeated transfusion results in accumulation of iron and resultant organ dysfunction [2].

Hepcidin, a peptide produced in the liver, is a key regulator of iron homeostasis [3]. It acts by inhibiting intestinal absorption of iron and its release from macrophages recycling senescent erythrocytes by binding to the iron exporter ferroportin and degrading it [4].

Transferrin donates iron to cells by interacting with a membrane receptor, transferrin receptor (TfR). Receptor density on proliferating cells is proportional to the availability of iron**.** Excess iron supresses the number of TfR [5]. Patients with BTM have iron overload which induces hepcidin synthesis and anemia which suppresses it [6]. Conflicting results have been observed in various studies on the level of serum hepcidin in patients with BTM. Hepcidin / ferritin ratio is a marker of the appropriateness of hepcidin expression relative to the degree of iron burden [7]. Measurement of serum hepcidin along with hepcidin/ferritin ratio can be a useful indicator of erythropoiesis and iron kinetics in patients with BTM and help in identifying patients at risk of iron overload.

This study measured the level of serum hepcidin in multi transfused children with BTM to assess the opposing effects of anemia and iron overload.

MATERIALS AND METHODS

This cross-sectional study was conducted on 52 multi-transfused patients of beta-thalassemia major enrolled from thalassemia day care centre. A detailed clinical history and physical examination was done. Patients with any clinical evidence of infection, fever or CRP >6.0 mg/L were excluded. Thirty-five non-anemic children with normal iron status were include as controls.

A pre-transfusion fasting venous blood sample (10ml) was collected from patients in EDTA and in plain iron free vials. Complete blood counts (LH 500) along with examination of stained peripheral blood film (wrights stain) and reticulocyte count were done [8, 9].

Serum iron (SI) [10], Total iron binding capacity (TIBC) [11] , transferrin saturation (%TS), serum ferritin (SF), serum transferrin receptor (sTfR), serum hepcidin (SH) and C-reactive protein (hsCRP) were performed using standard laboratory techniques. SF, sTfR, SH and CRP were determined using commercially available ELISA kit according to the manufacturer's protocol. Transferrin saturation was calculated as follows: SI/TIBC×100.

STATISTICS

Data was expressed as Mean ± SD, median, and range. Quantitative parameters between patients and controls were compared by 2 tailed t tests. Linear correlation using Pearson's correlation was obtained between all parameters. A 'p' value <0.05 was considered as significant.

RESULTS

The age of the patients ranged from 5-19 years with a Mean ± SD of 11.8 ± 4.0 years. There were 32 (61.5%) males and 20 (38.5%) females.

The haematological parameters of patients and controls are shown in Table-1. Hemoglobin was significantly ($p < 0.001$) lower in patients as compared to controls.

Table-2 shows parameters of iron status in patients and controls. SF was significantly ($p<0.001$) higher in patients (Mean ± SD 3476.5 ± 1794.2 µg/L) as compared to controls (Mean ± SD 39.1 ± 9.8 µg/L). sTfR was significantly ($p<0.001$)higher in patients (Mean ± SD 3.4 ± 2.2 µg/ml) as compared to controls (Mean ± SD 1.1 ± 0.6 µg/ml).

A significantly ($p<0.001$) higher level of serum hepcidin was seen in patients (28.3±3.2 ng/ml) as compared to controls (8.3±4.6 ng/ml). The ratio of hepcidin /ferritin which reflects the appropriateness of serum hepcidin for the degree of iron overload was calculated. Mean ± SD of the ratio was 0.0116 ± 0.0090 in patients, being 0.2266 ± 0.1399 in controls (Table-3). The difference was statistically significant ($p< 0.001$).

A negative correlation was seen between serum hepcidin and SI, TIBC and %TS, though not significant. No correlation was observed between hepcidin and SF as also sTfR.

Table-1: Hematological parameters in patients and controls

Parameter	Patients	Controls	
	Mean ± SD	Mean ± SD	p value
Hb (g/dl)	8.9 ± 0.8	12.7 ± 0.9	0.000*
RBC (x 10^{12}/L)	3.23 ± 0.35	4.70 ± 0.62	0.000*
MCV (fl)	84.2 ± 4.4	82.9 ± 10.2	0.502
MCH (pg)	27.6 ± 1.7	26.6 ± 4.2	0.222
MCHC (g/dl)	32.7 ± 1.3	31.6 ± 1.2	0.000*
TLC (×10^9/L)	7.8 ± 3.4	7.6 ± 1.9	0.810
Platelet count (×10^9/L)	283.3± 152.1	274.4 ± 71.6	0.717

*p<0.001

Table-2: Iron parameters in patients and controls

Parameter	Patients	Controls	
	Mean ± SD	Mean ± SD	p value
SI (µg/dl)	224.7 ± 57.6	90.9 ± 19.7	0.000*
TIBC(µg/dl)	296.5 ± 102.9	328.3± 65.1	0.098
%TS	82.0 ± 17.2	28.1 ± 7.8	0.000*
SF (µg/L)	3476.5 ± 1794.2	39.1 ± 9.8	0.000*
sTfR(µg/dl)	3.4 ± 2.2	1.1 ± 0.6	0.000*

*p<0.001

Table-3: Serum hepcidin and hepcidin /ferritin ratio in patients and controls

Parameter	Patients	Controls	p value
	Mean ± SD	Mean ± SD	
SH (ng/ml)	28.3 ± 3.2	8.3 ± 4.6	0.000*
Hepcidin/ferritin	0.0116 ± 0.0090	0.2266 ±0.1399	0.000*

*p<0.001

DISCUSSION

In this cross sectional, descriptive study parameters of iron status (SI, TIBC, %TS, SF and sTfR) and serum hepcidin (SH) were measured in 52 multitransfused patients of β-thalassemia major with a pretransfusion Hb of >8.0 g/dl. The study aimed to assess the effect of iron overload (SF) and enhanced erythropoiesis (sTfR) on the level of serum hepcidin in these patients.

Hb concentration was significantly (p<0.001) lower in patients as compared to controls. Anemia was present in all patients, being mild in 3.8% patients and moderate in severity in 96.2% patients. Anemia is usually the earliest symptom in patients with BTM. In a study on Indian patients with BTM, the Mean Hb concentration was 9.2 ± 1.0 g/dl and 13.0 ± 1.2 g/dl in patients and controls (p < 0.001) [7]. Guimaraes et al also observed a significantly (p<0.001) lower Hb concentration in BTM patients (9.1 g/dl) as compared to controls (14.3 g/dl) [12]. Studies from the west have reported a higher Hb concentration in these patients [13, 14]. Possibly due to better availability of blood transfusion.

In this study, serum iron and %TS were significantly (p<0.001) higher in patients as compared to controls, there was no significant difference in TIBC of patients and controls. Serum iron is usually elevated in patients with BTM. However, transferrin is completely saturated in majority of patients only after a few years of transfusion as was also seen in this study [2].

SF a measure of iron overload was significantly (p<0.001) higher in patients (3476.5 ± 1794.2 µg/L) as compared to controls (39.1 ± 9.8 µg/L) and was markedly increased in all patients. Other authors have also observed a significantly higher SF in patients with BTM as compared to controls [7, 13, 15]. Serum ferritin was elevated > 2500 µg/L in 37(71.1%) patients in this study. Levels of ferritin above 2500 ng/dl have been reported to be associated with a four-fold higher risk of death [2].

Iron overload remains a major cause of morbidity and mortality in these patients. Ineffective erythropoiesis (IE) and regular blood transfusions contribute to the iron overload in patients with BTM. The excess iron is removed with chelation. Most of the patients included in the present study were on chelation therapy with the drug being provided from the Hospital. Due to non-availability of continuous supply of chelators, they were advised to take chelators on their own. However, due to socioeconomic constraints, they were on irregular use of chelators explaining the high serum ferritin observed in this study.

The present study observed a significantly (p <0.001) higher level of sTfR in patients (Mean ± SD 3.4 ± 2.2 µg/ml) as compared to controls (Mean ± SD 1.1 ± 0.6 µg/ml). Similar results have been reported by other authors [7]. The most important determinant of receptor level is erythropoietic activity [16]. In patients with BTM, in whom erythropoiesis is markedly increased, the level of sTfR is high as was also seen in this study.

In a study on regularly and irregularly transfused patients with BTM, sTfR levels were significantly higher in the latter as compared to the former as also the controls. sTfR was the only statistically significant parameter of erythroid activity in regularly transfused thalassemics. The authors suggested that sTfR should be used as an indicator of effective suppression of erythropoiesis in these patients which will help in their better management [17].

The high reticulocyte count and elevated sTfR levels seen in patients with BTM in this study inspite of a median pretransfusion Hb of 8.8 g/dl indicates that the transfusion programme is not entirely satisfactory in supressing erythroid marrow activity effectively.

Serum hepcidin was significantly (p < 0.001) higher in patients (28.3 ± 3.2 ng/ml) as compared to controls (8.3 ± 4.6 ng/ml). Serum hepcidin was elevated (> 12.9 ng/ml) in all patients and in 3 (8.5 %) controls. Hepcidin, a hepatic peptide, inhibits iron absorption from the intestine by binding to the iron exporter ferroportin and causing its internalization. Multiple stimuli affect its expression such as iron availability, hepatic iron stores, erythropoietic activity, hypoxia and inflammation. Iron loading stimulates its synthesis while anemia suppresses it and both these opposing influences are concomitantly present in patients with thalassemia major.

The elevated hepcidin levels seen in this study is presumably due to multiple transfusions received by these patients. The transfusions reduce erythropoietic activity and facilitate iron overload. The markedly elevated level of serum ferritin seen in this study may also contribute to the elevation of serum hepcidin.

Conflicting results have been reported on the level of serum hepcidin in patients with BTM. An increase in the level of serum hepcidin has been reported in some studies [13, 18].

Origa *et al*., observed increased urinary hepcidin in 11 patients with BTM (median 218 ng /mg creatinine). The authors hypothesized that transfusion suppresses the erythropoietic drive and increase body iron load, both of which contribute to increased hepcidin levels [13].

In a study done on 6 patients with BTM, hepcidin mRNA expression was evaluated in liver biopsy specimens obtained 1-3 days after red cell transfusion. A 5 to 8 fold increase in hepcidin was observed in patient with BTM. They concluded that the increase in hepatic hepcidin mRNA expression reflects the dominance of iron overload over erythropoietic signals as the biopsy had been done 3 days after blood transfusion [18]. Other authors have also reported an increase in hepcidin levels [19].

In contrast, a decrease in the level of hepcidin has been observed in some studies [15, 20]. While other studies observed no difference in the level of serum hepcidin of patients with BTM and controls [7, 14].

Hepcidin / ferritin ratio was significantly ($p< 0.001$) lower in patients (0.0116 ± 0.0090) with BTM than in controls (0.2266 ± 0.1399). This markedly low value suggested that inspite of iron overload seen in these patients, hepcidin was not increased proportionately. Similar results have been observed by other authors [7, 13, 14].

In this study, a negative correlation was seen between serum hepcidin and serum iron ($r = -0.220$, $p= 0.117$), TIBC ($r = -0.023$,$p=0.883$) ,%TS ($r = -0.190$,$p=0.177$) but the correlation was not statistically significant. A positive correlation was seen between serum hepcidin and serum ferritin ($r= 0.022$, $p= 0.877$), though it was not statistically significant. Other authors also did not observe any significant correlation between SH and iron parameters [7, 15, 21]. They concluded that in disorders of massive erythroid production and ineffective erythropoiesis, the regulation of iron balance by iron stores collapses [7, 15]. In present the study, there was no statistically significant correlation between hepcidin and sTfR ($r = 0.090$, $p = 0.5270$). Similarly Pratummo et al did not observe any correlation between hepcidin mRNA and sTfR as also serum ferritin in regularly transfused patients of HbE/β thalassemia. They concluded that erythropoietic drive and body iron store do not play a key role in hepcidin expression [22].

CONCLUSION

The present study thus observed a significantly higher level of serum hepcidin in patients as compared to controls. It was elevated in all patients. No correlation was observed between serum hepcidin and sTfR as also serum ferritin, suggesting that erythropoietic drive and body iron store may not play a key role in hepcidin expression. The hepcidin to ferritin ratio was <1 in patients indicates suppression of hepcidin relative to the degree of iron overload. As most studies on hepcidin in patients with BTM have been done in the West and have yielded conflicting results, further studies are required on a larger number of patients to further validate these results in Indian patients.

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