International Journal of Contemporary Pediatrics
Chaithra P et al. Int J Contemp Pediatr. 2024 Jan;11(1):45-48
http://www.ijpediatrics.com
pISSN 2349-3283 | eISSN 2349-3291

DOI: https://dx.doi.org/10.18203/2349-3291.ijcp20233958

Original Research Article

Role of serum hepcidin as a marker of iron overload in beta thalassemia major

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Received: 20 October 2023
Revised: 14 November 2023
Accepted: 22 November 2023

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ABSTRACT

Background: Beta thalassemia major (BTM) is the most common hemolytic anemia. Regular blood transfusion is a basic treatment modality, recurrent blood transfusion which leads to iron overload and its complications. Hepcidin hormone is found to be a key regulator of iron homeostasis and is significantly increased in children of BTM with iron overload. The main objective of the study is to find out the role of serum hepcidin as a potential marker of iron overload in children with BTM, and to correlate the relationship between serum hepcidin and serum ferritin level in BTM children.
Methods: This was a hospital based prospective observational study conducted at Indira Gandhi institute of child health for 12 months from January 2019 to December 2019. Included 100 children between age group of 2 months to 18 years diagnosed with BTM on blood transfusion and 50 age and sex matched healthy controls.
Results: In the study group 70% children had >5 transfusions. The median serum hepcidin level (2.354 ng/ml) was significantly higher among those with higher number of total transfusions (>5 transfusions). In addition, hepcidin level showed good positive correlation with total number of transfusions ($r=0.608$, $p<0.001$). Also, serum hepcidin showed positive correlation with serum ferritin levels with 87% sensitivity and 88% specificity which was statistically significant ($r=0.749$, $p<0.001$).
Conclusions: In the present study, BTM children who received >5 transfusions serum hepcidin level was significantly elevated and serum hepcidin showed positive correlation with serum ferritin levels. Thus, hepcidin can be considered as a potential marker of iron overload in patients with BTM.

Keywords: BTM, Hepcidin, Iron Overload, Ferritin

INTRODUCTION

Thalassemia's are a group of blood disorders, caused by single gene mutations inherited in autosomal recessive pattern and results in reduced or absent synthesis of specific globin chains with concurrent accumulation of other unpaired globin chains leading to ineffective erythropoiesis with hemolysis.[1] In the year 1925, Cooley and Lee described Italian children with severe anemia, splenomegaly and hepatomegaly as Cooley's anemia and later to be called as thalassemia.[2]

Conventionally, thalassemia is treated with transfusions of packed red blood cells from a healthy donor at regular intervals and chelation therapy to prevent consequences of anemia and iron overload respectively. Improvement in standard of care by regular blood transfusion in a day-care center has increased the life expectancy in thalassemia children.[2] However, the iron overload in various organs leads to serious problems such as liver cell dysfunction, endocrine dysfunction including hypothyroidism, hypogonadotropic hypogonadism, growth hormone deficiency, hypoparathyroidism,

diabetes mellitus and cardiac dysfunction like cardiac failure and or arrhythmias, despite the expanded use of iron-chelator drugs.[3,4]

In BTM children, estimation of iron overload has relied on serum ferritin measurements, despite evidence that these do not always correlate well with liver iron concentration. Hepcidin hormone has been found to be a key regulator of iron homeostasis, increased plasma and stored iron levels stimulate hepcidin production in liver and is significantly increased in chronically transfused children with severe iron overload.[3-5]

Some studies have reported that children with BTM with iron overload have elevated serum hepcidin levels.[6-9] The gold standard for assessment of iron overload needs MRI T2/T2*relaxation images, which is very expensive and not easily available in all health care centers.[2] Therefore, a single, reliable cost-effective test is needed which can reliably predict iron overload.

Hence the present study was carried out to find out the role of serum hepcidin as a potential marker of iron overload in children with BTM.

METHODS

The present study was a prospective, observational study conducted over one year at Indira Gandhi institute of child health, Bangalore from January 2019 to December 2019.

Inclusion criteria included all confirmed BTM children by Hb fractionation by HPLC method between 2 months to 18years of age. Exclusion criteria included patients with anaemia other than BTM, BTM children on regular iron chelating therapy and those with concurrent infection.

The study was approved by the Institutional Ethical Committee; written informed consent was obtained from parents of each patient.

Sample size calculation

$n=Z^2P(1-P)/d^2$

Where P is prevalence of β-Thalassemia major=6.8% (6.8/100=0.068), Z is standard normal value @ 5% level of significance=1.96,

d is absolute precision=5% (5/100=0.05), $n=(1.96)^2$ *0.068*(1-0.068)/(0.05)2=97=100

Sample size was calculated based on the study conducted by Sujatha et al, the prevalence of β-thalassemia was 6.8% [34/100], assuming 5% level of significance and 5% absolute precision, the required sample size was hundred. One hundred β-Thalassemia cases and fifty age and sex matched healthy controls were enrolled for the study.[10]

One hundred confirmed cases of BTM between the age group of 2 months to 18 years formed the study group. Pre-designed standard proforma was used to record the relevant history like place of origin, age at onset of symptoms, progressive pallor, abdominal distension, number of transfusions, with or without chelation, detailed family history in children with BTM. Detailed examination of the children was carried out including severity of pallor, presence of splenohepatomegaly and any clinical features of iron overload.

The 2 ml of blood in gel tube (yellow cap) and 2 ml blood in EDTA tube (purple cap) was collected for cases while securing intravenous access intended for the routinely planned blood transfusion, thereby eliminating additional needle prick. These children were subjected for routine investigations including hemoglobin %, total WBC count, PCV, MCV, MCHC, and LFT. For estimation of serum hepcidin, 2 ml of blood collected in gel tubes, allowed to clot for 30 min, then it was centrifuged at 1500 revolutions per minute (rpm) for 15 min at room temperature and the serum was separated and analyzed for hepcidin level. Two ml blood in EDTA tube and 2 ml blood in gel tube were collected from healthy children to estimate serum hepcidin level for control.

These children with BTM were treated with regular transfusions and chelation therapy according to standard treatment protocol and counselling and follow up was done. Hepcidin level was measured by using an automated commercial immunoassay. Hepcidin levels were correlated with levels of serum ferritin.

Statistical analysis

Data was entered in MS excel version 2010. Data was analysed using R software version 4.0. All categorical data was expressed using frequency and percentages. All continuous data was expressed using mean and standard deviation or median and inter quartile range based on the distribution. To study the association of different levels of demographic and clinical parameters with Ferritin and Hepcidin, independent sample t-test or Mann Whitney U test after checking normality assumption was used. Receiver operating curve (ROC) analysis was carried out to find the best cut off discriminating BTM cases and controls. Diagnostic parameters were estimated for hepcidin levels keeping Ferritin levels as gold standard. P value was considered significant at 5% level of significance for all comparisons.

RESULTS

A total of one hundred children with BTM formed the study group, 59 (59%) children were >1 year age. The youngest was 4 months old and eldest being two years

two months. 58 (58%) were males with male to female ratio of 1.38:1. Majority of children 90 (90%) were from Karnataka State and remaining 10 (10%) around Karnataka. All children had pallor and splenomegaly, 70 (70%) children had >5 times packed red blood cell (PRBC) transfusion and remaining 30 (30%) had ≤5 PRBC transfusion.

In the study population, the mean pre-transfusion hemoglobin was 7.4 g/dL. The mean PCV was 22.3%. The mean RBC was 5 million/mm^3. The mean MCV was 59.22 fL. The mean MCH was 18.91 pg. HbF percentage varied from 69.6 to 96.5%. Median value of HbA_1 was 3.5 and HbA_2 was 2.8

The serum hepcidin median value for >5 transfusion was higher (2.354ng/ml) than ≤5 group (1.036ng/ml) which was statistically significant (p<0.001). The serum hepcidin level was directly proportional to the number of transfusions which was statistically significant (spearman rho-0.608, p<0.001) (Table 1).

The ROC shows the sensitivity and specificity of hepcidin and hepcidin and ferritin ratio, the area under the curve of hepcidin in predicting iron overload was 0.8930 which indicates that overall predictability of hepcidin which was statistically significant (p<0.001) with the best cut off of 0.8696 ng/ml with a sensitivity of 87% and specificity of 88%. In the study population, 71% had high serum hepcidin value with ferritin level>1000 ng/ml. The serum hepcidin and ferritin level correlation was statistically significant (spearman rho=0.60, p<0.001). BTM children with serum hepcidin level of ≥0.870 ng/ml are 5.94 times more likely to have serum ferritin level >1000 and the upper limit 95% C.I, of this increased odd can be as much as 19.76 times which showed statistical significance (p=0.004) (Figure 1) (Table 2).

Out of 50 age and sex matched controls, 22% children were ≤1 year and 28% were >1 year with male to female ratio was 1.38:1. Serum ferritin median value (35.98 ng/ml) and the serum hepcidin median value (0.516 ng/ml) was normal in control population.

Table 1: Comparison of hepcidin level in relation to number of PRBC transfusion and its correlation.

Variables	Serum hepcidin (ng/ml) Median (IQR)	P value
Number of PRBC transfusions ≤5	1.036 (564,1735)	<0.001*
Number of PRBC transfusions >5	2.354 (1766,3116)	
Spearman's rank correlation coefficient (rho)	0.608	<0.001*

*P value is significant.

Table 2: Results of the study conducted.

Variables	Results
Sample size (n)	100
Age group (In years)	0.2-18
Sex distribution (M: F)	1.38:1
Ferritin (ng/ml)	
Median	1235
P value	<0.001*
Hepcidin (ng/ml)	
Median (IQR)	1.875 (1.042-2.947)
P value	<0.001*
Number of transfusions with hepcidin correlation	
Median (ng/ml)	2.354
P value	<0.001*
Spearman rank correlation coefficient (rho)	0.608
Hepcidin / ferritin (ng/ml)	
Spearman's rank correlation coefficient (rho)	0.6
P value	<0.001*

*P value is significant

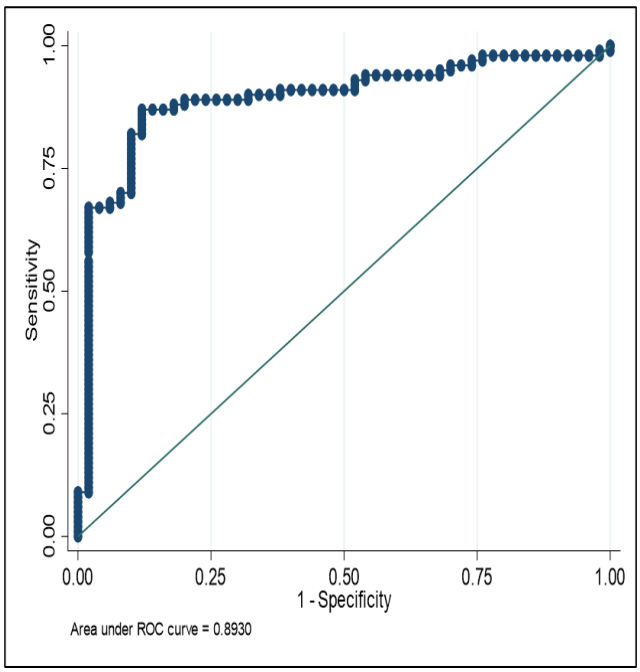

Figure 1: Sensitivity and specificity of hepcidin and hepcidin and ferritin ratio by ROC.

DISCUSSION

Management of Thalassemia has improved greatly in the past few years. Regular blood transfusion is a basic therapeutic regimen which has increased the life expectancy; however, the iron overload in various organs leads to complications. Hepcidin hormone is found to be a key regulator of iron homeostasis and is significantly increased in transfused children with iron overload in BTM.

The present study included 100 cases with BTM; the mean age of study population was 1 year 1 month. The sex distribution with male to female ratio was 1.38:1 which was comparable with the study done by Kaddah et al since majority of children with BTM manifests in infancy.[6] The median (IQR) serum ferritin value of the present study was 1235ng/ml which was statistically significant (p<0.001) and is par with study done by Kaddah et al since most of them had received >5 transfusions and were not on regular chelation therapy.[6] The median (IQR) of the serum hepcidin value was 1.875 ng/ml. Similar observation was seen by Kaddah et al study and was statistically significant (p<0.001).[6] This could be explained by the fact that more the number of blood transfusions, more severe the iron overload.

The median serum hepcidin level (2.354 ng/ml, p<0.001) was significantly higher among those with higher number of total transfusions, in addition hepcidin level showed good positive correlation with total number of transfusions (r=0.608). This confirms the fact that increase in number of transfusions will increase the production of hepcidin, as hepcidin is a key regulator of iron homeostasis which is stimulated by iron overload. Increased transfusions induce high tissue iron accumulation and inhibit erythropoietic drive there by increasing the hepcidin levels.

Serum hepcidin showed positive correlation with serum ferritin level which was statistically significant (r=0.60, p<0.001). Similar observation was found by Kaddah et al.[6]

Limitation

The main limitation of the present study is the lack of gold standard for the estimation of iron overload which is liver iron concentration by MRI T2/T2* relaxation images.

CONCLUSION

BTM is one of the common hemolytic anemia requiring regular blood transfusions which results in iron overload and its adverse effects on various organs. Serum hepcidin is one of the markers of serum iron overload. In the present study, BTM children who received >5 transfusions, serum hepcidin level is significantly elevated and serum hepcidin showed positive correlation with serum ferritin levels. Thus, hepcidin can be considered as a simple cost-effective potential marker of iron overload in patients with BTM.

ACKNOWLEDGEMENTS

The author would like to thank to all children and their parents for their co-operation and their trust.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee (IGICH/ACA/IEC-P-103/2020-21) of Indira Gandhi Institute of Child Health, Bengaluru

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Cite this article as: Chaithra P, Kumar PA, Aruna G, Hemalatha L. Role of serum hepcidin as a marker of iron overload in beta thalassemia major. Int J Contemp Pediatr 2024;11:45-8.