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Original Article

Hepcidin Levels in Multi Transfused β Thalassemia Major Patients

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Abstract

Background: To determine and compare the serum hepcidin levels and conventional markers of iron status in patients of β- thalassaemia major with controls.

Methods: Forty patients of β- thalassaemia major who were not on iron chelation therapy and 40 normal controls were included in this study. Hemoglobin level, red blood cell count and hematocrit of the study group were determined. Hepcidin levels along with serum iron, serum ferritin and total iron binding capacity were determined .

Results:Serum hepcidin level was found to be reduced in patients (426.21 pg/ml) as compared to controls (757.45 pg/ ml).

Conclusion: Determination of hepcidin concentration is a useful indicator for high risk of iron toxicity in patients of beta thalassaemia with multiple transfusions

Key Words: Hepcidin, Iron, β- thalassaemia major

Introduction

Thalassaemias are hereditary anemias resulting from defects in the production of haemoglobin. The World Health Organization (WHO) recognizes thalassaemia as the world's most prevalent genetic disorder. The estimated live births who suffer from β-thalassaemia in Pakistan are five to nine thousand per year with a carrier state of 9.8 million.[1]Iron overload is the main cause of mortality and morbidity in patients with β thalassaemia major.[2] Though frequent red cells transfusion has been identified as the leading cause of iron overload yet it is also established that non-transfusion dependent patients also develop iron overload.[3] This harmful iron over load results in many complications like growth retardation, delayed sexual maturation and later on involvement of liver, heart and endocrine glands.[4] Excess cellular iron is extremely harmful to cells by facilitating the production of reactive oxygen species which damage cellular proteins, lipids, and DNA.[5]

Hepcidin plays a vital role in iron homeostasis in humans, regulating iron absorption from the intestine and its recycling by macrophages.[6]There are three forms of hepcidin: 25aa (human), 22aa and 20aa peptide. Human hepcidin gene (HAMP) is located on chromosome 19q13.1 and is mainly expressed in the liver. [7,8] Hepcidin controls the dietary iron absorption and release of iron from macrophages.[9]

Several physiologic and pathologic processes regulate the production of hepcidin.[10] In iron-loading anaemias like thalassaemia, hepcidin synthesis is regulated by Growth Differentiation Factor 15 (GDF 15) which is secreted by marrow erythroblasts resulting in low Hepcidin expression and increased intestinal iron absorption. In patients of β-thalassaemia major with iron overload, serum hepcidin levels are lower than would be expected because of the increased but ineffective erythropoiesis. The reduction of erythropoietic activity by transfusions, partially relieve the suppression of hepcidin.[11]Although any hepcidin agonist is not yet available, but it can be valuable in prevention of iron overload in non- transfused β-thalassemia patients. Hepcidin therapy may also help in the intervals of transfusion when hepcidin falls and GI iron absorption increases.[12]

Patients and Methods

This case control study was conducted in the department of Haematology, University of Health Sciences Lahore in collaboration with Sundas Foundation Lahore and Capital Hospital Islamabad. Forty diagnosed patients of β-thalassemia major, who had received at least five transfusions and were not on iron chelation therapy (Group A) along with 40 healthy age and sex matched controls (Group B), were included. Three mL was delivered into the gel vacutainer for the estimation of serum iron, TIBC, ferritin and hepcidin and two ml into the EDTA vacutainer for the estimation of hemoglobin levels. Hemoglobin levels were measured by Sysmex Xi 1800 automated Hematology analyzer. Serum iron and TIBC were determined spectrophotometrically using Fer-Color kit from Wiener Lab Argentina and Serum

ferritin was determined by using human ferritin enzyme immunoassay test kit (Gen-Way Bio tech Inc. USA).Hepcidin levels were determined by using human hepcidin ELISA kit(Elabsciences, China).The methodology of given ELISA kit was based on Sandwich-ELISA.The micro ELISA plate provided in the kit was pre-coated with an antibody specific to hepcidin. Samples were added to the micro ELISA plate wells and bound by the specific antibody. Then a biotinylated detection antibody specific for hepcidin and Avidin-Horseradish Peroxidase (HRP) conjugate was added to each micro plate well successively and incubated. Free components were washed away. The substrate solution was added to each well. Blue color was observed. The enzyme-substrate reaction was terminated by the addition of a sulphuric acid solution and the color turned yellow. The optical density (OD) was measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The value of OD was proportional to the concentration of hepcidin.The comparisons of various variables for association or significance were done by using Independent student t -Test while correlation between different variables was established by using Pearson correlation test. Probability level less than or equal to 0.05 was considered as statistically significant.

Results

Forty transfusion dependent β-thalassemia major patients and 40 age and sex matched healthy controls were included in the study. Both groups comprised of 20 males and 20 females patients. The median age of patients was 36 (24- 42) months. Similarly in controls it was also 36 (33- 40) months.

Table 1-Haemoglobin, Serum Iron, TIBC, Ferritin and Hepcidin levels

Parameters	Patients (n=40)	Controls (n=40)	P-value
Hemoglobin g/dl	6.46± 1.63	12.92±0.69	-------
Iron (µg/dl)	1002.5 (622.5-2099.0)	296.5 (252.5-410.0)	0.001
TIBC (µg/dl)	197.5 (161.5-209.7)	276.00 (259.2-301.5)	0.001
Ferritin (ng/ml)	603.0 (317.7-897.7)	33.43(24.1-43.1)	0.001
Hepcidin (pg/ml)	426.2 (278.8-951.1)	757.4(424.4-1074.4)	0.001

Haemoglobin concentration was lower (6.46± 1.63 g/dL) in patients as compared to controls (12.92 ± 0.69 g/dL). In both groups serum iron profile was determined which comprised of serum iron, TIBC and ferritin. Serum iron level was found to be higher in patients (1002.50 µg/dl)as compared to controls i.e. (296.50 µg/dl), while serum TIBC was 197.50 and 276.0 µg/dl in patients and controls respectively.

Student t-test was applied on the given data of patients and controls. Serum ferritin levels were also higher in patients (603.03 ng/ml) as compared to controls (33.43 ng/ml). Serum hepcidin was reduced in patients 426.21 pg/ml as compared to controls i.e., 757.45 pg/ml(Table 1).Significant differences between two groups were observed (*p*-value <0.001) (Table 1).

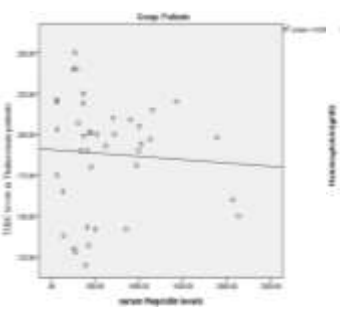

Fig-1:Scatter plot shows a negative correlation between serum hepcidin and TIBC

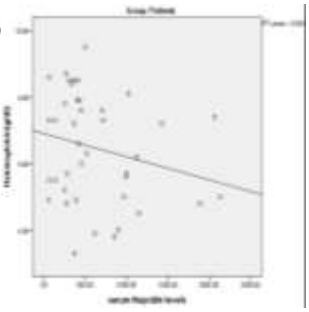

Fig-2: Scatter plot shows a positive correlation between serum hepcidin and ferritin.

Discussion

Iron overload in thalassaemia patients is fatal and a major cause of morbidity and mortality. Extraordinary iron deposition leads to marked cellular damage and organ dysfunction. [13,14] . It affects multiple organs and excessive iron accumulation results in progressive dysfunction of heart, endocrine glands, and liver. Extensive iron deposits are associated with cardiac hypertrophy and dilatation and rarely fibrosis. Iron induced hepatic fibrosis and cirrhosis can be seen in many patients. Iron toxicity in anterior pituitary gland results in disturbed sexual maturation and secondary amenorrhea. Diabetes mellitus is seen in 5% of adults. Long term iron deposition also damages thyroid, parathyroid, and adrenal glands. Iron overload is attributed mainly to blood transfusions, but it is also caused by increased iron absorption.[4]

There are different methods for detection of iron status in overload conditions. The liver is the main site of iron overload and liver iron correlates closely with total body iron. Estimation of liver iron concentration is the most precise method of estimation of body iron. But this facility is currently not common in Pakistan. Non-availability of MR T2 makes the assessment of cardiac iron virtually impossible. Serum ferritin

measured at regular intervals (at least 3 months) has some therapeutic and prognostic use. In this study we analyzed and compared serum hepcidin level and conventional markers of iron status in β Thalassemia major patients and controls.Hepcidin is a key regulator of iron homeostasis and a mediator of anemia of inflammation. It is a 25aa peptide, a regulatory protein produced by the hepatocytes, regulating intestinal iron absorption and its distribution throughout the body.[15] It is, therefore, emerging as an important diagnostic marker.

In the present study we found that serum hepcidin levels were lower in patients when compared to controls. A significant difference between two groups was observed (*p*-value 0.001).Similar observations have been made in another study, where serum hepcidin levels were low in thalassaemia major patients.[16] In another study the serum hepcidin levels were similar in both, patients and controls, because these patients were on chelation therapy.[17]

In this study we used Pearson correlation to find correlation of serum hepcidin with serum ferritin, TIBC and Hb. We did not find a significant correlation of hepcidin with serum ferritin, TIBC and Hb in patients (r= 0.224, p-value= 0.164), (r = -0.63, p-value=0.697) and (r= -0.224, p-value= 0.164) respectively. These results were in accordance with the previous report that showed no significant correlation between serum hepcidin and ferritin levels as a marker of iron overload in thalassaemia major patients.[18] Hepcidin is found to have low positive correlation with ferritin and negative correlation with Hb and TIBC. Similar result has been reported in another study where serum hepcidin had positive correlation with serum ferritin.[17]

Conclusion

1.Hepcidin concentration in patients with iron-loading anaemias is decreased and consequently leads to increased iron absorption.

2.Determination of hepcidin concentration is useful to identify the patients at higher risk of iron toxicity. 3. Developing hepcidin agonists will be useful in transfused thalassaemia patients

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