International Journal of Health Sciences and Research
www.ijhsr.org ISSN: 2249-9571

Original Research Article

Study of Iron Status in Multi-Transfused Thalassaemic Patients in a Referral Hospital in Manipur

Pratima Khoyumthem[1], A. Barindra Sharma[2], Th.Ibetombi Devi[3], K. Rachandra Singh[4], A. Meina Singh[5]

[1]Senior Resident, [2]Associate Professor, [3]Professor, [4]Medical Officer, [5]Professor and Head;
[1,2,4,5]Department of Immunohaematology & Blood Transfusion, [3]Department of Biochemistry,
Regional Institute of Medical Sciences, Imphal, Manipur, India

Corresponding Author: A. Barindra Sharma

Received: 07/03//2014 *Revised: 02/04/2014* *Accepted: 08/04/2014*

ABSTRACT

Thalassaemia remains one of the major health problems in Southeast Asia. Majority of the patients are dependent on frequent blood transfusions and iron overload is a life limiting complication. Excess iron is highly toxic to the tissues which may cause irreversible damage. Twenty multitransfused patients comprising 10 cases of HbE Thalassaemia, 8 cases of Thalassaemia major and 2 cases of Thalassaemia intermedia having fulfilled the inclusion criteria were selected for study. The aims of the study were to evaluate changes in iron profile and any contributing factor to the changes, and to assess iron overload. The parameters used were Serum iron, total iron binding capacity (TIBC), transferrin saturation (%) and ferritin. The measurements were done at least twice a year. Increased ferritin levels combined with increased transferrin saturation is usually taken as iron overload. There were significant increases of all the parameters in Thalassaemia major. The mean serum ferritin levels were 2496±1396.5ng/ml in the 1st reading and 2773.3±1190ng/ml in the 2nd reading above the recommended level of 1000ng/ml for initiating iron chelation. The mean transferrin saturation at two reading points was 220.3±121.6% and 515.7±132.8% with an increase of 37.9%. The correlation of changes in all the parameters between the two readings was highly significant with Pearson´s *(r)* value varying from 0.72 to 0.99 and p *value* being 0.00.Regular monitoring of iron status is essential in multi-transfused thalassaemic patients for early detection of iron overload and timely intervention to prevent from irreversible organ damage.

Key words: Thalassaemia, Iron profile, Serum ferritin, Transferrin saturation, Iron overload

INTRODUCTION

Thalassaemia is an autosomal genetic disease characterized by impaired synthesis of polypeptide chains of normal haemoglobin leading to anaemia. It remains one of the major health problems in Southeast Asia. [1] In severe cases, in order to improve survival and quality of life,multiple blood transfusions are required. Iron overload is the life limiting complication commonly found in thalassaemics, which may be due to ineffective erythropoiesis, increased gastrointestinal absorption, lack of physiologic mechanism for excreting excess iron, and above all multiple blood transfusions. A unit of red blood cells contains approximately 250mg of iron while the body cannot excrete more than 1 mg per day. [2,3]

Iron excretion is passive and cannot be actively upregulated. Therefore, patients with excessive iron absorption or with transfusion dependent congenital or acquired anemia are at increased risk for developing iron overload. [4] Iron is capable of producing free radicals which can cause cellular injury. Major organs affected by surplus iron include the heart, lung, liver and endocrine glands. In iron overload the export of iron from the cells overwhelms the ability of transferrin to bind iron (Fe^{3+}) leading to free, non-transferrin bound iron (NTBI). [5] The redox active component of NTBI, termed labile plasma iron (LPI), is the toxic compound and facilitates entry of iron into cells, causing a marked rise in labile cell iron (LCI). This in turn mediates tissue damage through superoxide generation, redox reactions, gene modulation, and direct interaction with ion channels. [6] Monitoring of iron status has become necessary in multi-transfused thalassaemic patients for early detection of iron overload.

The study was conducted with the aim to evaluate iron status among multi-transfused thalassaemics and to assess iron overload and changes in relation to the number of blood transfusions. The parameters used were serum iron, serum ferritin, total iron binding capacity (TIBC) and percentage saturation of transferrin. The findings are being highlighted.

MATERIALS AND METHODS

The study was a prospective, non-randomized and cross sectional one conducted in the Department of Immunohaematology and Blood Transfusion in collaboration with the Department of Biochemistry, Regional Institute of Medical Sciences, Imphal, Manipur from October 2010 to September 2012, after Institutional Ethical committee approval and written informed consent from the patients.

Inclusion criteria: Any multitransfused Thalassaemic patient confirmed by Haemoglobin Electrophoresis/ High performance liquid chromatography (HPLC) and having received at least two units of blood transfusion in the past one year.

Exclusion criteria: (a) Any patient on iron chelators (b) Patient with positive HIV, HBsAg and HCV (c) Patient with chronic inflammatory diseases (d) Patient with associated malignancy

Records of age, sex, weight and blood transfusion were taken. All the data collected were noted in a pre-designed proforma.

The laboratory investigations viz. Serum iron, Serum total iron binding capacity (TIBC), Transferrin saturation(%) and Serum Ferritin were carried out for all cases at least twice a year in addition to the routine investigations.

Serum iron was performed by Photocolorimetry using commercially available kit (Human, Germany) with procedures as per product manual. The reference range was 60-160ug/dl(male) and 35-145ug/dl(female). TIBC was also performed by Photocolorimetry using kit (Human, Germany), the reference range being 250-400ug/dl. Transferrin Saturation (%) was calculated as Serum iron/ TIBC x100, the reference range being 15-30%. Serum ferritin was done by ELISA (Monobind, USA), the reference range being 60-400ng/ml (male) and 10-150ng/ml (female).The observations were subjected to appropriate statistical analysis like mean, standard deviation for continuous variables, proportion for categorical data, etc. whichever were appropriate and $P \leq 0.05$ was deemed significant.

RESULTS

In our study, a total of 20 Thalassaemic patients fulfilling the criteria were enrolled during the study period. HbE

Thalassaemia (EThal), Thalassaemia Major (ThalM) and Thalassaemia Intermedia (ThalN) comprised 10(50%), 8(40%) and 2(10%) respectively and the male to female sex ratio was 1.2: 1 (Table 1). The mean blood units received at the time of 1st measurement were 59.3±28.4,26.0±21.7 and 18.0±21.2 respectively for ThalM, EThal and ThalN and during the period between 1st and 2nd readings were 3units for ThalM and 2 each for EThal and ThalN.The mean serum iron was 203.3±5.77ug/dl in ThalM in 1st reading and 253.3±58.5ug/dl in 2nd reading with an increase of 24.6%, 150±49.5ug/dl and 168.2±54.6ug/dl in EThal and the increase in ThalN was 8.1%(Table 2).The mean TIBC in ThalM was 58.66±18.03ug/dl in 1st reading and 50.6±12.85ug/dl in 2nd reading, 78.25±2544ug/dl and 68.87±21.38ug/dl in EThal, and 130±98.9ug/dl and 120±98.9ug/dl in ThalN respectively(Table 3).The mean transferrin saturation(%) was 373.82±135% in 1st reading and 515.7±132.8% in 2nd reading in ThalM, 220.3±121.6% and 274.6±145.1% in EThal followed by 197.5±187.3% and 242.3±251.2% in ThalN(Table 4).The mean serum ferritin level was 2496±1396.15ng/ml in 1st reading and 2773.3±1190ng/ml in 2nd reading in ThalM, 1202.1+1381.2ng/ml and 1335.5+1470ng/ml in EThal followed by 550+636.3ng/ml and 710+834.3ng/ml in ThalN (Table 5).The correlation of changes in the parameters with respect to the number of transfusion between the two readings was highly significant with Pearson´s (*r*) value varying from 0.72 to 0.99 and *P* value being 0.00.

Table 1. Age distribution.

Age interval (years)	HbE Thalassaemia	Thalassaemia Major	Thalassaemia intermedia	Total
0-10	3(15%)	2(10%)	0	5(25%)
11-20	3(15%)	3(15%)	2(10%)	8(40%)
21-30	2(10%)	3(15%)	0	5(25%)
31-40	2(10%)	0	0	2(10%)
Total	10	8	2	20

Table 2. Serum iron level (µg/dl) at two reading points.

Diagnosis	1st Reading Mean±SD(Range)	2nd Reading Mean±SD(Range)	Increase from the 1st reading (%)
Hb E Thalassaemia	150±49.5 (70-230)	168.2±54.6 (80-256)	18.2
Thalassaemia Major	203.3±5.77 (200-210)	253.3±58.5 (210-320)	24.6
Thalassaemia Intermedia	154±62.2 (110-198)	166.5±61.5 (123-210)	8.1

Table 3. Total iron binding capacity(µg/dl) at two reading points

Diagnosis	1st Reading Mean±SD(Range)	2nd Reading Mean±SD(Range)	Decrease from the 1st reading(%)
Hb E Thalassaemia	78.25±25.44 (46-110)	68.87±21.38 (40-100)	11.9
Thalassaemia Major	58.66±18.03 (40-76)	50.6±12.85 (38-60)	13.7
Thalassaemia Intermedia	130±98.9 (60-200)	120±98.9 (150-190)	7.6

.

Table 4. Transferrin saturation(%) at two reading points

Diagnosis	1st Reading Mean ±SD(Range)	2nd Reading Mean±SD(Range)	Increase from the 1st reading (%)
Hb E Thalassaemia	220.3±121.6 (70-434.7)	274.6±145.1 (96.4-550)	24.6
Thalassaemia Major	373.82±135 (236.2-525)	515.7±132.8 (375-638.9)	37.9
Thalassaemia Intermedia	197.5±187.3 (65-330)	242.3±251.2 (64.7-420)	22.6

Table 5. Serum ferritin level (ng/ml) at two reading points.

Diagnosis	1st reading Mean±SD(Range)	2nd reading Mean±SD(Range)	Increase from the 1st reading(%)
HbE Thalassaemia	1202.1±1381.2 (200- 3600)	1335.5±1470 (212-3800)	11.1
Thalassaemia Major	2496±1396.15 (888- 3400)	2773.3±1190 (1400- 3500)	11.1
Thalassaemia Intermedia	550±636.3 (100-1000)	710±834.3 (120- 1300)	29.0

DISCUSSION

The dynamics of iron regulation is multifaceted. In thalassaemia intermedia, high erythropoietic drive causes hepcidin deficiency resulting in hyperabsorption of dietary iron and body iron overload. In contrast, in thalM, transfusions decrease erythropoietic drive and increase the iron load, resulting in relatively higher hepcidin levels. In higher hepcidin levels, dietary iron absorption is moderated and macrophages retain iron, but body iron stores increase due to the inability to excrete iron in transfused red blood cells. [7] Ferritin is the main iron-storage protein in the body. It has a central in iron homeostasis, because it binds to and sequesters intracellular iron. [8] Serum ferritin has been used as an easily accessible marker of iron burden, however, a single measure may not provide reliable results. [9] Serum ferritin levels consistently above 1000ng/ml(1000ug/L) are indicative of iron overload and recommended for initiating iron chelation. [10,11] It has been reported that increased ferritin levels combined with increased transferrin saturation is a sign of iron overload. [12,13] The mean serum ferritin levels in ThalM in our study of 2496±1396ng/ml and 2777.3±1190ng/ml in two readings are close to the study conducted by Angulo IL et al. [14] with 2337±1012ng/ml but lower than 4236.5±2378.3ng/ml reported by Riaz H et al. [9] and 4098.67±1598.63ng/ml reported by Sultana N et al. [15] The mean serum ferritin level of 1202.1±1381.2ng/ml in EThal in our study is also lower than 2232.57±1598.63ng/ml in the study of Sultana N et al. [15] Serum iron is increased in iron overload and TIBC is decreased in which the relationship is nonlinear. [16] In this study the increase of serum iron from the 1st reading to the 2nd reading was 24.6% for ThalM, 18.2% for EThal and 8.1% for ThalN and the decrease of TIBC was 13.7% for ThalM, 11.9% for EThal and 7.6% for ThalN.

The transferrin saturation is a surrogate marker of NTBI, a toxic fraction of plasma iron, which usually appears when the saturation is above 70%. [17] A positive correlation between the presence of serum NTBI in thalassaemic patients with transfusional iron overload and increased risk of organopathy has been reported. [18] In our study, the percentage of increase in transferrin saturation from 1st to 2nd reading was 37.9% for ThalM, 24.6% for EThal and 22.6% for Thal N. In all the three types of thalassaemia, a progressive increase has been noted in serum ferritin, iron and transferrin saturation and decrease in TIBC across the two reading points.The correlation of changes in the parameters with respect to the number of transfusion between the two readings was highly significant, the Pearson´s (r) value being 0.72 for TIBC, 0.73 for transferrin saturation,0.94 for ferritin and 0.99 for iron, and p value being 0.00.

The liver contains most of the body iron stores (70-80%). The determination of liver iron concentration (LIC) after a liver biopsy was a reliable approach in the evaluation of iron overload which has been

gradually replaced by non-invasive techniques like magnetic resonance imaging(MRI) and more advanced superconducting quantum interference device (SQUID) susceptometry.[19,20] Measurement of NTBI, LPI and hepcidin have promising results and may be feasible markers in the future. [21,22]

CONCLUSION

In the present study, a significant increase has been found in serum ferritin and transferrin saturation percentage with increasing number of blood transfusions. The correlation of changes in the parameters of serum ferritin, serum iron, TIBC and transferrin saturation with the number of transfusions are also statistically highly significant. Regular monitoring of iron status is essential in multitransfused thalassaemic patients for early detection of iron overload and timely management to prevent from irreversible tissue damage.

REFERENCES

1. Fuchroen S, Winichagoon P. Thalassemia in Southeast Asia: problems and strategy for prevention and control. Southeast Asian J Tropical Medicine and Public Health 1992;23: 647-655
2. Giardina PJ, Grady RW. Chelation therapy in β-thalassemia: An optimistic update. Hematol 2001; 38: 360-366
3. Ikram N, Hassan K, Younas M, Amanat S. Ferritin levels in patients of Beta Thalassaemia Major. International Journal of Pathology 2004; 2(2): 71-74
4. Batts KP. Iron overload syndromes and the liver. Mod Pathol 2007; 20(Suppl 1): 31-39
5. Hershko C, Link G, Cabantchick I. Pathophysiology of iron overload. Ann N Y Acad Sci 1998; 850 : 191-201
6. Cabantchik ZI, Breuer W, Zanninelli G,Cianciulli P. LPI-labile plasma iron in iron overload. Best Pract Res Clin Haematol 2005; 18:277-287
7. Origa R, Galanello R, Ganz T et al. Liver iron concentrations and urinary hepcidin in beta thalassemia. Haematologica 2007; 92(5): 583-588
8. Estevao IF, Junior PP, Bonini-Domingos CR. Serum ferritin and transferrin saturation in β and β thalassemia. Genetics and Molecular Research 2011; 10(2): 632-639
9. Riaz H,Riaz T,Khan MU et al. Serum ferritin levels, socio-demographic factors and desferrioxamine therapy in multi-transfused thalassemia patients at a government hospital of Karachi, Pakistan. BMC Resaerch Notes 2011;4:287 doi:10.1186/1756-0500-4-287
10. Olivieri NF, Brittenham GM. Iron-chelating therapy and the treatment of thalassemia. Blood 1997;89(3): 739-761
11. Morrison ED, Brandhagen DJ, Phatak PD et al. Serum ferritin level predicts advanced hepatic fibrosis among U.S. patients with phenotypic hemochromatosis. Annals of Internal Medicine 2003; 138(8):627-633
12. Camaschella C, Poggiali E. Towards explaining "unexplained hyper-ferritinemia". Haematologica 2009; 94: 307-309
13. Brissot P, Troadec MB, Bardou-Jacquet E et al. Current approach to hemochromatosis. Blood Rev 2008; 22: 195-210
14. Angulo IL, Covas DT, Carneiro AA et al. Determination of iron overload in thalassaemia by hepatic MRI and

ferritin. Rev Bras Hematol Hemoter 2008; 30(6): 449-452
15. Sultana N, Sadiya S, Rahman MH. Correlation between serum bilirubin and serum ferritin level in thalassaemia patients. Bangladesh J Med Biochem 2011;4(2): 6-12
16. Wood JC. Diagnosis and management of transfusion iron overload: the role of imaging. Am J Hematol 2007;82(12 suppl):1132-1135
17. Cabantchik ZI, Breuer W, Zanninelli G,Cianciulli P. LPI-labile plasma iron in iron overload. Best Pract Res Clin Haematol 2005; 18:277-287
18. .Piga A, Longo F, Duca L et al. High nontransferrin bound iron levels and heart disease in thalassemia major. Am J Hematol 2009; 84: 29-33
19. Brittenham GM, Badman DG. Noninvasive measurement of iron: Report of an NIDDK workshop. Blood 2003; 101:15-19
20. Gandon Y, Olivie D, Guyader D et al. Non-invasive assessment of hepatic iron stores by MRI. Lancet 2004; 363: 357-362
21. Pootrakul P, Breuer W, Sametband M et al. Labile plasma iron(LPI) as an indicator of chelatable plasma redox activity in iron-overloaded beta-thalassemia/HbE patients treated with an oral chelator. Blood 2004;104(5): 1504-1510
22. Kemna EH, Tjalsma H,Podust VN et al. Mass spectrometry-based hepcidin measurements in serum and urine: analytical aspects and clinical implications. Clin Chem 2007; 53(4): 620-628.

How to cite this article: Khoyumthem P, Sharma AB, Devi TI et. al. Study of iron status in multi-transfused thalassaemic patients in a referral hospital in Manipur. Int J Health Sci Res. 2014;4(5):53-58.

****