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Iron Overload in Thalassemia Major: An Observational and Cross-sectional Study

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ABSTRACT

Objective: Managing iron overload in thalassemia requires a reliable assessment of excess iron load and organ iron distribution. Trends in serum ferritin have been most commonly used as an inexpensive guide to evaluate the body iron stores. Liver biopsy remains the gold standard for estimating iron overload. Here we have evaluated the liver iron in thalassemia major patients and compared it to the serum ferritin.

Method: In the present cross-sectional study, 55 patients of thalassemia major were enrolled. Serum ferritin estimate was compared to the iron status determined by histopathological examination of the liver biopsy specimens.

Result: Very high serum ferritin was present in these young thalassemics. Almost 90% of biopsy samples had grade 3-4 iron deposition. A good correlation was found between serum ferritin values and hemosiderosis of the liver. Significantly higher grades of liver fibrosis were observed in these Indian Thalassemics.

Conclusion: Serum ferritin can be used as a serum marker of somatic iron stores. However, it cannot replace invasive and non-invasive liver iron assessments for a more accurate estimation. Direct examination of liver specimens also allows us to grade fibrosis, which was present in almost 90% of the biopsy samples. Awareness needs to be created regarding the high iron overload and its consequences on the long- term survival of patients. Meanwhile, we need to optimize the chelation therapy and impress upon the need for regular monitoring of iron overload for better outcomes.

Keywords: *Thalassemia major, Serum Ferritin, Liver biopsy, Liver iron, Liver fibrosis.*

INTRODUCTION

Iron overload is a common clinical problem arising from multiple blood transfusions in beta thalassemic patients. Survival in iron overload syndromes has increased dramatically because of improved access to iron chelation therapy, availability of oral iron chelators and early recognition of life threatening organ iron deposition. [1] Liver is the dominant storage organ for excess iron. The plasma ferritin concentration and the amount of urinary iron excreted after the administration of chelating agents are mere qualitative indexes of iron loading and are also influenced by infection, inflammation, liver disease, ascorbate deficiency and other factors. [2] The reference method for evaluating the extent of body iron excess in systemic iron overload is measurement of the hepatic iron concentration. Measurement of hepatic iron concentration is the most quantitative, specific, and sensitive method for determining the body iron burden in patients with thalassemia major. [3] The biopsy also permits detailed morphological assessment that, apart from confirming the degree of iron overload, also identifies localizes and grades inflammation, as well as noting the presence of steatosis and other features like steatohepatitis. Finally, it allows for staging of hepatic fibrosis. Ishak staging [4] has been used in many studies for evaluating hepatic fibrosis and has given satisfactory results.

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Secondary iron overload like in thalassemia patients may have a predominantly though not exclusively, reticuloendothelial distribution. Although chemical measurement provides the most accurate means of assessing hepatic iron, [5] histochemical staining for iron in routine histological sections can be used to provide semiquatitative estimations.

Several investigators have described scoring systems for the assessment of stainable iron. [6,7] In our experience the method of Sciot et al [8] is the most useful and reproducible; values obtained using this approach have been shown to correlate well with chemical estimations. A study demonstrated that stainable iron could be measured using image analysis; values obtained show a linear relationship with those given by chemical estimations. [9]

Hence, considering liver iron to be the most accurate means of assessing iron load we performed semi-quantitative estimation due to the unavailability of Atomic absorption spectrophotometry for quantitative values.

MATERIALS AND METHOD

This was a prospective and interventional study conducted on randomly selected 55 patients of beta thalassemia major >2 years of age of either sex. Study was conducted in the Thalassemia Day Care Centre in the Department of Paediatrics, University College of Medical Sciences and Guru Tegh Bahadur Hospital, Delhi from December 2008 to April 2010. The study protocol was approved from Institutional Ethics Committee of University College of Medical Sciences, Delhi. Informed consent was taken from the parents or guardians of the patients.

Inclusion criteria:

Multi-transfused thalassaemic children >2 years of age and either sex.

Children with serum ferritin >1500ng/ml.

Exclusion Criteria: Patients who had been transfused less than 10 units of blood as a part of their management.

Method of Selection: 55 children getting repeated transfusions and chelation were randomly selected. Out of which 20 were on Deferoxamine, 20 were on Deferiprone and 15 were on Deferasirox.

Tests during the study: A single observer recorded all these parameters to minimize observer bias.

- **Proforma regarding personal and treatment details** were filled and patients were examined clinically and various parameters were done at start of the study such as
- **Anthropometry**
- **General physical examination** like presence of pallor, icterus, facial changes, liver and spleen size.
- **Baseline haematological profile-** Complete blood count and peripheral smear was done.
- **Biochemical profile** like
- **LFT:** Serum bilirubin, Serum glutamate aspartate transaminase (SGPT), and ALP was done.
- **KFT** (blood urea and serum creatinine) was done.
- **HIV, HCV and HBsAg status** were also determined.
- **Serum ferritin** was determined for all patients (n=55). Serum ferritin was a micro-particle enzyme linked immunoassay and was carried out on "AxSYM" automated machines manufactured by Abott Laboratories, U.S.A. The kits for carrying out the test were also supplied by Abott Laboratories.
- **Liver biopsy** was done with serum ferritin for those 35 subjects who gave their consent for the procedure. PT/PTTK was done for these patients prior to biopsy and after ascertaining that their coagulation profile was normal; the patients were admitted for the procedure. Biopsy sample was obtained from the right lobe of liver using a 14 G tru-cut biopsy needle under aseptic precautions, after adequate sedation. The sample was sent in 10% formalin to the histopathology laboratory. A biopsy specimen was considered adequate if two or more portal tracts were present. This was considered the absolute minimum necessary for the assessment of fibrosis and cirrhosis. Hematoxylin and Eosin (H&E) staining was done for all slides and the following features were noted- expansion, inflammation, bile duct proliferation, ballooning degeneration, steatosis, myofibroblasts and collagen deposition. For histological evaluation the 70 biopsy

slides were randomly arranged and each slide was assigned a unique number. The pathologist graded the findings according to the appropriate systems mentioned above. A scoring system for histochemical assessment of hepatic iron and zonal distribution of stains devised by **Sciot et al** [8] was used. The amount (0-4) and zonal distribution of stainable iron was assessed in hepatocytes, sinusoidal cells and in cells within the portal tract stroma and fibrous septa. A final score was obtained by multiplying the hepatocyte score by a co-efficient of 3 and the other scores by a co-efficient of 1. The total was scored on a scale of 0-20 is then divided into grades 0, 1, 2, 3 and 4. The fibrosis was scored on the basis of "**Ishak stage**". [4]

STATISTICAL ANALYSIS

Data was presented in tabular form and statistical analysis was done using Graph Pad software. Spearman's rank test was applied for comparison between serum ferritin and liver iron grades and a correlation was interpreted if the p value was <0.05. Results are expressed in median and range.

RESULTS

Table 1: Total cases, mean age and sex (n=55)

	Mean Age±SD (yrs)	Range(yrs)	Male(%)	Female(%)
Total (n= 55)	10.082±5.69	2.5-26	26(47.27)	29(52.73)

Table 2: Mean serum ferritin values

	Serum Ferritin (ng/ml)Mean±SD	Range(ng/ml)
Total (n=55)	4116.16±2240.4	1552.2-14067
Males (n=26)	3859±1858.9	1552.2-8025.9
Females (n=29)	4346.7±2545.3	1645-14067
Biopsy patients (n=35)	3872.61±1544.3	1552.2-8025.9

Only 20% patients had a serum ferritin between 1500-2500ng/ml. 80% cases had a ferritin >2500ng/ml.

Features noticed on H&E staining of biopsy specimens were changes in hepatocytes, kupffer cells and portal areas. Iron deposition and fibrosis, although seen on H&E were assessed semiquantitatively by Perl's stain and Masson Trichrome stain respectively. The hepatocytes in two of the four cases of viral hepatitis showed microsteatosis (Fig-1). Other changes seen in these cases were ballooning degeneration, widening of portal areas with inflammatory cells, bile duct proliferation and kupffer cell hyperplasia. Grade 4 iron deposition and Stage 4 fibrosis is depicted in Figure 2 and 3 respectively.

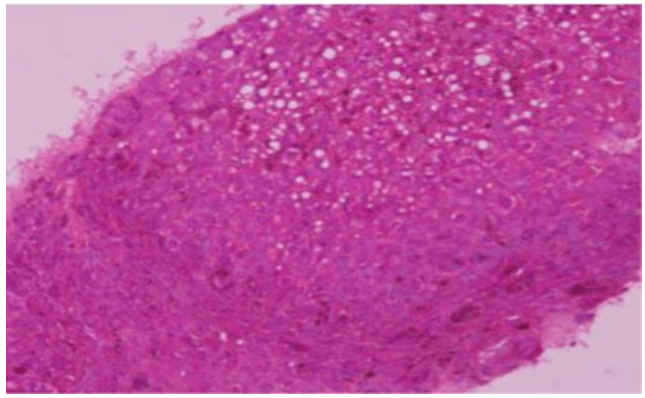

Figure 1: Hepatic Microsteatosis

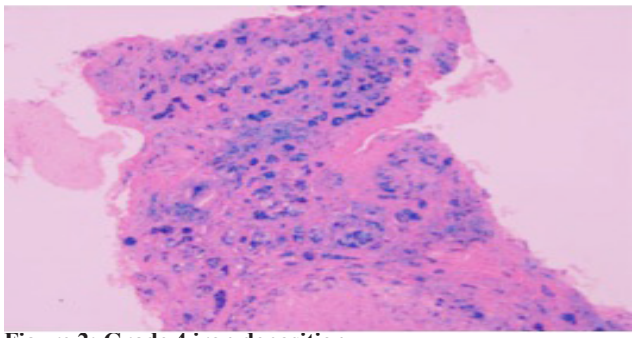

Figure 2: Grade 4 iron deposition

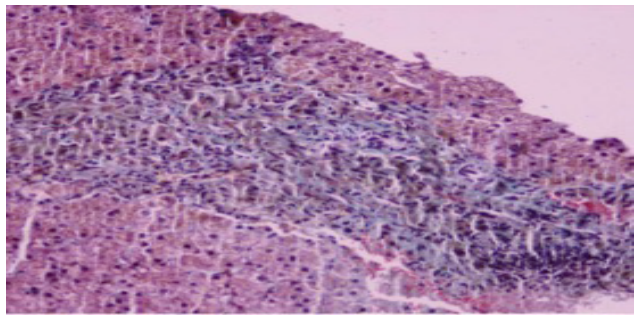

Figure 3: Stage 4 Fibrosis

Table 3: Grading of liver iron by Sciot's Scoring System

	Score		No. of patients in various grades				
	Mean±SD	Range	Grade 1	Grade 2	Grade 3	Grade 4	Median
Biopsy Patients (n=35)	17.3±3.2	5-20	0(0%)	2(5.7%)	4(11.43%)	29(83%)	4

Table 4: Staging of fibrosis by Ishak stage

	No. of patients in various stages (%)							Median
	Stage 0	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6	
Patients (n=35)	3(8.6% had no fibrosis)	5(14.3%)	5(17.1%)	13(37.14%)	5(14.3%)	1(2.9%)	2(5.7%)	3

Among the 35 patients 3 already had the setting of incomplete or definite cirrhosis. Surprisingly, that one child with incomplete cirrhosis was only 2.5 years. The other 2 were HCV positive which was a proven risk factor.

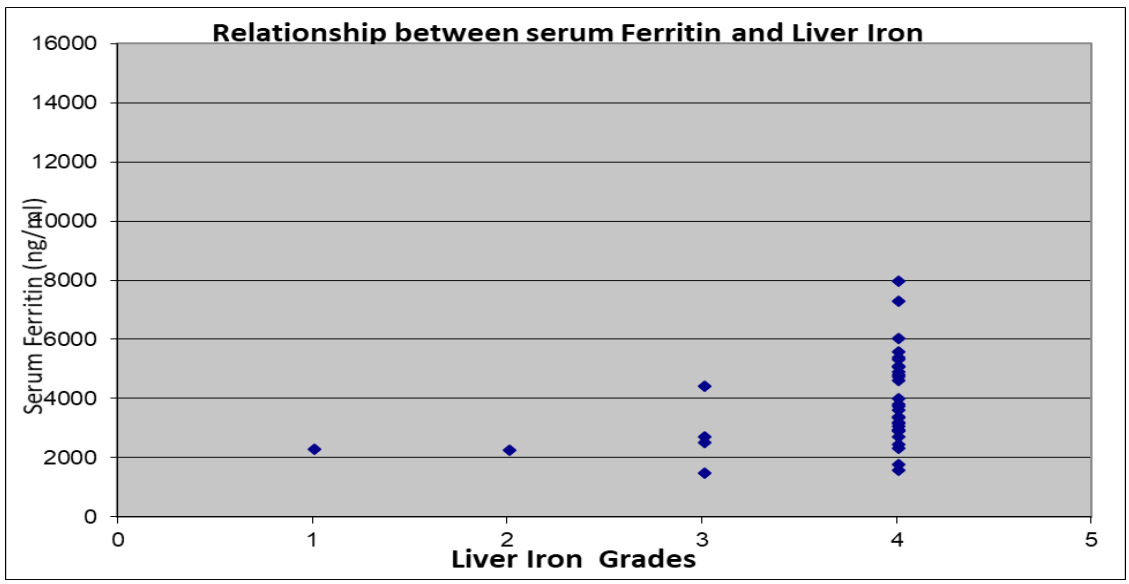

Figure 4: Relationship between serum Ferritin and Liver Iron

A correlation was proven between serum ferritin and liver iron grades (p=0.009). There was no relation between liver iron grades and fibrosis stages.

Hepatitis C infection had a prevalence of 14.45%(8/55) among the patients. One patient tested positive for HBsAg.

Table 5: Relation between HCV positivity, serum ferritin and SGPT.

HCV	S.Ferritin (Mean±SD)	SGPT (Mean ± SD)
Positive (n= 8)	5107.51±2495.3	116.13±91.63
Negative (n= 41)	3947.42±2178.22	97.60±72.25

The mean serum ferritin and SGPT was more in HCV positive patients than in HCV negative.

Table 6: Relation between HCV positivity and fibrosis

HCV	Fibrosis(Median)
Positive (n= 4)	4
Negative (n= 31)	3

Similar to the above results the four HCV positive patients who underwent biopsy had higher degree of fibrosis in comparison to HCV negative patients.

DISCUSSION

Liver biopsy and measurement of LIC in mg/gm liver tissue has been the gold standard for assessing body iron, as liver is the main organ of storage. Due to lack of facilities we used a semi-quantitative method of assessing liver iron by Sciot's score. [8] To the best of my knowledge no study has made use only of the semi-quantitative method of iron estimation in liver biopsy.

Reports of variability in the hepatic iron concentration in different areas of the liver are generally limited to cirrhotic liver. [10-12] There is lack of data in transfusional iron overload patients without cirrhosis. Although in a study by Angelucci et al. hepatic iron concentrations was a reliable indicator of total body iron stores in thalassemia major patients without cirrhosis, suggests that the extent of variability is limited. [13]

In our study, there was a direct correlation between the liver iron and serum ferritin values, which was in accordance with the observations of other workers. [14,15] 94% patients had grade 3- 4 iron loading which is ominous considering the mean age of study group was only 10 years. Li et al concluded that serum ferritin is the most significant predictor of moderate to severe hemosiderosis (p=0.003). [16] LIC appeared to be an even better predictor of moderate to severe hemosiderosis (p<0.001) in their study group. Although there was a correlation between serum ferritin and LIC in their study, it was less reliable at ferritin concentration above 5500ng/ml. Direct measurement of liver iron should be a good monitoring tool in those with high serum ferritin.

Secondary hemosiderosis as in thalassemia is one of the causes of liver fibrosis. Hepatic iron concentration and HCV positive status have been confirmed to be independent but mutually reinforcing risk factors predicting fibrosis progression. [17] The mean ferritin levels were above 4000ng/ml and most of the samples had a high iron score. 60% of the samples showed a fibrosis score of 3 or more. Almost 91% children already had liver fibrosis, which is an alarming number. Although, in the present study there was no correlation between the liver iron grades and fibrosis staging. Since fibrosis is an irreversible damage due to excess iron at the tissue levels, it does not have any relation with serum ferritin, which is a mobile iron pool. In a study by Ghavamzade et al it has been shown that the patients who had some degree of fibrosis before Hematopoietic Stem Cell Transplant (HSCT) had progression of fibrosis irrespective of the degree of iron overload even after HSCT. [18] Thus presence of higher grades of fibrosis can be detrimental to the success of HSCT, which is the only cure for thalassemia. Hepatic fibrosis is also a known prognostic factor for patient survival after BMT as reported previously. [19]

Unfortunately, patient acceptance is poor due to the invasive nature of the procedure. Therefore, considering the discomfort and risk of liver biopsy; it was never fully accepted as a standard monitoring tool.

The prevalence of Hepatitis C was 14.5% similar to earlier Indian studies. [20,21] As expected children who were HCV positive (14.5%) had higher mean values of liver enzymes as compared to HCV negative children. HCV is an independent risk factor for the development of fibrosis, which has been depicted in our observations.

Li et al also showed that hepatic fibrosis is more common in those with hepatitis C (p= 0.008). [16]

Limitations:

Study was done on a small sample size. Due to lack of facilities we used the semi-quantitative method of assessing liver iron. Liver biopsy is plagued by sampling variability and there are differences in the processing which can have an impact on the result. The comparative data of histopathological grading of iron is not available from Indian studies.

CONCLUSION

Indian thalassemic population has very high serum ferritin levels and severe hepatic iron overload. In the above context, implementation of direct monitoring of liver hemosiderosis is of importance to individualize chelation therapy. In centers where LIC estimation is not available, histological grading of liver hemosiderosis is reliable and is relatively simple to perform. Histological examination also gives us a direct of the fibrosis stage, which is a prognostic factor for successful HSCT. Liver fibrosis seems to occur at an early age in Indian thalassemic mainly due to poor chelation. This warrants the need for early initiation of iron chelation, with regular monitoring of iron overload to ensure an improved survival.

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Conflict of Interest: None

Ethical Clearance: Taken

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