

Morphological Study of Liver Biopsy in Thalassaemia Major

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Introduction

Thalassaemia major (b-thalassaemia) is one of the commonest inherited disorders in Pakistan. The carrier frequency varies from 4 to 5 percent in different ethnic groups and different parts of the country.^{1,2} It is statistically estimated that nearly 4000-5000 thalassaemia major children are born in Pakistan every year.^{3,4}

These patients need regular blood transfusions and so secondary iron overload may develop in many organs including liver, heart and endocrine glands. Secondary haemochromatosis causes liver damage in these children at an early age and is responsible for morbidity and mortality. The complications like chronic hepatitis, cirrhosis and even hepatocellular carcinoma may develop as a result of chronic hepatitis and severe iron overload (haemosiderosis).⁵

Iron-induced liver disease is a common cause of death in older patients and is often aggravated by infection with hepatitis C.⁶ Portal fibrosis and even cirrhosis may develop within 1-2 years after beginning of transfusions in spite of iron chelation therapy.⁷ Elevated serum ALT levels should alert the clinician about the possibility of hepatitis B and C due to multiple unscreened blood transfusions. The serum ferritin assays are also raised in thalassaemic children.⁸

Liver biopsy is important for assessment of iron load and its distribution within the liver and the severity of liver disease.^{9,10} The histological activity index (HAI) determines the aetiology, grading of activity (necroinflammatory) and staging of the disease and the hepatic fibrosis and haemosiderosis are the final outcome to be determined.¹¹ The two commonly scoring systems by Knodell and Scheuer are currently used for numerical assessment of the activity.¹²

The purpose of the study is to see the morphological changes and severity of hepatic

damage in transfusion dependent Thalassaemia major children undergoing bone marrow transplantation.

Materials and Methods

The study was carried out at Histopathology Department of Army Medical College and Paediatric Department of Military Hospital, Rawalpindi from January 2000 to October 2002. Liver biopsy specimens were obtained for histological examination from 40 cases of thalassaemia major children receiving 1-2 transfusions per month. Children selected for bone marrow transplantation were also included in the study. The children with history of biliary atresia, neonatal hepatitis and storage disorders were excluded from the study. The investigations included liver biopsy, serum ALT levels, Ferritin assays and serology for HBsAg and Anti- HCV antibody.

Biopsies were fixed in 10% buffered formalin and processed in automatic tissue processor- Sakura Japan. Five micrometers thick sections were stained with haematoxylin and eosin (H&E), Reticulin and Perl stains. Histological findings were noted and assessed by using Knodell.¹² According to Hepatitis activity index (HAI) scoring system (Table 1), total score (22), the lesions were divided into four groups having different grades as is shown in Table 2.

All biopsies were scored by three observers independently. The degree of haemosiderosis was categorized into five grades according to deposition in liver parenchymal and mesenchymal tissue (Kupffer cells) by Scheuler (modified by Rowe)¹¹ such as: Grade 0- Nil, Grade 1- minimal, Grade 2 - mild, Grade 3 - moderate and Grade 4 - severe.

The serology for HBsAg was done by ELISA Kit method and anti-HCV antibody was performed by fourth generation ELISA Kit. The serum ALT levels were done by UV kinetic method on Selectra- 2

Chemistry Autoanalyzer. The serum ferritin was measured by LIA mat ® system 300 Byk-Sangtec using two highly specific monoclonal antibodies for quantitative estimation.

Results

A total of 486 liver biopsies were received in the Pathology department of Army Medical College,

Table 1. Knodell histological activity index (HAI).

	Score (0-10)
A. Periportal necrosis (PN) with or without bridging necrosis	
a. None	0
b. Mild piecemeal necrosis	1
c. Moderate PN (<1/2 circum)	3
d. Marked PN (>1/2 circum)	4
e. Moderate PN + bridging necrosis	5
f. Marked PN + bridging necrosis	6
g. Multi-lobular necrosis	6
B. Intra lobular degeneration and focal necrosis	
a. None	0
b. Mild 1/3 of lobular	1
c. Moderate (1/3-2/3)	3
d. >2/3	4
C. Portal inflammation	
a. None	0
b. Mild (<1/3 of portal tract)	1
c. Moderate (1/3-2/3)	3
d. Marked (>2/3)	4
D. Fibrosis	
a. None	0
b. Fibrous portal expansion	1
c. Bridging fibrosis	3
d. Cirrhosis	4

Rawalpindi over a period of three years (July 2000 to August 2003) out of these 40 cases were of thalassaemic children and constituted a relative frequency of 8.2%. The children were seen between 1.5-10.5 years of age with an average age of 6.1 years. Male to female ratio was 1.2:1.

Eighteen (45%) cases were placed in Knodell HAI group III having grading score of 9-12 and ten (25%) cases were in group II with a grading score of 4-8 as presented in Table 2. Twenty two (55%) and 12 (30%) children had Knodell HAI stage 3 and 1 respectively while 6 cases (15%) showed cirrhotic changes (stage 4). Twenty four (60%) children revealed total Knodell HAI score between (13/22 to 18/22). Twenty eight (70%) of the patients had grade 3-4 haemosiderosis (Table 3).

Table 2. Knodell HAI - Grading score (n=40).

Grading score	No. of cases	%
1-3 (Group-I)	4	10
4-8 (Group-II)	10	25
9-12 (Group-III)	18	45
13-18 (Group-IV)	8	20

Table 3. Grades of haemosiderosis (n=40).

Grade	No. of cases	%
0	0	0
1	0	0
2	12	30
3	20	50
4	8	20

Table 4. Serum ferritin assays (ng/ml) (n=40).

Serum Ferritin Levels(ng/ml)	No. of Cases	%
500 - 1000	0	0
1000 - 1500	4	10
1500 - 2000	4	10
2000 - 2500	20	50
2500 - 3000	10	25
3000 - 3500	2	5

Twenty (50%) of these children showed serum ferritin assays between 2000 to 2500 ng/ml and while one

Figure1. Photomicrograph showing haemosiderin pigment deposition in the liver tissue (H&Ex40)

[[1]]

Figure2. Section of liver tissue shows Prussian blue reaction of iron pigment (Perl Stain x 40)

case had highest value of 3470 ng/ml as is presented in Table 3. Thirty two (80%) cases showed serum ALT values between (35-112 U/L) which is 2-3 times raised from the normal reference range (<30U/L). Six cases were positive for HBsAg and 14 for Anti- HCV antibody.

Discussion

Liver biopsy plays an important role in the assessment of haemosiderosis, histological changes of chronic hepatitis including fibrosis and cirrhosis in transfusion dependent thalassaemic children.⁹ These changes have been observed at an early age in our cases because proper and effective iron chelation therapy was not available. Moreover 3 cases were positive for HBs Ag and 7 for anti-HCV antibody as these children received repeated blood transfusions which sometimes were not thoroughly screened.⁵⁻⁷ The life expectancy of these children in our country is about 10-16 years in majority of the cases.¹³

The Knodell histological activity index (HAI)^{12,14} in this series has shown 18 patients (45%) in 9-12/18 grade while 6 of forty cases revealed fully developed cirrhotic changes. Twenty four cases (60%) were having HAI score between 13/22 to 18/22. Twenty eight (70%) children had 3-4 grade haemosiderosis. The study done by Li et al¹⁵ revealed 30% cases with HAI stage 3 and 44% patients showed grade 3-4 haemosiderosis in transfusion dependent thalassaemic children. No case of cirrhosis was seen in this study because screening program and facilities for effective and prompt iron chelation therapy were easily available. In the absence of liver iron content (LIC) assay facility histological grading of haemosiderosis was done on Haematoxylin and Eosin (H&E) stained sections and by doing special stain for iron (Perl Stain).

Another study by Jean et al¹⁶ for histological evaluation of liver biopsy in 86 children with thalassaemia indicated that some patients developed cirrhosis as early as 7-8 years of age. These findings are comparable to our study cases who developed cirrhosis as early as 6-7 years of age. The morphological study of liver biopsy in 30 cases by Thakerngpol et al⁷ noted that fibrosis and pattern of liver cell damage in thalassaemic children due to iron overload are similar to the cases of viral hepatitis. These changes are in conformity to those observed in our study.

In this study high Knodell HAI score has been found in patients having severe haemosiderosis, raised serum ferritin and ALT levels and those showing

positive serology for HBsAg and anti-HCV. The other studies have shown similar results.^{14,17} Majority of our patients were declared unfit for BMT due to higher Knodell grade and stage, as these cases did not fulfill the selection criteria (HAI grade <7/18 and stage 1/4) of Armed Forces Bone Marrow Transplantation Centre (AFBMT) Rawalpindi.

Liver disease is quite common in thalassaemic children. Moderate to severe haemosiderosis has been observed in majority of the patients. High Knodell HAI score has been found in patients having grade 3-4 haemosiderosis, raised levels of serum ALT and ferritin and positive serology for HBsAg and anti-HCV antibody. Liver biopsy should therefore be performed in these cases to document hepatic necrosis, inflammation, fibrosis/cirrhosis and to assess the grading of stainable iron in the liver tissue. Liver histological examination also gives information about severity of the disease in thalassaemia major children and thus selecting the suitable cases before undergoing bone marrow transplantation (BMT).

References

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Abstract

Objective: To see the morphological changes in liver in transfusion dependent Thalassaemia major children undergoing bone marrow transplantation.

Methods: This retrospective cross-sectional survey was conducted at Pathology department of Army Medical College and Paediatric department of Military Hospital, Rawalpindi from Jul 2000 to Aug 2003. Liver biopsies were done in 40 thalassaemic major children and histological changes including Knodell Histological activity index (HAI); grade, stage and score along with degree of haemosiderosis were noted. Serum ALT levels, ferritin assays and screening for HBsAg and Anti-HCV antibody were also carried out in these cases.

Results: Forty children 1.5 - 10.5 years of age (mean 6.1 years) with a male to female ratio of 1.2:1 were included in the study. According to Knodell HAI scoring, 24 (60%) cases had Knodell HAI score between 13/22 to 18/22 and 18 patients (45%) in grade 9-12/18. Six children had fully developed cirrhotic changes whereas 22 and 12 patients showed stage 3 and 1 respectively. Twenty eight (70%) patients had grade 3-4 haemosiderosis. HBsAg was positive in 6 and anti-HCV antibody in 14 patients. Serum ferritin and ALT levels were markedly raised in most of the patients.

Conclusion: Seventy percent patients had moderate

to severe haemosiderosis and high Knodell HAI score was found in children with severe haemosiderosis, raised ALT and Ferritin levels and with positive serology for HBsAg and anti- HCV antibody. Liver biopsy is useful in thalassaemic

children to assess the stage of liver disease and selection of suitable cases for bone marrow transplantation (BMT) (JPMA 54:415;2004).