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Original Article

Fibroscan as a Non-invasive Tool for Assessment of Hepatic Fibrosis in Children with Beta Thalassemia Major

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Abstract

Introduction: Fibroscan is a noninvasive tool for measuring liver fibrosis. This study was done to assess liver fibrosis in thalassemic children and to correlate fibroscan findings with supportive markers of increased iron store such as serum ferritin.

Methods: This prospective observational study was conducted in children between five and 18 years of age diagnosed with beta thalassemia major who were on regular blood transfusion. The fibroscan findings of liver were correlated with serum ferritin, liver enzymes, and blood transfusion requirements.

Results: Among the 34 children who satisfied the inclusion criteria, 22 children (64%) had no fibrosis, 10 (30%) had mild fibrosis, and two (6%) had moderate fibrosis. Higher mean duration of blood transfusion and greater median ferritin levels (3420 vs 1997 μg / L; p-value 0.006) were observed in children with fibrosis. A greater number of transfusions were significantly associated with a greater degree of hepatic fibrosis which was statistically significant ($p < 0.05$). No association was observed between liver fibrosis and mean age of the study population, gender, and mean age at first transfusion, AST or ALT levels. Spearman's correlation coefficient (rho) measured to estimate the degree and direction of the relationship between degrees of fibrosis with ferritin levels was 0.549, indicating a moderately positive relationship between serum ferritin levels and hepatic fibrosis (p-value - 0.0007).

Conclusions: Fibroscan is an affordable, noninvasive, and faster alternative tool to estimate liver fibrosis in children with beta thalassemia major and is directly related to serum ferritin levels and transfusion requirements in children.

Introduction

Thalassemia is a common hereditary blood disorder characterized by reduced or absent production of β-globin chains resulting in ineffective erythropoiesis. Children with β-thalassemia major are chronically anaemic and transfusion dependent. Iron overload secondary to repeated transfusions and increased gut absorption involves various organs causing significant morbidity and mortality.[1] Iron accumulation in liver leads to direct toxic injury to hepatocytes resulting in hepatic fibrosis. Activated lymphocytes produce several cytokines and growth factors, such as TNF-a, TNF-b, IFN-G, IL-1, IL-10, and TGF-b1, causing the activation of hepatic stellate cells, which are thought to be the major source of excess collagen in liver fibrosis.[2]

Traditionally, serum ferritin levels reflect the total body iron store and also have

correlated well with hepatic iron overload.[3] However, its clinical significance in hepatic fibrosis is not well established.[4] Estimation of liver iron concentration (LIC) by liver biopsy was considered the gold standard in the past. Presently, it is not preferred due to invasiveness, sample variability because of uneven iron distribution, and inter-observer variability.[5] Magnetic resonance imaging (MRI) transverse relaxation time (T2*) is considered the method of choice for detecting iron overload in the liver, however, it is expensive, requires expert radiologist and is still not widely available.[6]

Fibroscan is a widely available, noninvasive, and inexpensive tool for measuring liver stiffness caused by fibrosis.[7] Liver stiffness measurement (LSM) by transient elastography (TE) has been shown to be closely related to the degree of hepatic fibrosis assessed by biopsy in thalassemic patients.[8] Therefore, this study aimed to study the role of fibroscan for detecting fibrosis of the liver in children with beta thalassemia major. This would enable more efficient resource utilization and allow more frequent monitoring of iron status in patients requiring frequent blood transfusions, which could improve clinical outcomes by detecting iron overload earlier.

Methods

This was a single centre, cross-sectional study conducted at the Department of Paediatrics of a tertiary care centre in south India. The study population included children between five and 18 years of age who were diagnosed with beta thalassemia major on regular blood transfusions and admitted between April 2021 and September 2022. A written informed consent was obtained from the parents before inclusion in the study. Children with beta thalassemia major with any other liver pathology, such as Hepatitis A, Hepatitis B or C, Wilsons disease, or autoimmune hepatitis, and obese children with BMI > the 95th percentile for that age and sex were excluded, as these conditions can cause liver fibrosis and interfere with the fibroscan findings.[9] Clearance was obtained from the Institutional Ethics Committee (IEC number: 941/2020; CTRI registration number: CTRI/2021/04/033008). A detailed history regarding the symptoms related to iron overload such as hyperpigmentation, delayed puberty, growth retardation, breathlessness, and orthopnea were taken. History of age at first transfusion, duration and frequency of transfusion, type and duration of chelation therapy and compliance, and any history of transfusion reactions were taken. Examination findings including, anthropometry, general and systemic examination findings were recorded. Liver function tests, thyroid function tests, and serum ferritin levels were performed. The mean pre-transfusion hemoglobin (g / dL) and the mean transfusion requirement (ml / kg / year) were calculated. Children with other conditions which could cause increased serum ferritin levels like metabolic syndrome, obesity, septic shock, HLH and any apparent preexisting chronic liver disease were excluded. Fibroscan was performed in all children by an experienced radiologist who was blinded to the clinical data of the children. The patient was kept in a supine position / left lateral decubitus position with the right arm fully abducted to obtain a good intercostal window. With the assistance of fibroscan ultrasound, a liver portion of at least 6 cm thickness and free of large vessels was identified for examination. The patient was advised to breathe normally and hold their breath under inspiration. The mean of 10 readings was taken as the final value of liver stiffness in kilo Pascal. LSM was graded as follows: No fibrosis-LSM < 5.5 kPa; mild fibrosis - LSM ≥ 5.5 kPa to 7.7 kPa; moderate fibrosis - LSM ≥ 7.8 kPa to 13.4 kPa, and severe fibrosis - LSM ≥ 13.5 kPa.[10,11] The statistical analysis was performed with SPSS (Statistical package for social science) v23 software. Descriptive data was expressed as frequencies and percentages. Mean and standard deviation were computed for the variables following normal distribution curve, while median and inter-quartile range were computed for non-parametric data. Spearman's correlation analysis was used to correlate between serum ferritin levels and LSM. Statistical significance was considered with p-value < 0.05.

Results

Table 1: Baseline parameters of children with beta thalassemia major

Characteristics	Values
Mean age ± SD years	10.32 ± 3.40
Male / Female (N)	21 / 13
Splenomegaly (N)	22
Splenectomy (N)	12
Mean duration of chelation therapy (Years) ± SD	4.8 ± 1.2
Mean pre-transfusion Hemoglobin (g / dl) ± SD	6.77 ± 0.48
Age at 1st blood transfusion (Months) ± SD	0.9 ± 0.4
Median annual transfusion requirements in ml / kg / year (IQR)	131 (84, 240)
Median serum ferritin (mcg / L) (IQR)	2852 (358, 9874)
Median serum AST (IU / L) (IQR)	48 (20, 125)
Median serum ALT (IU / L) (IQR)	45 (25, 130)

A total of 34 children with beta thalassemia major between the age of five years and 18 years were admitted during the study period. None were excluded. The mean age of the study population was 10.32 ± 3.40 years. There were 21 (63%) boys and 13 (37%) girls with male : female ratio of 1.6:1. Hepatomegaly was present in all children. Splenectomy

was performed in 12 children. All children were on regular chelation therapy. The baseline demographic, clinical and laboratory characteristics are depicted in Table 1.

Among 34 children, 22 (64%) had no fibrosis, 10 (30%) had mild fibrosis, and two (6%) had moderate fibrosis. None of the included children had severe fibrosis. Patient's characteristics compared between those children with fibrosis and those without fibrosis is depicted in Table 2. Higher mean duration of transfusion and greater median ferritin levels (3420 vs 1997 μg / L; P-value 0.006) were observed in children with fibrosis and was statistically significant. Thus, the incidence of liver fibrosis increases progressively as the duration of transfusion increases. Children with increased serum ferritin levels had a significantly increased risk of iron overload and hepatic fibrosis. Additionally, no association was observed between liver fibrosis and mean age of the study population, gender, and mean age at first transfusion, AST or ALT levels.

Fibrosis was observed in 12 children. Two children had moderate fibrosis while mild fibrosis was observed in 10 children. Among those with fibrosis, five children had received transfusions for five to 10 years, compared to seven children who had received transfusion for more than 10 years. Among the children whose serum ferritin levels were less than 2000 mcg / L, three had fibrosis (mild); between 2000 and 4000 mcg / L, four had fibrosis; and those with values greater than 4000 mcg / L, five had fibrosis. Among the children with a mean transfusion requirement between 100 - 200 ml / kg / year, 10 had mild fibrosis. Among those with a mean transfusion requirement of more than 200 ml / kg / year, two had moderate fibrosis, and none of the children with a requirement of < 100 ml / kg / year had fibrosis.

It was concluded that a greater volume of transfusion and a greater annual mean transfusion requirement were associated with a significantly greater incidence of hepatic fibrosis. There was no significant relationship between age at first transfusion and incidence of hepatic fibrosis. There was no significant association between abnormal liver function tests, age of initiation of chelation therapy, or mean pre-transfusion hemoglobin and hepatic fibrosis in the present study.

Table 2: Clinical and laboratory characteristics of children with fibrosis and those without fibrosis

Characteristic	Children with fibrosis (N = 12)	Children without fibrosis (N = 22)	p-value
Mean age ± SD years	12.2 ± 2.2	8.02 ± 1.50	*0.062
Gender			
-Males	7	14	#0.6
-Females	5	6	
Mean age at first transfusion (Years) ± SD	0.8 ± 0.2	0.9 ± 0.4	*0.32
Mean duration of transfusion (Years) ± SD	8.24 ± 2.44	5.20 ± 2.18	*0.038
Mean pre-transfusion Hb (g / dL) ± SD	5.78 ± 0.89	6.75 ± 0.77	*0.96
Mean transfusion requirement (ml / kg / year) ± SD	171 ± 37.80	114.2 ± 19.22	*0.002
Median serum ferritin (μ / L) (IQR)	3420 (3204, 4548)	1997 (1650, 2908)	$0.006
Median serum AST (IU / L) (IQR)	53.5 (32.7, 73)	33 (26, 50)	0.2
Median serum ALT (IU / L) (IQR)	42 (17.7, 84.5)	20 (11, 48.7)	0.415

* Student T-test, $ Mann-Whitney U test, SD- Standard deviation, IQR- Interquartile range

Spearman's correlation coefficient (r) was measured to estimate the degree and direction of the relationship between degrees of fibrosis with ferritin levels (Figure 1). Spearman correlation coefficient (rho) (R_s) was 0.549, indicating moderately positive relationship between serum ferritin levels and hepatic fibrosis, with the p-value of < 0.001 (highly significant), indicating higher serum ferritin levels associated with greater incidence of hepatic fibrosis.

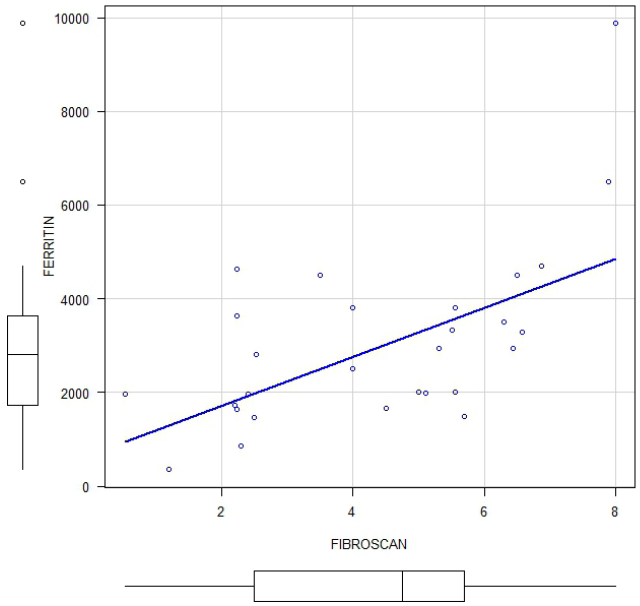

Figure 1: Serum ferritin levels and degree of fibrosis in children (Spearman's correlation coefficient: 0.549; p-value 0.0007)

X- axis: Liver thickness measurement in kilo Pascal; Y-axis: Serum ferritin level in mcg/L

Discussion

Fibroscan is a widely available, noninvasive, and inexpensive tool for LSM caused by fibrosis. The basic principle of fibroscan is that the transmission velocity of a wave through homogenous tissue is proportional to its elasticity, which is strongly correlated with the degree of fibrosis in the liver. In the present study, 34 patients aged five to18 years who were diagnosed with beta thalassemia major were included. The male: female ratio was 1.6:1. In a study by Fraquelli et al[12] among 154 children with beta thalassemia, the mean age was 12 ± 3.6 years, and the male: female ratio was 1.7:1, which is comparable to the present study population. Among the children in the study, four had deranged LFTs, and 30 had normal LFTs. In the present study, the median AST and ALT levels were 48 (20 - 125) and 45 (25 - 130) IU / L respectively which were comparable to those in the study by Fraquelli et al. (the mean ALT level was 65.0 ± 51.8 U / L, and the mean AST level was 62.9 ± 44 U / L). Fibroscan was performed in all children who satisfied the inclusion criteria.

In the present study, three children aged between five and 10 years, two children aged between 10 and 15 years and seven children aged between 15 and 18 years were found to have fibrosis. Hence, it was interpreted that older children are at increased risk of developing iron overload and hepatic fibrosis, but the association was not statistically significant. Among the children for whom chelation therapy was started between three and five years after diagnosis, six had fibrosis, and among those children for whom chelation therapy started between five and 10 years after diagnosis, two had fibrosis. There was no significant association between the age of initiation of chelation therapy and fibroscan findings. However, the sample size was small, and the dose and type of chelation were not considered for the analysis.

In a study performed by Fraquelli et al[12] (N = 115), the mean serum ferritin level was 4712 ± 3301 (mcg / L), and increased values of fibroscan were observed in beta-thalassemia major patients with higher serum ferritin levels. The positive correlations observed between fibroscan findings and serum ferritin levels in our study were also observed by MS Elalfy et al[13] (N = 51), who reported progressively increasing fibrosis in thalassemia major patients with higher ferritin levels. In a study done in Canada by George Ou et al[14] a weak positive correlation was found between serum ferritin levels and fibroscan values. However, a strong positive relationship was obtained in the current study. A prospective study done by Nirav Pipaliya et al[15] (N = 154) (mean age 12 ± 3.6 years) beta Mediterranean anaemia children who required chronic transfusion and on iron chelator therapy were assessed for serum levels of ferritin, and fibroscan within one month of MRI T2*. Fibroscan LSM correlated well with MRI R2* values (r [0.85; P < .001). Fibroscan results identified and divided the patients into severe, moderate, and mild hemochromatosis with area under the receiver operating graph values of 94.8%, 84.5%, and 84.7% respectively. LSM greater than 13.5, 7.8 and 5.5 kPa identified patients with severe, moderate, and mild hemochromatosis, respectively. The sensitivity and specificity values were 92% and 93% for severe overload, 82% and 82% for moderate overload, and 73% and 75% for mild overload. Similar findings have been reported in studies performed by Musallam KM et al and Di Marco V et al.[16,17] Limitations of this study include, a small sample size and single centric study. The standardized chelation therapy was not observed due to financial restraints, and there was no method for identifying the adequacy of chelation therapy.

Conclusions

Thus, fibroscan is a more affordable, non-invasive, and faster alternative tool compared to MRI to estimate liver fibrosis in children with beta thalassemia major and is directly related to serum ferritin levels and transfusion requirements in children.

However, there was no significant association between abnormal liver function tests, age of initiation of chelation therapy, or mean pre-transfusion hemoglobin and hepatic fibrosis in the present study.

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