Original Article

The Utility of Transient Elastography (Fibro-Scan) as an Indicator of Hepatic Iron Overload in Transfusion Dependent Thalassemia Patients (TDT) from a Tertiary Care Hospital from Eastern India

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Background : Progressive iron overload leads to liver fibrosis is a well-known complication in TDT patients. This study was done to evaluate the liveriron load in correlation to Serum Ferritin. This is not a new endeavor however there we tried to see the effect of other factors besides iron overload in the progression of liver fibrosis with a non-invasive test.

Material and methods : A retrospective cross-sectional study from December 2018-December 2019 on sixty (n=60) transfusion dependent thalassemia patients who were on regular iron chelation underwent liver function and Serum Ferritin by the automated analyzer and liver stiffness measured by transient elastography. The severity of fibrosis by TE is graded as ≤7.9kPa; Significant (F2) >7.9-10.3kPa; Severe (F3) >10.3-12.0kPa; cirrhosis (F4) >12.0kPa as per previous studies.

Results : Out of a total of 60 TDT patients with primary diagnosis Hb E-β thalassemia, median age 15 years (11-25years), mean transfusion requirement 12.9±3.68 Units/year and median ferritin was 1933ng/ml. There were 13 patients with anti-HCV positive, two were HIV Seropositive and one Hepatitis B Positive. On fibroscan, there were 43.34%(n=26), 13.34%(n=8), 6.66%(n=4),36.66%(n=22), patients with Mild, Significant, Severe and Cirrhosis Fibrosis respectively. The correlation between the grade of fibrosis and ferritin level was found to be statistically insignificant. Out of 13 HCV Seropositive, 7(53.8%) were found to have liver stiffness of >12.0PKa (cirrhosis), with a median ferritin value of 2291ng/ml.

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Key words **: Fibroscan, Ferritin, Liver Stiffness, Thalassemia.**

The Transfusion Dependent Thalassemia (TDT) patient requires a regular blood transfusion for growth and survival from a very early age. With regular blood transfusion and ineffective erythropoiesis, there is the gradual and continuous iron accumulation in TDT patients at a rate of approximately 0.3-0.6 mg/Kg/day[1]. As human lacks the efficient mechanism to excrete excess iron, the management of iron overload requires regular Chelation Therapy to remove excess iron[2]. The Serum Ferritin concentration is the most commonly used test to estimate iron overload in TDT patient[3]. The quantification of Liver iron by using Liver Biopsy (LIC) is still the gold standard to diagnose iron overload which is an invasive and painful procedure. Magnetic Resonance Imaging (MRI) using R2 or T2* techniques is another non-invasive and accurate evaluation of liver iron overload but it is not cost-effective and also not universally available[4]. Transient Elastography (Fibroscan) is used for the assessment of Liver Stiffness in patients with chronic liver disease. It is non-invasive, can be performed in the out-patient clinic with immediate results and good reproducibility[5]. Here we tried to see the correlation of Liver Stiffness with Serum Ferritin Levels in transfusion dependent

Editor's Comment :

- Fibroscan is a non-invasive test to detect liver stiffness.
- Iron overload is common in TDT patients and the major brunt of injury is in the liver.
- Monitoring of serial serum ferritin level along with fibro scan (TE) might help us to diagnose the early stage of liver fibrosis.
- The correlation between the grade of fibrosis and ferritin level was found to be statistically insignificant in our study.
- HCV seropositive patients have higher liver stiffness at their corresponding serum ferritin levels.

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Thalassemia patients in the tertiary centre of Eastern India.

Material and Methods

Design : This is a cross-sectional, single centre, retrospective study conducted at Nil Ratan Sircar Medical College and hospital, Kolkata, in the period from December 2018 and December 2019. Ethical clearance for the study was taken from the Institutional Ethical Committee. Informed and written consent was taken from all the patients/guardians, after discussing in his/her language.

Patients : A total of sixty (n=60) Transfusion Dependent Thalassemia patients were enrolled, who were on regular blood transfusion support and attendees of the Thalassemia Clinic of our tertiary centre. All patients were diagnosed with high performance Liquid Chromatography (Bio Rad Variant III) and mutation study (if required).There were fifty-eight (n=58) Hb E-Beta Thalassemia and two (n=2) Beta Thalassemia patients. All patients were on regular blood transfusion within 5 years of life at intervals of 3-5 weeks with pre transfusion Hb kept as ≥9gm/dl. Patients enrolled were on regular iron chelators once Serum Ferritin increased >1000ng/ml.

Inclusion Criteria : All the TDT patients ≤10 years of age who were on regular iron chelation

Exclusion criteria : Patients with decompensated Cardiac Insufficiency, Chronic Kidney Disease Decompensated Liver Cirrhosis, pregnancy, and age younger than 10 years were excluded from the study.

Evaluation : All the included patients were subjected to thorough history taking and complete clinical examination with a series of investigations that are routinely performed, including Complete Blood Counts, Liver Function Tests; Hepatitis B Surface Antigen, Circulating Anti-HCV Antibodies, were tested using Enzyme Immunoassay Kits. Patients who were sero-reactive for anti HCV were undergone HCV RNA levels using RT PCR.

All the patients recruited were subjected to:

(1) Serum Ferritin Level determined using standard Laboratory Standards, using an Access Ferritin Kit in the Chemiluminescence Immunoassay analyzer (Beckman Coulter Access2). An average of two Ferritin levels of a patient was taken for analysis.

(2) Transient Elastography (Fibroscan) was carried out by experienced examiner in all patients, within the same week of Ferritin estimation. Liver Stiffness measurement Fibroscan (Echosens fibroscan 430 mini) was based on one dimensional TE that uses both ultrasound (5MHz) and low-frequency (50 Hz) elastic waves. Liver Stiffness results were expressed in kilopascal (kPa) by the software.

The severity of Fibrosis by TE is graded as ≤7.9kPa; Significant (F2) >7.9-10.3kPa; Severe (F3) >10.3-12.0kPa; Cirrhosis (F4) >12.0kPa as per previous studies[6].

Statistical analysis : The data was entered in MS EXCEL spread sheet and analysis was done using Statistical Package for Social Sciences (SPSS) version 21.0. The patient's background characteristics and laboratory investigation data were expressed as mean and SD. Qualitative variables were correlated using the Chi-Square test and quantitative variables were compared using the t-test. A p-value of <0.05 was considered statistically significant.

Results

A total of 60 TDT patients were enrolled in the study during the period December 2018-December 2019. The median age of the patients in the study was 15 years (11-25 years) with the male predominance (M: F-1.5:1). The median age of the receiving first blood transfusion was 2.5 years and the mean transfusion requirement in the cohort was 12.9 units per year. All patients were on regular iron chelators as per standard Guidelines[1]. There were 13 (21.67%) patients who were HCV seropositive and 2 (3.34%) patients were HIV seropositive and were on treatment as per the standard care. Table 1 shows the clinical characteristics of all the patients. The mean Serum Ferritin level was 2174 ng/ml with an SD of 897.2 ng/ml (IQR = 1595.1-2557.7 ng/ml).

On Fibroscan, there were 26 (43.34%), 08(13.34%), 04(6.66%) and 22(36.66%) patients with Mild, Significant, Severe and Cirrhosis fibrosis grade respectively.

The significant fibrosis grade (F2-3) were seen in 12(20%) of patients while cirrhotic (F4) was seen in 22(36.66%) of patients. There were 26 patients

Table 1 — Various clinical characteristics

Characteristics	Results
Age in years (mean±SD)	15.4 ±4.65 years
Median age in years	15 (6-25) years
Gender	
Male (%)	60 %
Female (%)	40%
Age of first transfusion (mean±SD)	3.35±2.97 years
Median age of the first transfusion (years)	2.5 years
Transfusion requirement Units/year (Mean ±SD)	12.9±3.68 Units/year
Serum Ferritin ng/ml (mean±SD)	2174.138±897.2 ng/ml
Anti HCV seropositive (%)	13 (21.67%)
HBsAg positive (%)	1 (1.67 %)
HIV seropositive (%)	2 (3.34%)

(43.34%), who were having mild/no fibrosis as assessed by TE. The mean±SD Ferritin level between the different grade of Fibrosis mild (F1), moderate (F2), severe (F3) and cirrhotic (F4) were 1910.8±677.8 ng/ml, 2069.42±610.2 ng/ml, 1865.9±652.9 ng/ml, 2443.1±930.4 ng/ml respectively. Tables 2 & 3 Summarizes the distribution of various grades of liver stiffness with mean Ferritin levels. The correlation (r=0.08) between Ferritin levels and the grade of fibrosis were statistically insignificant (p-value >0.05).

Out of 13 HCV seropositive, 7 were found to have Fibro Scan of >12.0PKa (cirrhosis), with the mean ferritin level of 2443.1±930.4 ng/ml and 3 out of 13 (23.07%) patients had the mild grade of Fibrosis with a mean Ferritin level of 2013.5±1009 ng/ml. There was a statistically insignificant difference (p-value>0.05) between mean Ferritin levels and Fibrosis grades (r=-0.2) in HCV sero-reactive TDT patients in the study.

In two groups HCV reactive and HCV free group there was no significant difference in the mean Ferritin levels (p-value >0.05) or mean Liver Stiffness was seen between two groups (p-value >0.05) (Table 4).

Fig 1 shows the linear regression graph of Ferritin levels (ng/ml) with respective TE value in TDT patients with concurrent HCV seropositivity.

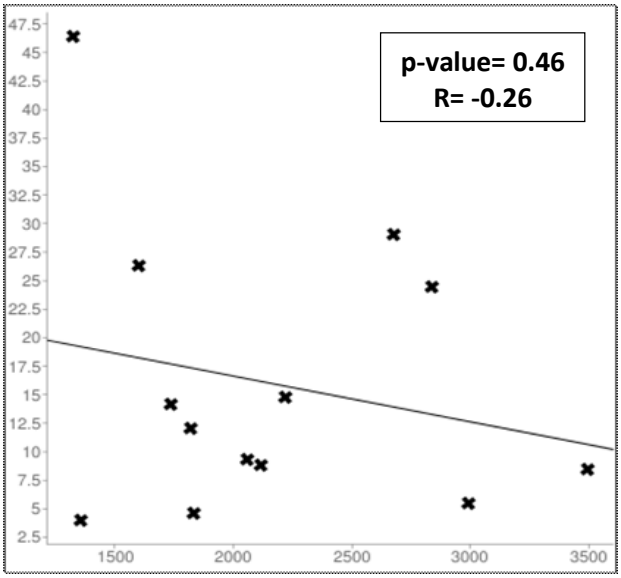

Fig 1 — Linear regression curve between mean serum ferritin levels and fibrosis grade (Pka) in HCV seropositive TDT patients

Discussion

Iron overload and its consequences are the main concern in the management of TDT patients, requiring frequent monitoring and timely intervention to prevent irreversible damage to the Liver and other organs of the body. Our study was an attempt to analyse the iron overload state in TDT patients as there is limited data regarding hepatic iron overload in Hb-E beta-thalassemia from the Eastern part of India[7]. The median age in our study cohort was 15 years (11-25 years) almost equivalent to that in other studies[8,9]. There was a male predominance in our study with M:F-1.5:1 which might not represent the general population because of small sample size[10], however it didn't found to influence the iron overload status as main factors involved in liver iron overload in TDT patients is regular blood transfusion with ineffective Erythropoiesis[11].

In our study, the mean Liver Stiffness Measurements (LSM) was found significantly increase with 22(36.66%) of patients having LSM >12Kpa and 34 (56.66%) patients showed LSMs >7.9 kPa, which is a cut-off value for significant fibrosis[12].

There is a definite higher risk of HCV infection due to repeated blood transfusion in TDT patients. In our study, there is a statistically significant in the mean LSM in HCV infected and HCV free TDT patients15.99Pka and 10.62Kpa respectively, however, mean ferritin of 2158.36ng/ml and 2086.52 ng/ml statistically insignificant (Table 4). In the series of Di Marco, *et al* the TM patients free of HCV infection showed a mean LSM of 5.2 kPa in Thalassemia major patients and found in their study that Liver Stiffness is proportional to liver fibrosis and not on Liver Iron status[13].

No statistically significant difference in LSM score between HCV infected and HCV free TDT patients (p value->0.05) or with different Serum Ferritin levels were

Table 2 — *Distribution of TE grading and Ferritin Levels*

Characteristics	F1 (≤7.9Kpa)	F2 (>7.9-10.3Kpa)	F3 (>10.3-12 Kpa)	F4 (>12Kpa)
No of patients	26	8	4	22
Patients (%)	43.34 %	13.34 %	6.66 %	36.66 %
Median ferritin (ng/ml)	1843.96	1925.01	2022.46	2291.96
Mean ferritin±SD (ng/ml)	1910.8±677.8	2069.42±610.2	1865.9±652.9	2443.1±930.4
HCV Seropositive	3	3	0	7

Table 3 — *Different Ferritin levels and LSM score*

Ferritin Level (ng/ml)	No of Patients (%) (n)	Mean LSM score (Pka)
>1000-≤2000	33 (55%)	10.66
>2000-≤3000	20 (33.34%)	11.8
>3000	07 (11.66%)	17.58

Table 4 — *Mean serum Ferritin Levels and LSM score in HCV infected and HCV free*

	HCV Infected (n=13)	HCV Free (n=44)	p-value
Ferritin Level	2158.36ng/ml	2086.52 ng/ml	>0.05
Mean LSM score (pka)	15.99	10.62	

found in these two groups (Table 4), indicating that Liver Stiffness is independent of iron overload[13].

Assessment of Liver Fibrosis stage and LIC in TDT patients is most crucial for the long-term prediction of liver-related adverse outcomes. Although Liver Biopsy remains the gold standard in assessing hepatic injury[14,15] the Coagulopathy associated or the concomitant HCV infection, biopsy related complications can be problematic. These and several previous studies[16-18] had shown that TE has promising results as the non-invasive diagnostic tool with moderate to high accuracy for the assessment of Liver Fibrosis in HCV-infected and HCV free TDT patients.

This study is unique in providing data on the utility of TE in assessing Liver Stiffness in TDT patients of the Eastern part of India.

Limitations of our Study :

The limitations of our study include being a cross-sectional study with a small sample size influence of other factors on the Liver Stiffness cannot be ascertained. Secondly, we did not compare the results of the non-invasive assessment of Liver Fibrosis to the gold standard (liver biopsy). Therefore, a prospective study with a large number of TDT patients and especially on paediatric patients (<10years) is required to understand the utility and effectiveness of TE as a diagnostic tool for the early diagnosis of hepatic iron load and its related longterm adverse outcomes.

Conclusion :

Iron overload is common in TDT patients and the major brunt of injury is in the liver. Fibroscan is a non-invasive test to detect liver stiffness. Monitoring of serial serum ferritin level along with Fibro Scan (TE) might help us to diagnose the early stage of liver fibrosis. HCV Seropositive patients were found to have higher liver stiffness at their corresponding Serum Ferritin levels. The correlation between the grade of fibrosis and ferritin level was found to be statistically insignificant in our study because of smaller sample size.

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