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Noninvasive assessment and risk factors of liver fibrosis in pediatric patients with beta thalassemia major using transient elastography

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Abstract

Background: In beta-thalassemia major (TM) patients, levels of liver iron overload and the presence of chronic hepatitis C are directly correlated with the onset and severity of liver fibrosis. A noninvasive approach that can evaluate cirrhosis and liver fibrosis in these patients is transient elastography (TE). We aimed to find out the role of noninvasive elastography in the assessment of liver fibrosis in young beta-thalassemia major patients receiving frequent blood transfusions. Identifying the patients' risk factors for liver fibrosis is another goal. The study comprised 53 patients, all of whom had a thorough history-taking procedure, clinical examination, and investigations such as CBC, serum ferritin, HCV and HBV serology, and liver function testing. It was carried out transient elastography to find liver fibrosis.

Results: According to transient elastography, 52.8% of the patients had severe fibrosis (F2 and higher). 9.4% of people had positive HCV serology results. Significant liver fibrosis was correlated with all serum ferritin levels (708.2 ± 182.1, 3213.5 ± 1272.9, nonsignificant vs. significant fibrosis), HCV infection, age, blood transfusion frequency, and irregular chelation therapy. But no significant correlation regarding sex and BMI was detected.

Conclusion: Transient elastography is an alternate noninvasive approach that assesses liver fibrosis in beta-thalassemia major patients. The risk of liver fibrosis is related to iron overload, HCV seropositivity, advanced age, frequent blood transfusion, and irregular chelation therapy.

Key messages

- For individuals with beta-thalassemia major, transient elastography is a noninvasive method for assessing liver fibrosis
- A further contributing factor to liver fibrosis in these patients is HCV infection acquired during transfusional therapy.
- Patients' advanced age and a history of numerous transfusions may increase their chance of developing liver fibrosis.

Keywords: Transient elastography, Beta-thalassemia major, Iron overload, HCV infection

1 Background

A hereditary blood illness known as beta-thalassemia major is characterized by reduced or absent β-globin chain synthesis, which lowers the amount of hemoglobin in red blood cells (RBCs), reduces RBC production, and

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ultimately causes anemia [1, 2]. For patients with beta-thalassemia major, lifelong transfusion and iron chelation are the cornerstones of therapy. Anemia is treated with transfusion in an effort to reduce inefficient erythropoiesis [3]. Regular blood transfusions would more than double the body's typical iron reserves. Serious side effects from iron overload include cirrhosis, diabetes, heart disease, and hypogonadism [4, 5]. We have to keep an eye out for both short- and long-term effects of repeated transfusions, such as iron excess and liver damage [6].

Children who are thalassemic frequently develop the hepatitis C virus (HCV), which is spread through blood transfusions. The amount of the liver iron overload and the existence of chronic hepatitis C are directly connected to the development and severity of liver fibrosis in those kids. Most infected patients with chronic hepatitis C develop cirrhosis, hepatocellular cancer, liver fibrosis, and portal hypertension [7]. The gold standard for determining the stage of liver fibrosis has always been liver biopsy. Patients with viral hepatitis have been evaluated specifically using liver biopsy. The drawbacks of biopsy include the fact that it is an invasive test, that the patient must spend half a day in the hospital, that it is expensive, and that it has some risks, including discomfort and bleeding [8]. Researchers have worked hard over the past 10 years to create noninvasive assays that can evaluate liver fibrosis because of these restrictions and patients' desire to avoid invasive testing. One such test is the Fibroscan, which has a number of benefits over liver biopsy. Fibroscan is a noninvasive test that may be carried out at the point of treatment, causes no pain, and does not call for sedation. The test is also substantially less expensive than a liver biopsy, only takes 5–7 min to do, and has not been linked to any negative side effects [8]. With pulse-echo ultrasound acquisitions, Fibroscan's transient elastography (Echosens, Paris) monitors the velocity of an elastic shear wave as it travels through the liver parenchyma to determine the stiffness of the liver. Results from a Fibroscan range from 2.5 to 75 kilopascal (kPa). The best cutoff values were > 7 kPa, 85% probability of METAVIR F2-F4, and > 13–14 kPa, 90% risk of cirrhosis (METAVIR F4) [9]. Our study aimed to identify the significance of noninvasive transient elastography in the assessment of liver fibrosis in pediatric beta-thalassemia major patients, as well as the risk factors for liver fibrosis in these patients who get regular blood transfusions.

2 Methods

This was a prospective study involving 53 children (26 boys and 27 females) who had been identified as having beta-thalassemia major by hemoglobin electrophoresis. It was carried out in the pediatric hematology unit of Beni Suef university hospital between June 2020 and December 2021. Inclusion criteria; ages of the included children ranged from 6 to 18 years, and all were receiving regular transfusions of packed red blood cells every 14 to 40 days, iron chelation therapy with deferasirox tablets at a dose of 14–28 mg per kilogram per day, as well as other supporting drugs (folic acid, L-carnitine, ca supplementation). Exclusion criteria; age over 18 and other forms of thalassemias. All participants and the parents of the children who were being studied gave their informed consent. The local ethical committee (FMB-SUREC/05032018) gave its approval to the study. A thorough history was taken of each of the children who were included, including information on each child's age at disease beginning, frequency of blood transfusions, consanguinity, family history, and frequency of chelation therapy. Complete clinical examination includes a general checkup and an abdominal checkup for the liver and spleen. Laboratory tests such as the complete blood count (CBC), serum ferritin, serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), serum albumin, serum bilirubin, prothrombin time (PT), hepatitis c virus antibody (HCV Ab), and hepatitis B surface antigen (HB s Ag) were done.

2.1 Collection of samples

Sterile venipunctures were used to take blood samples. Hemoglobin and platelet samples were taken and placed in vacutainers with EDTA (ethylenediamine tetraacetic acid) so they could be examined right away. After centrifuging samples for ALT, AST, PT, albumin, and ferritin for 20 min at a speed of 2000–3000 rpm, the samples were stored at − 80 C until testing. Prior to giving packed red blood cell transfusions, all samples were collected. Lala and co [10].

(4) Abdominal ultrasonography was performed to evaluate the liver echo pattern.
(5) Transient elastography: The tool was utilized to evaluate liver fibrosis (Fibroscan-502, Echosens, Paris, France).

Technique: The patient is positioned in a supine position, and an ultrasound-like probe is applied to the skin over the liver region, usually in the right mid-axillary line. Every time the probe generates a vibration wave, the patient feels a slight "flick." The procedure typically lasts 10 min and is painless for the patient. Patients should typically fast for at least two hours before to the operation. An interquartile range of no more than 30% of the median value and a minimum of 10 accurate readings are required to increase test reliability. The results are presented in kilopascals (kPa).

Results interpretation:

1. Fibrosis score F0 to F1 indicates either no liver scarring or minimal liver scarring (2–7 kPa)
2. Fibrosis score F2: Moderate scarring of the liver (8–9 kPa)
3. Fibrosis score F3: Severe scarring of the liver (9–14 kPa)
4. Fibrosis score F4: Cirrhosis (advanced liver scarring) (14 kPa or higher)

The highest outcome is 75 kPa.

2.2 Statistical analysis

- The SPSS version 25 was used to code, input, and analyze the obtained data (Statistical package for social science). For numerical variables in the form of mean and standard deviation (mean SD), as well as categorical variables expressed as frequency and percentage, descriptive statistics were performed.
- Appropriate statistical tests of significance were applied, including:
  Spearman's correlation analysis; *r* values: 0–0.3 either positive or negative (weak), 0.3–0.6 (moderate), and more than 0.6–1 (strong); independent sample *t* test for two unrelated samples; Chi-square (2) test or Fisher's exact test for categorical data; *p* values of 0.05 or less were regarded as statistically significant.
- Some information was illustrated using simple graphs.
- ROC curve was employed to determine the ideal ferritin cutoff for detecting substantial fibrosis.

3 Result

A total of 53 beta-thalassemia major patients with a mean age of 11.3 ± 3.1 years were enrolled in this study; 49.1% of them were males, and 50.9% of them were girls. Additionally, 26.4% of the patients exhibited positive consanguinity, and 41.5% had a positive family history. Body mass index (BMI) was 15.7 ± 1.9. There was just one patient with diabetes (DM). In 73.6% of the study group, splenectomy was performed. Only 47.5% of cases regularly used chelating agents. The blood transfusion frequency was 1 ± 0.3 (months). Table 1 shows the demographic details of the patients who were the subject of the study.

The studied group's mean ferritin level was 2031.75, while its mean hemoglobin level was 7.3170. Table 2 shows the laboratory features of the individuals of the study. Only one case (1.9%) exhibited positive HB s Ag, while 9.4% of cases had positive HCV Ab.

Every subject showed normal echo results. On abdominal ultrasonography, hepatomegaly was present in all involved children, with a homogeneous texture in 25 patients (47.2%) and a coarse texture in 28 patients (52.8%).

Table 1 Demographic characteristics of the studied patients

Characteristics	Values (no = 53)
Age (mean ± SD)	11.3 ± 3.1
Sex	
Males	26 (49.1%)
Females	27 (50.9%)
Consanguinity	14 (26.4%)
Family history	22 (41.5%)
BMI	15.7 ± 1.9
DM	1 (1.9%)
Spleen	
Splenectomy	39 (73.6%)
Splenomegaly	14 (26.4%)
Hepatomegaly	53 (100%)
Use of chelating agents	
Regular	25 (47.2%)
Irregular	28 (52.8%)
Age at 1st blood transfusion (months)	0.6 ± 0.4(median = 0.5)
Frequency of blood transfusion (months)	1 ± 0.3 (median = 1)

BMI body mass index, *DM* diabetes mellitus

Table 2 Laboratory characteristics of the studied patients

Labs	Mean	Sd	Minimum	Maximum
Ferritin	2031.75	1565.498	500.00	6249.00
Hb	7.3170	1.10935	5.00	9.70
TLC	7.3113	3.69738	3.10	20.00
PLT	286.7170	104.65279	125.00	567.00
PT	11.8868	.75091	11.00	15.00
Albumin	4.3187	.53914	3.00	5.50
Bilirubin	.9400	.45750	.30	3.40
ALT	58.2075	25.68549	15.00	115.00
AST	46.0170	22.62928	11.00	103.00
Urea	22.5830	7.90252	3.00	44.00
Creatinine	.4509	.16597	.20	1.20

Hb hemoglobin, *TLC* total leukocyte count, *PLT* platelets, *PT* prothrombin time, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase

3.1 Liver fibrosis and evaluation of risk factors

Fifty-three patients underwent TE in total. Patients with F0, F1, F2, F3, and F4 made up, respectively, 6 (11.3%), 19 (35.8%), 15 (28.3%), 10 (18.9%), and 3 (5.7%). Twenty-eight individuals (52.8%) had significant fibrosis, according to the classification (F2 and higher). Data between people with liver fibrosis and people without fibrosis are compared in Table 3. Additionally, the

Table 3 Comparison between patients with fibrosis and patients without fibrosis regarding patients' characteristics

Characteristics	No fibrosis (no = 25)	Fibrosis (no = 28)	*P* value
Age (mean ± SD)	8.6 ± 1.5	13.7 ± 2.1	< 0.001
Sex			0.685
Males	13 (52.0%)	13 (46.4%)	
Females	12 (48.0%)	15 (53.6%)	
Consanguinity			0.805
No	18 (72.0%)	21 (75.0%)	
Yes	7 (28.0%)	7 (25.0%)	
Family history			0.442
No	16 (64.0%)	15 (53.6%)	
Yes	9 (36.0%)	13 (46.4%)	
BMI	15.9 ± 2.1	15.5 ± 1.8	0.326
DM			> 0.999
No	25 (100.0%)	27 (96.4%)	
Yes	0 (0.0%)	1 (3.6%)	
Hb	7.6 ± 1.2	7.1 ± 1	0.098
TLC	7.3 ± 4.0	7.3 ± 3.4	0.976
PLT	320.0 ± 97.7	256.9 ± 103.2	0.027
PT	11.7 ± 0.5	12 ± 0.9	0.128
Albumin	4.4 ± .46699	4.2 ± .59338	0.261
Bilirubin	0.9 ± 0.3	0.9 ± 0.5	0.991
AST	40.2 ± 15.9	74.3 ± 21.8	< 0.001
ALT	32.9 ± 12.6	57.7 ± 23.3	< 0.001
Urea	20.8 ± 8.6	24.2 ± 6.9	0.121
Creatinine	0.5 ± 0.1	0.4 ± 0.2	0.836
Age at 1st blood transfusion (months)	0.7 ± 0.5	0.6 ± 0.2	0.439
Frequency of blood transfusion (months)	1 ± 0.1	0.8 ± 0.3	0.001
Hepatomegaly			< 0.001
Homogenous	25 (100.0%)	0 (0.0%)	
Coarse	0 (0.0%)	28(100.0%)	
Spleen			0.317
Splenectomy	20 (80.0%)	19 (67.9%)	
Splenomegaly	5 (20.0%)	9 (32.1%)	
HBsAg			0.340
−ve	25 (100.0%)	27 (96.4%)	
+ve	0 (0.0%)	1 (3.6%)	
HCV Ab			0.05
−ve	25 (100.0%)	23 (82.1%)	
+ve	0 (0.0%)	5 (17.9%)	
Chelating agents			< 0.001
Regular	25 (100.0%)	0 (0.0%)	
Irregular	0 (0.0%)	28 (100.0%)	

BMI body mass index, *DM* diabetes mellitus, *Hb* hemoglobin, *TLC* total leukocyte count, *PLT* platelets, *PT* prothrombin time, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *HCV Ab* hepatitis c virus antibody, *HB s Ag* hepatitis B surface antigen

correlation between Fibroscan grades and patients' data (Table 4) revealed that liver fibrosis was strongly related to age, older age (13.7 ± 2.1 years) was a risk factor for liver fibrosis. Another risk factor for liver fibrosis in our patients was an increase in the frequency of blood transfusions (0.8 ± 0.3 months). Furthermore, patients receiving irregular chelation therapy had a higher risk of developing liver fibrosis than those receiving regular chelation therapy (*P* value 0.001).

Table 4 Correlation between Fibroscan grades and different parameters (non parametric correlation)

	Fibroscan
Age	
R	.889
P value	.000
BMI	
R	−.014
P value	.920
1st blood transfusion	
R	−.030
P value	.834
Interval of transfusion	
R	−.660
P value	.000
Ferritin	
R	.951
P value	.000
Hb	
R	−.065
P value	.644
TLC	
R	.040
P value	.777
PLT	
R	−.420
P value	.002
PT	
R	.434
P value	.001
Albumin	
R	−.346
P value	.011
Bilirubin	
R	−.047
P value	.736
ALT	
R	.788
P value	.000
AST	
R	.664
P value	.000
Urea	
R	.142
P value	.311
Creatinine	
R	.071
P value	.612

BMI body mass index, *DM* diabetes mellitus, *HB* hemoglobin, *TLC* total leukocyte count, *PLT* platelets, *PT* prothrombin time, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase

Additionally, liver fibrosis was linked to HCV seropositivity (*P* value 0.05). Regarding the serum ferritin level, it was a significant risk factor for liver fibrosis in these individuals; a serum ferritin level ≥ 1500 was associated with significant liver fibrosis (F2,3,4) compared to < 1500 which showed nonsignificant fibrosis (F0,1) (Table 5).

The ferritin level was (708.2 ± 182.1) in patients with stages (F0, F1) compared to stages F2, F3, F4 (3213.5 ± 1272.9), and there was a significant *P* value (0.001). Also, Fig. 1 demonstrated a strong positive correlation between serum ferritin level and liver fibrosis grades detected by fibroscan.

Additionally, there were statistically significant changes in serum ferritin levels between stages F2, F3, and F4 (2202.9 240.3, 3955.2 450.0, and 5794.0399.6 correspondingly) (*P* value 0.001).

Ferritin played a substantial impact in the prediction of the existence of significant fibrosis, with 100% sensitivity and specificity at a cutoff of > 1900 as demonstrated in Fig. 2. So serum ferritin level can be used as a predictor of significant liver fibrosis in beta-thalassemia major patients.

Additionally, there was a significant association between fibrosis grades and PT, albumin, ALT, AST, and coarse liver texture on abdominal ultrasonography, although the correlation with platelet levels was negative. However, there was no connection between sex, consanguinity, family history, BMI, diabetes, or splenectomy and liver fibrosis.

Additionally, no association between liver fibrosis and Hb, TLC, bilirubin, urea, or creatinine was discovered.

4 Discussion

We aimed to use transient elastography to evaluate the liver fibrosis in pediatric beta-thalassemia major patients and identify the risk factors for fibrosis in these patients.

Our patients had a high rate of fibrosis/cirrhosis; twenty-eight of them (or 52.8%) were deemed to have significant fibrosis (F2, F3, F4). Al-Khabori et al. [11] reported significant fibrosis in 60% of patients, Elalfy et al. [12] found a very high number of TM patients with both fibrosis (35%) and cirrhosis (24%), and two Italian studies Ferraioli et al. [13] and Fraquelli et al. [14] found significant fibrosis in 33.6% and 35% of their TM patients, respectively.

Our study found a robust relationship between serum ferritin level and the progression and severity of liver fibrosis (*p* value 0.0001). An increase in serum ferritin level was linked to a higher risk of both hepatic fibrosis and cirrhosis.

Increased iron burden from transfusion therapy in transfusion-dependent thalassemia is the main factor

Table 5 Relation between significant fibrosis (2–4) by Fibroscan and ferritin level categories

Fibrosis by Fibroscan	Ferritin level			Total	*P* value
	500 to < 1000	1000 to < 1500	≥ 1500		
No fibrosis (0, 1)	21	4	0	25	< 0.001*
	100.0%	100.0%	0.0%	47.2%	
Fibrosis (2–4)	0	0	28	28	
	0.0%	0.0%	100.0%	52.8%	
Total	21	4	28	53	
	100.0%	100.0%	100.0%	100.0%	

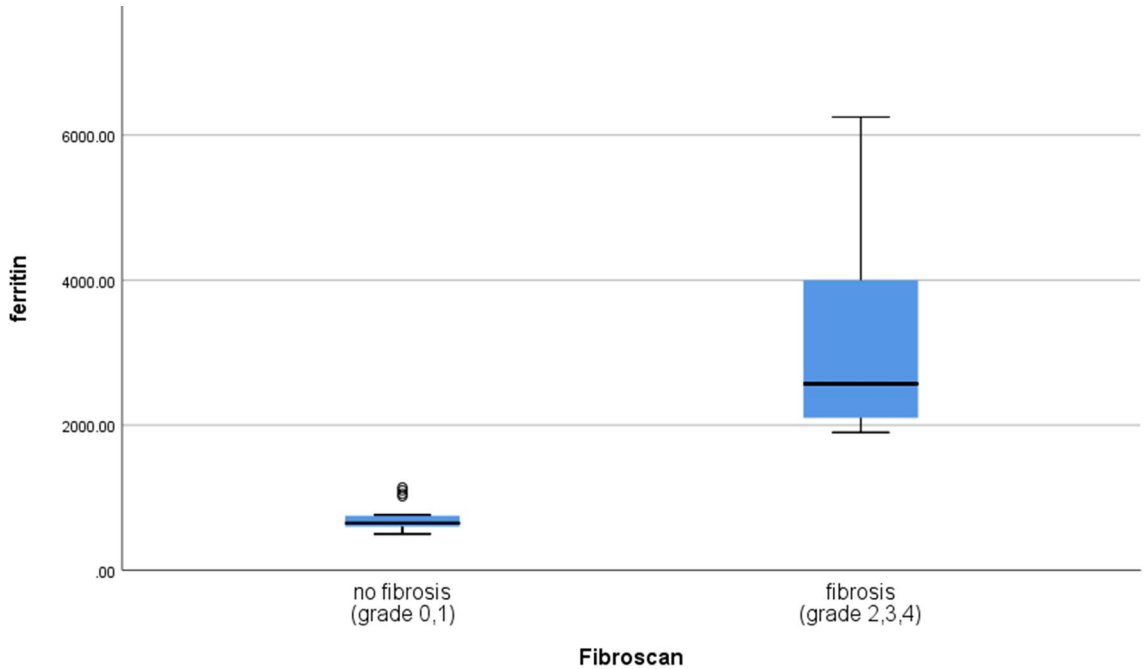

Fig. 1 Comparison between patients with fibrosis and patients without fibrosis regarding the ferritin level

driving the iron loading process. Due to the underlying mechanism of iron loading and the pace of iron accumulation, different organs are impacted by iron overload in different ways.

Iron buildup in the liver can result in cirrhosis and liver fibrosis [15].

This was in line with the findings of Angelucci et al. [16] and Al-Khabori et al. [11], who both observed an association between an iron overload and an increased risk of liver fibrosis.

9.4% of the cases in our study had HCV serology that was positive. While Mirzaei et al. [17] reported a lesser percentage (3.4%), Al-Khabori et al. [11], Elalfy et al. [12], and Kandi et al. [18] recorded greater percentages (38%, 82%, and 28%, respectively) of HCV antibody positive individuals. According to our research, having a positive HCV serology was associated with the severity of liver fibrosis (p value 0.05). We discovered that all individuals with HCV antibodies had severe liver fibrosis (F3, F4).

Elalfy et al. [12] reached the same conclusion, noting that beta-thalassemia patients with active hepatitis C infection may develop cirrhosis or fibrosis of the liver at a young age.

In contrast, three studies, two Italian [13, 14] and one Egyptian [11], failed to identify a link between liver fibrosis and HCV seropositivity.

Additionally, a higher incidence of liver fibrosis was linked to our patients' older ages (p value 0.001). This might be brought on by increased iron load with age brought on by exposure to additional blood transfusions. Additionally, HCV infection was a contributing factor to liver fibrosis in these patients; we discovered

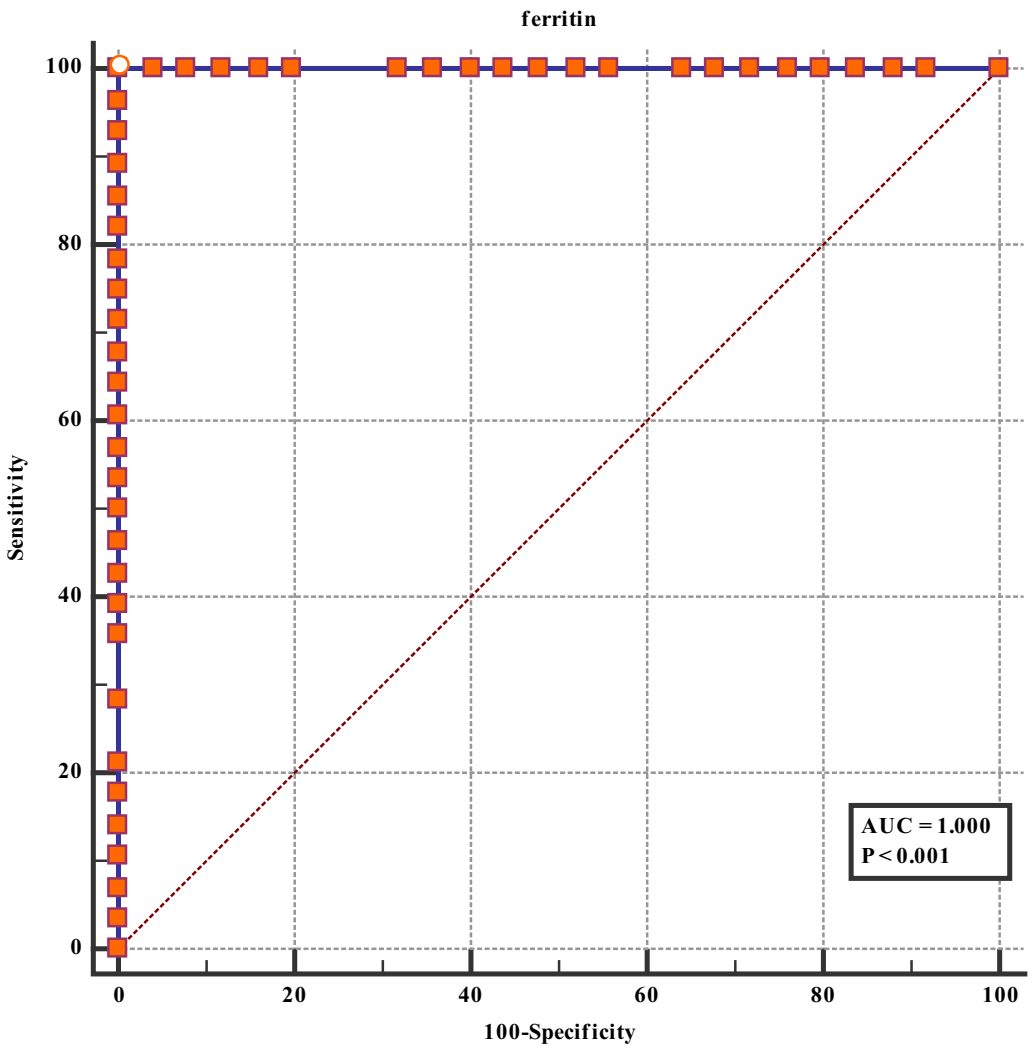

Fig. 2 Receiver operating characteristic curve for prediction of significant fibrosis (Fibroscan, F 2, 3, 4) from ferritin level

that positive HCV serology was identified at an older age (16 ± 2 years). On the other hand, Al-Khabori et al. [11] recorded that age had no bearing on the likelihood of substantial liver fibrosis developing.

Additionally, we discovered that there was a strong positive link between the frequency of blood transfusions and liver fibrosis; patients who got more transfusions were at a higher risk of developing liver fibrosis, which may be brought on by an excess of iron from transfusions.

According to Remacha et al. [19], the quantity of blood transfusions has a direct correlation with transfusion iron excess. A transfusion of blood typically contains 200–250 mg of iron.

Patients who receive between 10 and 20 units of blood are often at high risk for iron overload.

Additionally, Tari et al. [20] reported that receiving numerous blood transfusions might lead to iron excess, which further disrupts metabolism and damages tissue and organs.

The irregular use of iron chelation therapy contributed to the development of liver fibrosis in our study, this is due to the important role of chelation therapy which prevents the body from absorbing too much iron and causing problems like hepatic dysfunction, by binding to the iron and allowing the body to eliminate the bound particle. Therefore, regular chelation therapy is necessary for transfusion-dependent thalassemic patients in order to prevent iron overload and tissue damage. Porter et al. [21] also mentioned that chelation therapy aims to balance the rate of iron accumulation from blood transfusion by increasing iron excretion in urine, chelation

therapy is to achieve regular adherence to treatment regimens throughout a lifetime, as even short periods of interruption to treatment can have damaging effects.

Moreover, our patients' liver fibrosis was unrelated to their sex, consanguinity, family history, BMI, diabetes, or splenectomy. Contrarily, Al-Khabori et al. [11] discovered a link between liver fibrosis and gender in TM patients.

Additionally, high grades of liver fibrosis (F3, F4) in our patients were linked to liver function impairment (PT, albumin, ALT, AST), coarse liver texture on abdominal ultrasonography, and low platelet counts. According to Ahmed et al. [22], scar tissue caused by fibrosis can obstruct or restrict blood flow within the liver, this can starve and ultimately kill healthy liver cells, leading to the formation of more scar tissue, which impairs liver function and allows enzymes from damaged tissue to leak.

There was no significant correlation between liver fibrosis and any of the Hb levels, TLC counts, or bilirubin levels. Only 1.9% of patients exhibited positive HB s Ag and there was no conclusive evidence of a connection with liver fibrosis.

In conclusion, transient elastography is a different noninvasive technique that assesses liver fibrosis in people with thalassemia major. In these patients, iron overload, HCV seropositivity, frequent blood transfusions, age, and irregular chelation therapy are all risk factors for liver fibrosis. However, there is no correlation between liver fibrosis and sex, consanguinity, family history, BMI, diabetes, or splenectomy.

4.1 Limitations

The study's limited sample size could have an impact on the findings. So, further research on a larger sample of children is required to corroborate the findings.

Abbreviations

TM: Thalassemia major; TE: Transient elastography; CBC: Complete blood count; HB: Hemoglobin; TLC: Total leukocyte count; PLT: Platelets; PT: Prothrombin time; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; DM: Diabetes mellitus; BMI: Body mass index.; HCV Ab: Hepatitis c virus antibody; HB s Ag: Hepatitis B surface antigen.

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Author contributions

YA and AA analyzed and interpreted the patient data. DS supervised data collection and analysis. AM helped in data analysis. AM and YA collected the data. All authors have read and approved the manuscript.

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Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate

This study was approved by Ethics Committee of Beni-Suef University, Faculty of medicine and the ethics code was FWA00015574 FMBSUREC/01102019. Also, written consent was obtained from the care givers of the participating children.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflict of interest.

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