

Liver fibrosis in young Egyptian beta-thalassemia major patients: relation to hepatitis C virus and compliance with chelation

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ABSTRACT

Background. The main causes of liver fibrosis in transfusion-dependent thalassemia major are hepatitis C virus (HCV) infection and hepatic iron overload. The study aimed to assess liver fibrosis in Egyptian adolescents and young adult poly-transfused beta thalassemia patients infected with HCV using liver FibroScan in relation to iron overload and Liver iron concentration (LIC). **Material and methods.** Fifty-one regularly transfused beta thalassemia patients above 12 years old were subjected to measurement of serum alanine transaminase (ALT), serum ferritin (SF), HCV (antibody and RNA), LIC assessed by hepatic R2* and transient elastography (TE) (FibroScan). FibroTest and liver biopsy were done to 25 patients. **Results.** Eighty two% of studied thalassemia patients were HCV antibody positive; 21(49%) of them were viremic (HCV RNA positive); median LIC was 12 mg/gm dry weight. There were strong positive correlation between the degree of liver stiffness and Ishak fibrosis score assessed in liver biopsy specimens ($P = 0.002$) and between FibroScan and FibroTest results ($P < 0.001$). Patients with HCV viremia showed significantly higher ALT, γ -glutamyl transpeptidase (GGT), SF, LIC and increased liver stiffness compared to patients with no viremia ($P = 0.0001$, 0.001 , 0.012 , 0.006 and 0.001) respectively. Liver cirrhosis (TE values > 12.5 kPa) was encountered in 23.5% and variable degrees of liver fibrosis (TE values $> 6-12.5$ kPa) in 35% of studied thalassemic patients. **Conclusion.** Young beta thalassemia patients with active hepatitis C infection may have hepatic cirrhosis or fibrosis at young age when accompanied with hepatic siderosis. Non invasive Liver FibroScan and FibroTest were reliable methods to assess liver fibrosis in young thalassemic-patients.

Key words. Iron overload. Liver iron concentration. Cirrhosis. FibroScan. FibroTest.

INTRODUCTION

Worldwide, no patients get more red cell products than those with thalassemia major. The life-long need for transfusion renders patients vulnerable to transfusion-transmitted viral infections. Hepatitis C virus infection has emerged as the major risk in the last decades.¹

Hepatitis virus C infection is the main risk factor for liver fibrosis in transfusion-dependent thalassemics. Excess liver iron is now clearly recognized as a cofactor for the development of ad-

vanced fibrosis and cirrhosis in patients with HCV infection.²⁻⁴

In adults, the non-invasive assessment of fibrosis in chronic hepatitis, especially of viral etiology, is more and more accepted, partially replacing liver biopsy in some countries. Guidelines from France recommend that the first-line test for untreated patients with HCV chronic hepatitis, with no comorbidities, should be a non invasive procedure (either FibroTest® or FibroScan®).⁵

The vast majority of studies assessing transient elastography (TE) as compared to liver biopsy were performed in patients with HCV chronic hepatitis.⁶⁻⁹

In beta-thalassemia patients (especially HCV infected ones) at higher risk of liver biopsy related complications as compared to other chronically HCV-infected patients, the availability of a noninvasive method to measure hepatic fibrosis is crucial.¹⁰ However, FibroScan; a medical device based on TE and FibroTest; were not extensively investigated in young beta thalassemia patients infected with HCV.

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The purpose of this study was to assess liver fibrosis status in young polytransfused beta thalassemia patients infected with HCV using both FibroScan and FibroTest in relation to iron overload and liver iron concentration (LIC).

MATERIAL AND METHODS

This cross sectional study included fifty one regularly transfused beta thalassemia patients (26 males and 25 females with age range 12-24 years from Thalassemia Center, Ain Shams University during the period from March 2009 to March 2010. Written informed consent was obtained from adult patients, patients' parents or their legal guardians after approval of the study by the Local Ethical Committee, Ain Shams University. The study protocol conformed to the ethical guidelines of Declaration of Helsinki 1975.

Inclusion criteria

Beta thalassemia major patients aged ≥ 12 years, poly-transfused > 100 transfusion, with liver siderosis; LIC > 2 mg Fe/g liver dry weight [dw], HCV antibody positive patients have had their infection for at least 10 years as recorded from their files. All patients received iron chelation therapy either in the form of subcutaneous infusion of desferoxamine (Desferal; Sigma, Saint Louis, Missouri, US) with a dose of 40 mg/kg for 5 days/week. Alternatively, oral chelation therapy Deferiprone (Ferriprox®; ApoPharma, Toronto, Canada) was administered at 75 mg/kg/d for 7 days/week.

Compliance defined as the extent to which patients take medications as prescribed by their health care providers; was assessed by the following questions:

- How many doses were missed per day during the preceding 4 weeks, and
- What is the amount of medication remaining?

At each clinic visit, the old vials were brought and the remaining tablets were counted.

Exclusion criteria

Non applicability conditions of FT: diabetes mellitus, patients with ALT > 10 times ULN (Upper Limit of Normal), serum bilirubin > 5 mg/dL.

Thalassemic patients were categorized on the basis of risk to develop liver fibrosis i.e. both LIC values and HCV viremia into 3 subgroups:

- **Group I.** Patients with both HCV-RNA positivity and LIC > 14 mg/gm dw.
- **Group II.** Patients with either HCV-RNA positivity or LIC > 14 mg/gm dw, and
- **Group III.** Patients with negative viremia with LIC < 14 mg/gm dw.

Blood samples were taken 4 weeks from last blood transfusion; Complete blood count was performed using coulter B66 (Miami, Florida, USA), Liver function tests including AST, ALT using Synchron CX9 autoanalyzer (Brea, California, USA), serum ferritin on Immulite instrument (Diagnostic products corporation 5700 West 96 St. Los Angeles, USA), hepatitis B surface antigen, human immunodeficiency virus antibody using ELISA technique (R & D system, USA) and HCV antibody using ELISA technique (R & D system, USA) and HCV RNA by polymerase chain reaction (Amplicor HCV; Roche Molecular Systems, Basel, Switzerland) were done to all patients according to manufacturer's instructions. Transfusional iron intake was calculated in mg/kg/day.¹¹

FibroTest (Biopredictive, France, [FT]): this test consists of an algorithm of five fibrosis markers (alfa2-macroglobulin, apolipoprotein A1, haptoglobin, GGT, bilirubin).¹²⁻¹³ It has been evaluated in 25 patients suitable for FT who performed also liver biopsy. Gamma-glutamyltranspeptidase (GGT), total bilirubin, apolipoprotein A1, and haptoglobin were measured with a Cobas Integra 400 analyzer (Roche, Indianapolis, Indiana, USA) and Roche Diagnostics reagents (Roche, USA). Alpha2-macroglobulin was assayed with a Cobas Integra 400 Turbidimetry with Dako utility channel reagents (Glostrup, Denmark). All tests were performed by personnel blinded to all patients' data, including biopsy results.

Liver iron concentration (LIC) was assessed by Magnetic resonance imaging (MRI) measurements of the proton transverse relaxation parameter R2 using 5 mm axial slices. R2 scans were performed using FerriScan® technology (Resonance Health).¹⁴ Results are expressed as mg/gm liver dry weight.

Liver histology and quantification of liver fibrosis

Liver biopsy was performed in 25 patients with chronic HCV hepatitis who accepted to do the biopsy within one month from TE by senior operators using the Menghini technique with a 1.6-mm-diameter needle (Hepafix, Braun, Melsungen, Germany). All biopsy specimens were analyzed by the same

trained pathologist blinded to the results of non-invasive methods. Fibrosis was scored by Ishak classification.¹⁵ None of the patients experienced biopsy complications.

Transient elastography (TE)

All patients were examined by TE (FibroScan®; Echosens, Paris, France). The procedures were performed by the same investigator who was blind to clinical, serological, and histological data. Details of the technical background and examination procedure have been previously described.¹⁶ The results were expressed in kilopascals (kPa). The median value was considered representative of the elastic modulus of the liver. Only procedures with at least 10 successful acquisitions and a success rate of at least 60% with interquartile range (IQR) of all validated measurements less than 30% of the median value were considered reliable. Twenty five patients who had the liver biopsy and FT were retested after 2 weeks to verify the results. Cirrhosis was defined according to the published cut-offs in patient with hepatitis C: 12.5 kPa.¹⁷ The cut-off value of TE 6 kPa for diagnosing $F \geq 1$ (18) was used to exclude hepatic fibrosis with smaller values. No liver stiffness measurement failure was observed in the present study.

Statistical analysis

Analysis of data was performed by using SPSS (version 15). Comparison between 2 groups of patients was made using Student's t-test for parametric measures and Wilcoxon signed-rank test (Z value) for non parametric measures. Spearman's rank correlation coefficient was used to correlate between two quantitative variables. P value < 0.05 was considered the cut-off value for significance.

RESULTS

Fifty-one consecutive patients (26 males and 25 females; mean age 15.92 ± 3.11 years); 25 with a suitable liver biopsy entered the study. All beta thalassemia major patients were transfusion-dependent: they transfused packed red cells every 15-21 days with pretransfusional Hb levels from 7.4 to 9.4 g/dL. Median BMI was 19.5 (range 14.5-28.3 kg/m²). Eighty-two percent of thalassemia patients were HCV antibody positive; 21 of them (49%) were viremic (HCV RNA positive), 4% were hepatitis B surface antigen positive and none of them was human immunodeficiency virus positive. Median LIC was

12 mg/gm liver dw. Ishak fibrosis score was 6 in 4 patients, 5 in 3 patients, 4 in 4 patients, 3 in 4 patients and 2 in 10 patients. Mean stiffness value was 10.75 ± 10.41 kPa (median 6.8, range 2.8-49.7 kPa).

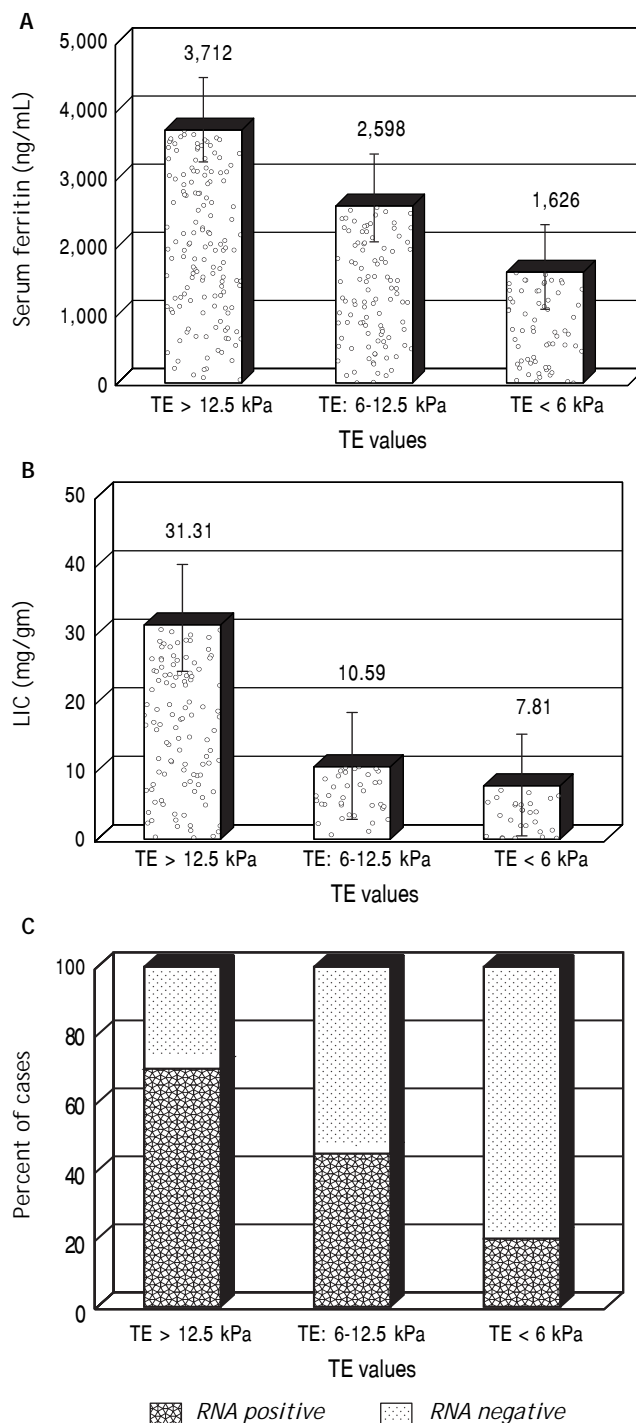


Figure 1. Serum ferritin (A), LIC (B) and RNA positive cases (C) among beta thalassemia major patients with different TE values.

Table 1. Demographic and biochemical markers of HCV viremic vs. non viremic Beta Thalassemia major patients.

	HCV Viremia		Non viremic HCV		P
Age (years)	16.67±3.45	(N = 21)	15.53± 0.39	(N = 30)	n.s.
TI (mg/kg)	0.45±0.10	(N = 21)	0.38± 0.08	(N = 30)	n.s.
ALT (IU/L)	126.38±49.16	(N = 21)	53.60± 18.49	(N = 30)	0.0001*
AST (IU/L)	95.05±49.31	(N = 21)	38.00± 20.45	(N = 30)	0.0001*
GGT (IU/L)	48.83±19.47	(N = 12)	23.46± 8.24	(N = 13)	0.001*
S.F. (ng/mL)	3081±1329	(N = 21)	2063± 1317	(N = 30)	0.012*
LIC (mg/gm liver dw)	20.86±15.27	(N = 21)	10.43± 6.21	(N = 30)	0.006*
FT	0.71±0.21	(N = 12)	0.54± 0.22	(N = 13)	n.s.
TE (kPa)	15.95±13.77	(N = 21)	7.11± 4.79	(N = 30)	0.001*

*Significant. TI: transfused iron. ALT: alanine aminotransferase. AST: aspartate aminotransferase. GGT: γ -glutamyl transpeptidase. SF: serum ferritin. LIC: liver iron concentration. dw: dry weight. FT: FibroTest. TE: transient elastography. kPa: kilopascal.

Table 2. Risk of liver fibrosis in relation to HCV RNA positivity and LIC in Beta Thalassemia major patients.

	(Risk) Liver fibrosis (HCV RNA & LIC values)				
	Group I (N = 9)	Group II (N = 16)	Group III (N = 26)	P1	P2
TI (mg/kg)	0.50 ±0.12 (N = 9)	0.43±0.08 (N=16)	0.38± 0.08 (N = 26)	n.s.	0.047*
Compliance with chelator	0.11 ±0.33 (N = 9)	0.31±0.48 (N=16)	0.73± 0.45 (N = 26)	n.s.	0.030*
FT	0.81 ±0.22 (N = 6)	0.68±0.20 (N=7)	0.47± 0.13 (N = 12)	0.004*	0.002*
TE (kPa)	28.19 ±13.23 (N = 9)	9.46±5.27 (N=16)	5.51± 2.28 (N = 26)	0.0001*	0.0001*

Group I: HCV RNA +ve & LIC \geq 14. Group II: HCV RNA +ve & LIC < 14 OR HCV RNA -ve & LIC \geq 14. Group III: HCV RNA -ve & LIC < 14. *Significant, P1: comparison between column 1 and column 2 p values. P2: comparison between column 2 and column 3 p values. RNA: ribonucleic acid. LIC: liver iron concentration. TI: transfused iron. FT: FibroTest. TE: transient elastography.

Using FibroScan; liver cirrhosis (TE values > 12.5 kPa) was encountered in 23.5% and variable degrees of liver fibrosis (TE values > 6-12.5 kPa) in 35% of studied thalassemic patients. There was strong positive correlation between degree of liver stiffness and Ishak fibrosis score detected by liver biopsy ($r = 0.40$, $P = 0.002$). All patients with liver cirrhosis (TE values > 12.5 kPa) were HCV antibody positive and showed highest HCV RNA positivity (Figure 1). Patients with HCV viremia showed significantly higher ALT, GGT, SF, LIC and increased liver stiffness compared to patients with no viremia ($P = 0.0001$, 0.001 , 0.012 , 0.006 and 0.001) respectively (Table 1). Non viremic HCV antibody positive patients showed significantly higher LIC (12.10 ± 6.56 vs. 6.56 ± 2.79 mg/gm liver dry weight, $P = 0.006$) and increased liver stiffness (8.26 ± 5.30 vs. 4.44 ± 1.175 kPa, $P = 0.006$) compared to HCV antibody negative patients. Highest serum ferritin (SF), LIC and RNA positivity were observed among beta thalassemia major patients with TE values in the cirrhotic range (above 12.5kPa) (Figure 1).

Lowest liver stiffness, fibrotest results and best compliance with chelation therapy were obtained in

thalassemic patients with negative viremia and LIC less than 14 mg/gm dw whereas highest TE values, worst compliance with chelation and highest fibrotest results were shown in patients with both HCV-RNA positivity and LIC above 14 mg/gm dw followed by patients with either HCV-RNA positivity or LIC above 14 mg/gm dw (Table 2). Details of compliance of each subgroup were shown in figure 2, where 57% of the patients compliant with Deferoxamine had LIC below 14 mg/gm dw and negative viremia. Most of the non-compliant patients to Deferoxamine were HCV RNA positive with LIC above 14 mg/gm dw and 83% of the patients compliant with Deferiprone had LIC below 14 mg/gm dw and negative viremia.

Relation between compliance with chelators and TE values showed that most non compliant patients with Deferoxamine had TE more than 12.5 kPa whereas most compliant patients with Deferiprone had TE less than 6 kPa (Figure 3). Strong positive correlations were shown between TE values and both SF levels and LIC (Figure 4), between TE values and fibrotest ($r = 0.935$, $p < 0.001$) and also between SF levels and LIC ($r = 0.538$, $P < 0.001$).

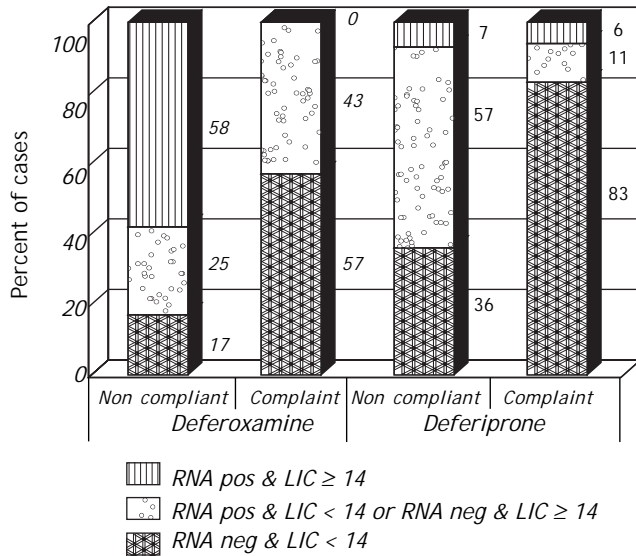


Figure 2. Risk of Liver fibrosis/cirrhosis (RNA Positivity and LIC) in beta thalassemia major patients according to compliance with iron chelation therapy.

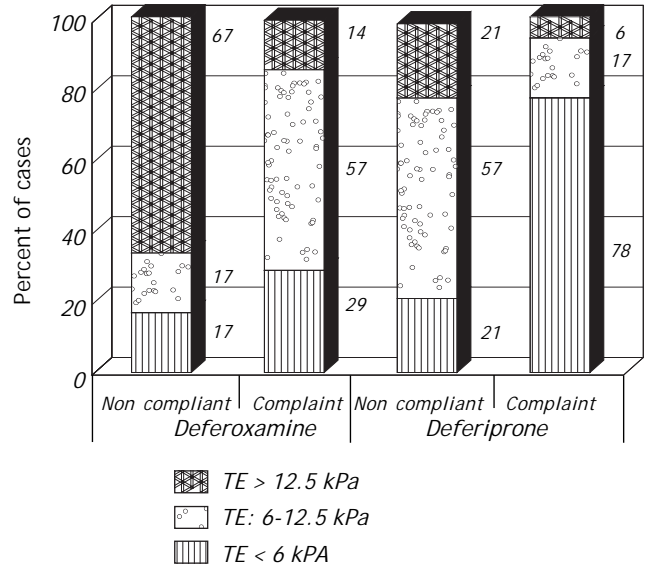


Figure 3. Compliance with iron chelation therapy among beta thalassemia major patients with different TE values.

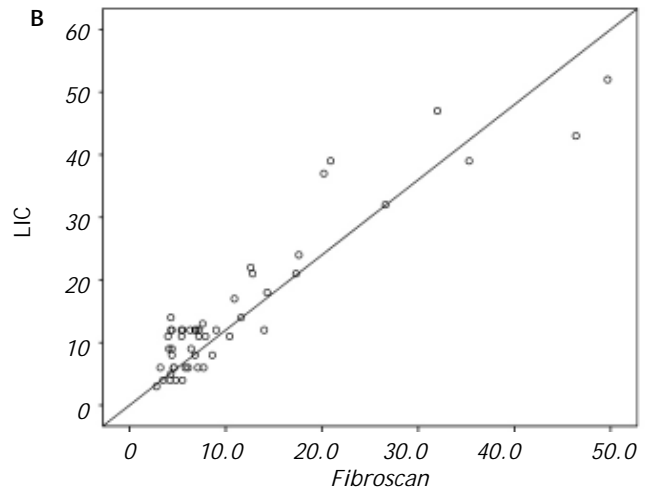
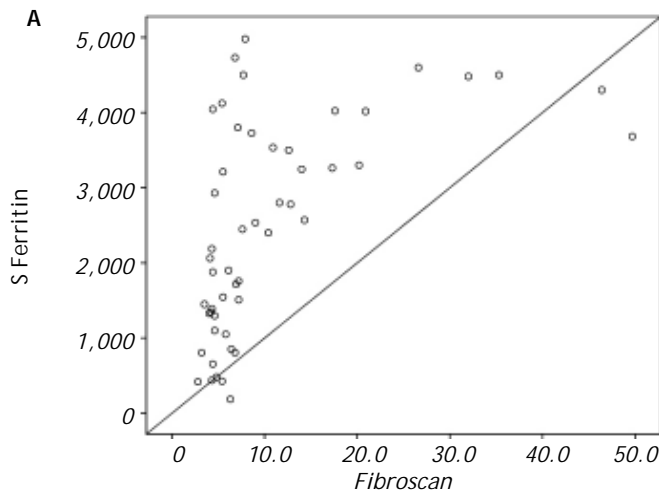


Figure 4. Correlation between Fibro Scan and both serum Ferritin (A) ($r = 0.555^{**}$, $P < 0.001$) and LIC (B) ($r = 0.929$, $P < 0.0001$).

DISCUSSION

The present study revealed that 82% of thalassemia patients were HCV antibody positive; 21(49%) of them were viremic (HCV RNA positive). Previous studies on the prevalence of hepatitis C in Egyptian thalassemic children reported that 44%¹⁹ to 75.6%²⁰ had hepatitis C antibodies. HCV-PCR was positive in 64% of studied Egyptian patients.²¹ While in Italian multicenter study,²² anti-HCV antibodies were found in 91% of thalassemic patients and 72% of them were viremic.

A high percentage of fibrosis/cirrhosis was encountered in the studied young beta thalassemia major patients. The development and the severity of liver fibrosis were strongly related to the presence of chronic HCV infection and to the extent of liver iron overload. Patients with liver cirrhosis (TE values > 12.5kPa) had multiple risk factors; all were HCV antibody positive; had high LIC (≥ 14 mg/gm) and SF levels above 2,500 ng/mL. Several studies²²⁻²⁶ reported similar data. Patients with HCV viremia showed higher TE values and increased fibrosis. However; two non viremic patients showed TE

values in the cirrhotic range and some non viremic patients had fibrosis; this might be explained by the undetectable persistent low level of HCV viremia for long duration with high liver iron due to poor compliance with chelation therapy. This observation is in concordance with previous study²⁷ who reported fibrosis by liver biopsy in non viremic HCV antibody-positive patients suggesting that HCV may persist in the liver in the majority of HCV RNA-negative cases.

Liver iron concentration is related to transfusional iron intake, type and compliance with chelation therapy. In transfusion-dependent thalassemia major, hepatic iron overload is one of the major problems for the progression of the liver disease and is due to regular transfusion regimen that leads to iron overload.²⁸ Previous studies suggested the role of iron loading as a factor in fibrosis progression in hepatitis C.^{22,29-31} The positive correlations observed between TE values and both SF levels and LIC in our study are in agreement with Fraquelli, *et al.*,¹⁰ who reported that in thalassemia major patients with higher ferritin levels, TE increased progressively; viremic patients with higher ferritin levels showed a higher increase of TE as compared with non viremic ones suggesting a possible synergistic effect of iron overload and ongoing HCV on hepatic fibrosis.

In this study; serum ferritin (SF) levels were assessed as a potential surrogate marker for LIC and showed positive correlation with LIC. There are controversies on the relation between SF and hepatic fibrosis. Data on a strong correlation between SF and the degree of hepatic fibrosis was observed in thalassemia major patients not infected with HCV; however, SF levels alone were not sufficient to assess the degree of fibrosis in HCV positive thalassemia major patients.³² Meanwhile; others found no correlation between TE values and the degree of iron overload in beta-thalassemia major patients.³³⁻³⁴

The positive correlation between Fibrotest results and TE values in studied thalassemic patients was in concordance with previous studies in other hepatic disorders.³⁵⁻³⁶ In patients with FibroScan and Fibrotest concordant results liver biopsy might be avoided. FibroScan and Fibrotest appear to be valuable methods for detecting early stages of fibrosis among patients with chronic HCV infection, allowing avoiding the progression of liver damage.³⁶ Combinations of two modalities of non-invasive methods can reliably differentiate between minimal and significant fibrosis, and there-

by avoid liver biopsy in a significant percentage of patients.³⁷

In the current study, cirrhosis was revealed by FibroScan and confirmed by biopsy in young thalassemics as early as 12 years old. The relatively long duration of infection and poor compliance with chelation may explain this observation.

TE values increased proportionally according to the Ishak stage. This goes in agreement with previous reports.^{10,33-34} Highest TE values observed in patients with both HCV-RNA positivity and high LIC (> 14 mg/gm dw) may confirm that thalassemic patients with active HCV replication and severe iron overload develop severe fibrosis or cirrhosis more frequently. Their concomitant presence results in a striking increase in risk for liver fibrosis progression.³⁸ Difference in compliance with chelators in our patient subgroups may help explain these findings. Moreover, strong correlation between TE and fibrosis stage detected by liver biopsy may denote that high LIC did not affect the usefulness of TE. Liver Iron, in association with HCV viremia, may lead to an increased rate of fibrosis detected by TE, but further studies are required on wider scale before this can be determined.

FibroScan should be verified on thalassemic patients of younger age group as we had cirrhotic thalassemic patients who died early in the second decade of life with liver cell failure.

In the current study, most compliant patients with deferoxamine or deferiprone had the lowest TE values whereas most non compliant patients had higher TE values. It is noteworthy that most compliant patients with deferiprone showed TE values within normal range. These results can confirm previous results³⁹⁻⁴¹ which demonstrated no evidence of hepatic fibrosis induced by deferiprone. Non compliance with chelation in the group with HCV viremia and high LIC was marked. Adherence to adequate chelation therapy can prevent the development of liver fibrosis in thalassemics free of HCV-infection and may reduce the risks of developing severe fibrosis in thalassemics with chronic hepatitis C.⁴²

CONCLUSIONS

Liver cirrhosis and/or fibrosis were commonly encountered in young beta thalassemia patients with chronic active hepatitis C infection and heavy iron overload. Thalassemic patients compliant with adequate chelation may have normal liver

stiffness with reduced LIC. Liver FibroScan and FibroTest were reliable methods as surrogate for liver biopsy to assess fibrosis progression in thalassemic patients.

ABBREVIATIONS

- **HCV:** hepatitis C virus.
- **LIC:** liver iron concentration.
- **ALT:** alanine aminotransferase.
- **SF:** serum ferritin.
- **RNA:** ribonucleic acid.
- **TE:** transient elastography.
- **FT:** FibroTest.
- **GGT:** γ -glutamyl transpeptidase.
- **kPa:** kilopascal.
- **dw:** dry weight.
- **ULN:** upper limit of normal.
- **MRI:** magnetic resonance imaging.
- **IQR:** inter-quartile range.
- **BMI:** body mass index.
- **PCR:** polymerase chain reaction.
- **AST:** aspartate aminotransferase.
- **TI:** transfused iron.

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