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Using FIB-4 score as a screening tool in the assessment of significant liver fibrosis (F2) in patients with transfusion-dependent beta thalassaemia: a cross-sectional study

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ABSTRACT

Objective To evaluate the performance of the fibrosis-4 (FIB-4) score as a screening tool to detect significant liver fibrosis (F2) compared with transient elastography (TE), among chronic transfusion-dependent beta-thalassaemia (TDT) patients in a resource-poor setting.

Design A cross-sectional study.

Setting Adolescent and Adult Thalassaemia Care Centre (University Medical Unit), Kiribathgoda, Sri Lanka.

Participants 45 TDT patients who had undergone more than 100 blood transfusions with elevated serum ferritin >2000 ng/mL were selected for the study. Patients who were serologically positive for hepatitis C antibodies were excluded.

Outcome measures TE and FIB-4 scores were estimated at the time of recruitment in all participants. Predefined cut-off values for F2, extracted from previous TE and FIB-4 scores studies, were compared. A new cut-off value for the FIB-4 score was estimated using receiver operating characteristics curve analysis to improve the sensitivity for F2 prediction.

Results Of the selected 45 TDT patients, 22 (49%) were males. FIB-4 score showed a significant linear correlation with TE (r=0.52;p<0.0003). The FIB-4 score was improbable to lead to a false classification of TDT patients to have F2 when the FIB-4 cut-off value was 1.3. On the other hand, it had a very low diagnostic yield in missing almost all (except one) of those who had F2. Using a much-lowered cut-off point of 0.32 for FIB-4, we improved the pick-up rate of F2 to 72%.

Conclusions Regardless of the cut-off point, the FIB-4 score cannot be used as a good screening tool to pick up F2 in patients with TDT, irrespective of their splenectomy status. On the contrary, at a 1.3 cut-off value, though FIB-4 is a very poor detector for F2 fibrosis, it will not erroneously diagnose F2 fibrosis in those who do not have it.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This study assessed liver fibrosis by fibrosis-4 (FIB-4) score biomarker compared with transient elastography (TE) among a Sri Lankan transfusion-dependent beta-thalassaemia population using TE as the reference standard for liver fibrosis.
⇒ With predefined cut-off values, the study estimated the sensitivity and specificity of the FIB-4 score as a screening tool to rule out significant liver fibrosis (F2).
⇒ Receiver operating characteristics curve analysis was carried out to estimate a new cut-off value with better sensitivity for the FIB-4 score for ruling in significant liver fibrosis (F2).
⇒ The small sample size of the study is a major limitation.
⇒ MRI elastography or liver biopsy was not used to better assess the degree of liver fibrosis.

INTRODUCTION

Liver disease is the third most common cause of morbidity and mortality in transfusion-dependent thalassaemia (TDT) patients. Though infections and cardiac failure are the leading causes of death among these patients, the liver is yet another major target organ susceptible to damage.[1] Transfusional iron overload and transfusion-transmitted hepatitis infections are the leading causes of hepatic fibrogenesis, triggering liver cell dysfunction in these patients.[2 3] On chronic exposure to excess iron, myofibroblasts activate and secrete extracellular matrix protein, predominantly collagen type I and III, assisting in scar tissue formation, causing liver fibrosis.[4 5]

Assessing liver fibrosis using liver biopsy is the gold-standard method. Owing to its procedure-related mortality (<1 in 10 000 cases) and its invasive nature, liver biopsy is not favoured by clinicians or patients.[6–8] Transient elastography (TE) estimates liver fibrosis/stiffness non-invasively. This technology was first introduced by Sandrin *et al*. It

is a rapid bedside tool with remarkable reproducibility.[9] TE is based on measuring the velocity of a mechanical shear wave generated by a transducer placed on the skin. The shear wave velocity is decided by the time the shear wave takes to travel through the liver tissue, and the velocity is then converted to liver stiffness measurement and is expressed in kilopascals (kPa).[7 10–13] Despite being simple, safe, and efficient, routine use of TE is restricted by the cost of the device, primarily in resource-poor settings.

Consequently, the desire to develop clinical scores to detect liver fibrosis using inexpensive point-of-care tests has always been explored.[8] Of the several unique biomarkers that have been developed, the fibrosis-4 score (FIB-4 score) has been widely used. This was initially developed in patients with HIV, and Hepatitis C Virus (HCV) coinfection to predict liver fibrosis and has been validated in HCV and non-alcoholic fatty liver disease (NAFLD) patients. It is a simple model based on biochemical parameters of alanine aminotransferase (ALT), aspartate aminotransferase (AST), platelet count, and the patient's age.[14 15] Though its interpretation is cautious in splenectomised thalassaemia patients due to post splenectomised thrombocytosis, the FIB-4 score, is a cost-effective bedside biomarker for liver fibrosis assessment in primary care. However, there is limited information available on the applicability of this score in patients with TDT.[16 17] Moreover, data regarding the diagnostic performance of the FIB-4 score and its comparability with TE in TDT patients is scarce.

The objective of this study was to evaluate the performance of the FIB-4 score in assessing liver fibrosis compared with TE and evaluate if the FIB-4 score could be used as a screening tool to detect significant liver fibrosis (F2), among chronic TDT patients, in a resource-poor setting.

METHODOLOGY

Study design

We prospectively followed up a selected cohort of patients with TDT undergoing aggressive chelation therapy over 2 years to assess the variation of liver fibrosis. This paper is a cross-sectional study on the baseline characteristics of the study participants at the time of recruitment.

Study setting and the participants

Patients in our cohort were registered at the Adult and Adolescent Thalassaemia Unit, Kiribathgoda, Sri Lanka. Written informed consent was obtained from each study participant/guardian at the time of recruitment.

TDT patients who have undergone more than 100 blood transfusions with elevated serum ferritin >2000 ng/mL on three consecutive occasions, 3 months apart, were selected for the study. Patients who were serologically positive for hepatitis C antibody and patients with ultrasound evidence of established cirrhosis/portal hypertension were excluded from the study. At the time of enrolment, blood was taken for the laboratory evaluation of full blood count (FBC), ALT, and AST. All the patients underwent TE to quantify liver fibrosis which the same operator carried out.

Transient elastography

TE was performed as per the manufacturer's instructions using FibroScan 502 touch (Echosens, Paris, France). Relevant clinical guidelines for the elastography assessment were referenced in accordance with Ferraioli *et al*.[12] The median value of at least 12 valid measurements with a >60% success rate (ratio of valid measures to the total number of measures) and an IQR of <30% of the median liver stiffness measurement were considered successful TE scores. A cut-off value for TE scores to estimate significant liver fibrosis (F2) was predefined as per the previously published literature. According to Ferraioli *et al*, in a study done on patients with beta thalassaemia TE value of 7.0 kPa was considered the cut-off value for significant liver fibrosis (F2).[12] Therefore, the current study followed the same threshold of 7 kPa to estimate F2 fibrosis in patients with TDT.

FIB-4 score

FBC, AST and ALT values at recruitment were considered for the FIB-4 score estimation. The age of the patient was calculated according to their last birthday. FIB-4 score was estimated in all patients according to the following published formula; age (years) * AST [U/L] / (platelets [109/L] * (ALT [U/L])1/2).[15 18 19] Though this score was initially developed in HIV/HCV coinfected patients FIB-4 score has been validated in both HCV and NAFLD patients. According to Castera *et al*[20] the prespecified cut-off value to rule out F2 fibrosis in patients with NAFLD is 1.3.[20] Conversely, Sterling *et al*[15] reported that a cut-off value of 1.45 with a negative predictive value of 90% was considered the threshold level for predicting F2 fibrosis in patients with HIV/HCV coinfection.[15] When we recruited the study participants, we excluded TDT patients who had been infected with the HCV. Hence, our study followed the threshold level of 1.3, similar to NAFLD patients, to rule out significant liver fibrosis.

Statistical analysis

Data analysis was carried out in two stages. During the first stage, the Spearman correlation coefficient was estimated between the FIB-4 and TE scores to find an association between the two measurements in the study group. To assess the FIB-4 score as a screening tool to detect F2 fibrosis with reference to TE findings, cut-off values were predefined for FIB-4 and TE scores as 1.3 and 7 kPa, respectively. The sensitivity and specificity of the FIB-4 score as a screening tool to detect significant fibrosis were calculated and tabulated in the entire study population. During the second stage, receiver operating characteristics (ROC) curve analysis was undertaken to identify a new cut-off value with good sensitivity for the FIB-4 score.

Table 1 Demographic and biochemical characteristics, FIB-4 and TE scores of the study participants

Variable (unit measure)	Entire group Mean (SD)	Unsplenectomised group Mean (SD)	Splenectomy group Mean (SD)	P value
Age (years)	18.9 (4.8)	17.7 (4.8)	21.1 (3.97)	0.02
AST (IU/L)	47 (27.5)	41.8 (26.5)	57.73 (27.3)	0.007
ALT (IU/L)	60 (46.35)	55.7 (50.4)	68.96 (36.8)	0.08
Platelet count(cells/*10^3 mm3)	329 (186)	272 (100)	442 (259)	0.11
FIB-4 score	0.46 (0.32)	0.43 (0.22)	0.53 (0.46)	0.97
TE score (kPa)	10.22 (6.42)	7.75 (2.46)	15.2 (8.8)	0.002

FIB-4=age (years) * AST [U/L]/(platelets [109/L] * (ALT [U/L])1/2).

ALT, alanine aminotransferase; AST, aspartate aminotransferase; FIB-4, fibrosis-4; IU/L, international units per liter ; TE, transient elastography.

The distribution of continuous variables was expressed as mean (SD), and categorical variables were presented as frequencies. The p<0.05 was considered to be statistically significant. All descriptive and analytical statistics were calculated with R programming language V,3.4.2.

Patient and public involvement

Patients or the public were not involved in our study design, conduct, reporting or dissemination plans of our research.

RESULTS

Of the 45-TDT patients we studied, 22 (49%) were males. The mean age of the patients was 18.9 years (SD=4.8). Thirty (67%) patients have not undergone splenectomy in our study group. Of the 15 (33%) patients who had undergone splenectomy, seven (47%) patients had thrombocytosis (defined as >450 000) following postsplenectomy. Demographic, biochemical characteristics, FIB-4, and TE scores of the entire group, the unsplenectomised group, and the splenectomised group are summarised in table 1. There were no failures recorded in the TE assessment.

The FIB-4 score showed a significant linear correlation with TE scores in the entire study group (r=0.52; p<0.0003).

At the time of recruitment, 29 (64%) patients had significant liver fibrosis as per the TE results. Conversely, the FIB-4 score detected only 1 out of 29 patients with significant liver fibrosis in the study group; hence sensitivity is 3.4% (table 2). In other words, when a predefined cut-off value of 1.3 was set for the FIB4 score, only 1 (1/29) patient was correctly classified as having significant liver fibrosis in our study cohort (true positives). Twenty-eight out of 29 patients (28/29) were misclassified as not having significant liver fibrosis (false negative). Similarly, 16 patients did not have significant liver fibrosis at the time of recruitment as per the TE assessment (true negatives). Of that group, the FIB-4 score was able to classify 100% as not having significant liver fibrosis; hence specificity is 100%. Not a single patient without significant liver fibrosis was misclassified as having significant liver fibrosis (false positives). At this cut-off point, it is a very poor detector for fibrosis, but it will not erroneously diagnose fibrosis in those who do not have it.

Table 3 shows the sensitivity, specificity and area under the curve (AUC) for the FIB-4 score with a new cut-off value to detect F2 fibrosis compared with the predefined cut-off value of 1.3.

The best new cut-off point to detect significant liver fibrosis in this study group is 0.32 (AUC=66%) (figure 1). With the suggested new cut-off value of 0.32, the FIB-4 score was able to detect 21 out of 29 patients (72%) with F2 fibrosis in the study cohort. Hence sensitivity improved from 3.4% to 72%. Only 8 out of 29 patients (8/29) were misclassified as not having significant liver fibrosis (false negative). As per the new cut-off value, nine patients were correctly classified as having F2 fibrosis (true negatives), whereas seven patients were misclassified as not having F2 fibrosis (false positives). Hence the new cut-off value cannot rule out patients with significant liver fibrosis 100%.

Table 2 At the time of recruitment, the number of patients with significant liver fibrosis (F2) by FIB-4 score and TE assessment

	Entire group N (%)	Un-splenectomised group N (%)	Splenectomy group N (%)
F2 by FIB-4 score (FIB-4 score >1.3)	01 (2.22)	0	01 (2.22)
F2 by TE (TE>7 kPa)	29 (64)	17 (58.6)	12 (41.4)

FIB-4, fibrosis-4; TE, transient elastography.

Table 3 ROC curve analysis data in the total study participants in comparison with the previously published FIB-4 cut-off value of 1.3

Study group	FIB-4 cut-off value		AUROC	Sensitivity	Specificity
Entire group	As per the pre-defined cut-off value	1.3	–	3.4%	100%
	New cut-off value from ROC curve analysis	0.32	66%	72%	56%

AUROC, area under receiver operating characteristics; FIB-4, fibrosis-4; ROC, receiver operating characteristics.

DISCUSSION

Hepatic TE is one of the most useful imaging modalities for liver fibrosis assessment. Though this technology was first validated in patients with chronic viral hepatitis C infection, it has not been validated for use in the thalassaemia population.[21] Nevertheless, TE has been recommended as a reliable tool in assessing liver fibrosis in patients with TDT.[22–25] While the FIB-4 score was initially developed and validated in patients with HIV/HCV coinfection to predict liver fibrosis, this score has previously been tested only on a few occasions in patients with TDT.[15–17] Yet as per the WHO recommendation, the FIB-4 score can be applied to assess hepatic fibrosis in resource-poor settings when TE is not feasible.[26]

Of the 15 patients who had undergone splenectomy in our study cohort, 7 (47%) patients had thrombocytosis following post-splenectomy. Postoperative thrombocytosis is a known complication in beta thalassaemia major patients following splenectomy. Approximately 75% of splenectomised patients develop thrombocytosis, even reaching values as high as 1 000 000 cells/mm^3 is known.[22 27] In our study cohort, there is no statistically significant difference between the mean platelet count of the splenectomised and un-splenectomised patient groups (p=0.11). Therefore, in the FIB-4 calculator, though the platelet count is a crucial component, changes would unlikely affect this study cohort's final result.

With the ROC curve analysis, we propose a better cut-off value of 0.32 with 72% sensitivity and 56% specificity (table 3; figure 1) for the FIB-4 score in patients with TDT. Yet the area under receiver operating characteristics (AUROC) of 66% does not satisfactorily predict F2 fibrosis in this patient cohort. The findings of this study are consistent with the study done by Hamidieh *et al* on 83 paediatric patients with TDT who have been selected for haematopoietic stem cell transplantation. The study has concluded that TE was superior to the FIB-4 score for evaluating liver fibrosis in this paediatric TDT population. The best cut-off value for the FIB-4 score for assessing liver fibrosis is 0.699 (AUROC of 61%) with a 93.5% sensitivity and 45% specificity.[17] In addition, a study done on 76 HCV-infected patients with beta thalassaemia, Poustchi *et al*[16] disclosed a FIB-4 score cut-off value of 0.25 with an AUROC of 51% to detect significant liver fibrosis.[16] However, authors have concluded that TE was superior to FIB4-score in liver fibrosis prediction. Combining TE with the FIB-4 score improves the performance of liver fibrosis prediction in chronic hepatitis patients with beta-thalassaemia.

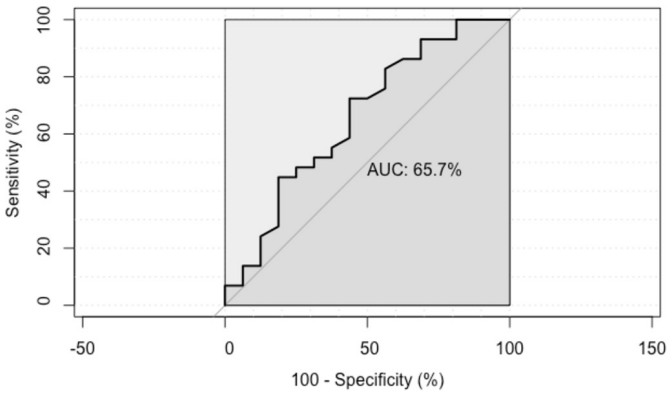

Figure 1 ROC curve analysis of the total study participants (AUC=66% with the best new cut-off for FIB-4 score=0.32). AUC, area under the curve; FIB-4, fibrosis-4; ROC, receiver operating characteristics.

The data from this study suggest that when using TE as the standard (in the absence of liver biopsy) and using the FIB-4 score cut-off value of 1.3, the FIB-4 score is improbable to lead to a false classification of thalassaemia patients to have significant fibrosis (F2). On the other hand, it had a very low diagnostic yield in missing almost all (except one) of those who had significant fibrosis. Using a much-lowered cut-off point for the FIB-4 score at 0.32, we were able to improve the pick-up rate of F2 fibrosis using the FIB-4 score to 72%. Yet the new cut-off point misclassified seven patients as not having significant liver fibrosis, hence unable to exclude patients with F2 100%.

Regardless of the FIB-4 cut-off point, it is clear that in clinical practice FIB-4 score cannot be used as a good screening tool to pick up significant liver fibrosis in patients with TDT, irrespective of their splenectomy status. On the contrary, at a 1.3 cut-off value, though FIB-4 is a very poor detector of F2 fibrosis, it will not erroneously diagnose F2 fibrosis in those who do not have it.

We acknowledge that there are several limitations in our study. The small sample size of our study is one of the significant limitations. A similar study with a larger sample size would be required to justify the findings of this study. We acknowledge that clinicians should be cautious in using this formula in the liver fibrosis assessment in individuals with markedly elevated platelet counts, especially in splenectomised patients. Even though liver biopsy is the gold standard for assessing liver fibrosis, our study used TE as the reference standard. There is

limited information on the effects of splenomegaly on the measurements of TE in patients with thalassaemia. Hence part of the variability of our study findings would have been accounted for these. Yet we believe that TE has been validated in various other clinical conditions. It has shown a perfect correlation with the histological gradings of liver fibrosis.[28 29]

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Contributors AP contributed to design and perform the research, data analysis, interpretation and draft the paper and critically revised the paper. PP was involving in designing and performing the research, data analysing and drafting the paper. MAN was contributing to design the research and critically revised the paper. DSE was helping in data analysing and interpretation and drafting the paper. ADS critically revised the paper. PP is responsible for the overall content as the guarantor. All the authors approved the final version of the article.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by Name of the Ethics Committee-Ethics Review Committee (ERC) of the Faculty of Medicine, University of Kelaniya, Sri Lanka,reference number or ID - P/89/02/2017. Participants gave informed consent to participate in the study before taking part.

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Data availability statement Data are available on reasonable request.

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