



Utility and accuracy of transient elastography in determining liver fibrosis: a case-control study

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Received: 22 August 2019 / Revised: 19 December 2019 / Accepted: 23 December 2019 / Published online: 21 January 2020
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Abstract

The objectives of this prospective case-control study were to determine liver stiffness (LSM) by transient elastography (TE) in children with newly diagnosed chronic liver disease (CLD) and to find out normal values in healthy Indian children. Two groups (A: 50 CLD who underwent liver biopsy and B: 50 healthy) aged 5–18 years were recruited prospectively. Liver biopsies were scored as per Metavir scoring and compared with TE. The median age of 100 recruited children was 13.6 years. In group B, normal LSM was 4.9 (2.5–7.3) kPa with significantly higher LSM in adolescent males (5.6 (4.1–7.3) kPa) as compared with females (4.3 (3.7–4.9) kPa), $p = 0.001$. In group A, TE was excellent in discriminating significant fibrosis ($\geq F2$) ($P = 0.001$) at a cut-off value of 10.6 kPa with area under receiver operating characteristic curve of 0.96. Metavir fibrosis stage ($\beta = 0.611$; $R2 = 0.586$) and age ($\beta = 0.230$; $R2 = 0.586$) were independent variables associated with higher LSM in stepwise multiple logistic regression analysis.

Conclusions: TE is an excellent non-invasive tool to assess significant liver fibrosis and can be used as an alternative to liver biopsy. Normative value of TE in adolescent males is higher than in females.

What is Known:

- Transient elastography is a good non-invasive test for liver fibrosis assessment.
- Normal liver stiffness depends on race, gender, and age.

What is New:

- This is the first study from India to show the normative data of transient elastography in healthy Indian children.
- We have documented that liver stiffness measurement by fibroscan in treatment naïve chronic liver disease has excellent correlation in significant fibrosis, severe fibrosis, and cirrhosis.

Keywords Transient elastography · Chronic liver disease · Liver fibrosis · Children

Communicated by Peter de Winter

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Abbreviations

AUROC	Area under the receiver operating characteristic
ANA	Anti-nuclear antibody
Anti HBe	Antibody to hepatitis B e-antigen
BMI	Body mass index
CLD	Chronic liver disease
CTP	Child–Turcotte–Pugh
EGD	Esophagogastroduodenoscopy
HBsAg	Hepatitis B surface antigen
HBeAg	Hepatitis B e-antigen
HBV-DNA	Hepatitis B deoxyribonucleic acid
HCV RNA	Hepatitis C ribonucleic acid
IQR	Interquartile range
LSM	Liver stiffness measurement
LKM	Liver kidney microsomal antibody
MELD	Model for end-stage liver disease
NAFLD	Non-alcoholic fatty liver disease
PELD	Pediatric end-stage liver disease
ROC	Receiver operating characteristic
SMA	Anti-smooth muscle antibody
TE	Transient elastography

Introduction

Liver fibrosis is the result of dynamic reaction of a healthy liver towards chronic cell injury [1]. It is observed in a large proportion of children with chronic liver disease (CLD) regardless of its etiology [2]. Early treatment of the underlying disease may limit or even reverse the progression of fibrosis; hence, early detection of fibrosis is crucial for timely treatment [3]. Currently, liver biopsy is the accepted gold standard for evaluating the fibrosis in liver disease; however, it is limited by its invasive nature with inherent risk of serious complications and sampling error and often it is not possible to get a liver biopsy due to coagulopathy and/or thrombocytopenia [4, 5]. Moreover, due to its invasive nature, repeated liver biopsies to assess the progression of liver disease are practically impossible. Transient elastography (TE) is a non-invasive, rapid, painless, and reproducible tool for assessment of liver fibrosis. It has the potential to reduce or replace the need of liver biopsy for assessment of the progression of liver disease in children. The utility of TE has been documented in several studies in adults [6–10]. A number of studies in children [11–19] have addressed the feasibility of TE in healthy children and its utility in chronic liver disease; however, there is scarcity of studies in children from developing countries. The aim of this study was to evaluate the utility and accuracy of liver stiffness measurement (LSM) by TE in children with newly diagnosed CLD and also to determine normative values of LSM in healthy Indian children.

Materials and methods

This case-control prospective study was conducted between May 2014 and May 2016 in the Department of Pediatric Gastroenterology of Sanjay Gandhi Post Graduate Institute Medical Sciences, Lucknow, India. Two groups (group A and group B) of children aged 5 to 18 years were enrolled after obtaining informed written consent from either parent. Group A was comprised of consecutive children with CLD who underwent liver biopsy during the study period. CLD was diagnosed on the basis of history, physical examination, biochemical parameters, Doppler ultrasonography findings such as nodular liver with portosystemic collaterals, dilated portal vein, and/or esophagogastroduodenoscopy (EGD) showing esophageal varices (grade II or more). Group B was comprised of healthy age- and sex-matched children (healthy siblings of either admitted CLD cases or children of hospital employees). At the time of recruitment, data related to detailed clinical history and physical examination findings including anthropometry of all children were recorded. In group B, siblings of admitted CLD cases were subjected to liver function tests, prothrombin time, complete blood counts, HBsAg, Kayser Fleischer ring, serum ceruloplasmin, 24-h urine copper, etc. depending upon the diagnosis of admitted CLD proband. However, due to ethical issues, healthy children of hospital employees did not go for any blood investigations. Children in group A underwent liver function tests, prothrombin time, hemogram, abdominal Doppler ultrasonography, EGD, and if required, ANA/LKM/SMA antibody, serum IgG level, HBsAg (if necessary HBV DNA, HBe, anti HBe), anti HCV (if necessary genotyping, quantitative HCV RNA), Kayser Fleischer ring, serum ceruloplasmin, 24-h urine copper, and percutaneous liver biopsy. Transjugular liver biopsy was done in children with coagulopathy and/or thrombocytopenia. The etiology of CLD was determined as per standard criteria [20–24]. The severity of liver disease was assessed by the Child–Turcotte–Pugh (CTP) score for all ages, model for end-stage liver disease (MELD) in children ≥ 12 years of age, and pediatric end-stage liver disease (PELD) in children of less than 12 years of age. In group A, children with more than minimal ascites (uncontrolled on salt restriction, diuretics, and paracentesis) and obesity (BMI z score > 2) were excluded. In group B, children with growth failure (weight and height z score < -2), obesity (BMI z score > 2), pallor (which indicates malnutrition and/or underlying chronic disease), any suspicious of systemic illness, or liver disease (on the basis of detailed history and clinical examination) were excluded.

Transient elastography

TE was performed by a single experienced technician who was blinded of histopathological findings. It was done in

children of both groups after 4 h of fasting. In group A, TE was performed without any sedative medication after adequate control of ascites by salt restriction, diuretic, or paracentesis whenever needed. However, mild ascites in dependent position was not a contraindication as it did not interfere with TE [25]. TE was performed by using fibroscan touch 502 France version, in dorsal decubitus position with right arm in maximal abduction using the right lobe of the liver through the intercostal space at the site of biopsy. S2 probe was used for chest circumference 45–75 cm and M probe for chest circumference above 75 cm. Ten-millimeter diameter core of tissue with a depth between 20 and 40 mm from skin surface was measured. Ten shots within 3–5 min were performed (at least 60% valid shots). The median liver stiffness measured by shots represents LSM. Ratio of interquartile range (range in which 50% of all shots, IQR) to LSM < 0.3 was taken as valid results.

Liver Biopsy

Liver biopsy was performed in all group A children either through percutaneous or transjugular route after sedation and local anesthesia. The liver biopsy specimen was fixed in formalin followed by paraffin embedding and sectioning with a microtome to 4 mm sections. Histopathology slides were stained with hematoxylin and eosin for routine diagnosis; however, for grading of fibrosis, reticulin/Masson's trichrome stain was used. The fibrosis in liver histology was graded as per METAVIR scoring system and by a single histopathologist who was blinded to TE results [26]. Different stages of fibrosis as per METAVIR scoring system were F0 = no fibrosis, F1 = portal fibrosis without septa, F2 = portal fibrosis and few septa, F3 = numerous septa without cirrhosis, and F4 = cirrhosis. As per METAVIR scoring system, the different grades of inflammation were A0 = no activity, A1 = mild activity, A2 = moderate activity, and A3 = severe activity.

Statistical analysis

Data analysis was carried out by using SPSS software (version 15.0; SPSS Inc., Chicago, Illinois, USA). Continuous data was expressed as median and categorical data as number (percentage). Mann Whitney *U* test was used for comparison of continuous variables and Fisher's exact test for categorical variables. Correlation was tested by Spearman test. A stepwise diagnostic performance of non-invasive methods was assessed by the area under the receiver operating characteristic (AUROC) curve analysis. The receiver operating characteristic (ROC) curve was used to determine the optimal cut-off values (optimal sensitivity and specificity simultaneously) for the presence of any/significant/severe fibrosis and cirrhosis. A *p* value of < 0.05 was taken as significant.

Results

A total of 100 children were recruited during the study period; 50 children each in group A (CLD) and group B (healthy controls). Clinical details of both groups are given in Table 1. Children in group A and group B were age and sex matched. Though the liver was palpable equally in both the groups, the size (palpable below costal margin) was significantly more in group A than that in group B. As expected, none of the children in group B had pallor, jaundice, ascites, and splenomegaly. Median LSM was significantly higher in group A than in group B.

Chronic liver disease children (group A, *n* = 50)

Transient elastography was done in all 50 children (31 boys) with CLD. The median duration of symptoms at diagnosis was 15.5 (range, 1–192) months. Etiology of chronic liver disease includes autoimmune hepatitis (AIH) (*n* = 18), immune tolerant phase of chronic hepatitis B = 15; chronic hepatitis C = 2; Wilson's disease = 4, NAFLD = 3, and cryptogenic liver disease = 8. Baseline characteristics and LSM values of all children are shown in Table 1.

Liver histology

Liver biopsy was performed percutaneously in 38 children and via transjugular route in 12 children and none had any complications. In the histopathology, fibrosis was absent (F0) in 6 (12%), some degree of fibrosis (\geq F1) was evident in 44 (88%) (F1 = 13, F2 = 10, F3 = 15, F4 = 6). Grades of necroinflammation (activity) were A0 in 9, A1 in 11, A2 in 19, and A3 in 11.

Transient elastography in group A

TE was performed successfully in all children; time taken was less than 5 min and without any complications. The comparisons between histological grading and TE results showed that TE is a moderate discriminator for any fibrosis (\geq F1) from no fibrosis (F0) with AUROC for TE was 0.76 (*p* = 0.04) and a cut-off value of 7.6 kPa (sensitivity 77.3%, specificity 66.7%) but good discriminator for significant fibrosis (\geq F2) from minimal/no fibrosis (< F2) with AUROC of 0.96 (*p* = 0.001) and a cut-off value of 10.6 kPa (sensitivity 90.3%, specificity 89.5%); for severe fibrosis (\geq F3), AUROC was 0.96 (*p* = 0.001) with a cut-off was 15.05 kPa (sensitivity 95.3%, specificity 89.7%) and lastly, for cirrhosis (F4) from no cirrhosis (< F4), AUROC was 0.88 (*p* = 0.001) with a cut-off was 21.8 kPa (sensitivity 83.3%, specificity 77.3%) (Fig. 1a–d). TE was not correlated with necroinflammatory activity in liver biopsies according to METAVIR scoring system (*r* = 0.167, *p* = 0.246). Stepwise multiple logistic regression analysis

Table 1 Comparisons between cases and controls

Clinical features	Cases (<i>n</i> = 50)	Controls (<i>n</i> = 50)	<i>p</i> value
Median age (years)	12 (5–17.3)	12 (5.66 to 17)	0.65
Male:female	31:19	31:19	1.00
Jaundice (number)	19	0	0.0001
Edema (number)	8	0	0.006
Pallor (number) [#]	13	0	0.0001
Median BMI (kg/m ²)	15.28 (12–27.68)	16.6 (12–21.6)	0.235
Median BMI <i>z</i> score	− 1.17 (− 4.58 to 1.96)	− 0.29 (− 1.47 to 1.9)	0.03
Median height <i>z</i> score	− 1.24 (− 4.45 to 1.54)	− 0.93 (− 1.98 to 1.20)	0.15
Palpable liver (number)	40	42	0.79
Liver: size below costal margin (cm)	2 (1–5)	1 (0.5 to 2)	0.04
Palpable spleen (number)	28	0	0.0001
Spleen: median size below costal margin (cm)	2 (1–16)	-	
Median liver stiffness (kPa)	13.80 (4–71)	5.00 (2.8–8.0)	0.0001

Italic values are significant

[#] Paleness of either conjunctivae, tongue, palms, or nailbed [27].

showed METAVIR fibrosis stage ($\beta = 0.611$; $R^2 = 0.586$) and age ($\beta = 0.230$; $R^2 = 0.586$) were the two independent variables associated with higher LSM.

Healthy children (group B, *n* = 50)

Out of 50 children, 26 were healthy siblings of CLD patients and the remaining 24 were healthy children of hospital employees. The success rate of TE in healthy children was 100%. The median LSM was 4.9 (range, 2.5–7.3) kPa with 5th centile was 3.1 kPa and 95th percentile is 7.2 kPa. Median LSM in boys were significantly higher ($n = 31$; 5.2 (range, 2.8–7.3) kPa) as compared with girls ($n = 19$; 4.3 (range, 2.5–7.1) kPa), $p = 0.02$. Children were divided into two age groups; young children ($n = 26$; between 5 and 11 years) and adolescents ($n = 24$; between 12 and 18 years). There was no significant difference in LSM values between two age groups (4.4 (range, 2.5–7.3) vs. 5.1 (range, 3.7–7.3) kPa, $p = 0.111$, respectively). In adolescents (12–18 years) group, median LSM value in boys was significantly high ($n = 17$; 5.6 (range, 4.1–7.3) kPa) as compared with girls ($n = 7$; 4.3 (range, 3.7–4.9) kPa), $p = 0.001$ (Table 1). The 5th and 95th percentile of LSM (kPa) in both age groups are shown in Table 2.

Discussion

This is for the first time we have generated the normative data of LSM in Indian children between 5 and 18 years of age. In our study, LSM upper limit of normal (95th percentile) across all age groups of healthy children was 7.2 kPa which is higher than the value obtained by Engelman et al. from Germany, in a study of 240 healthy children (3.5 to 4.5 kPa in 0 to 18 years

age groups) [16]. Difference in upper limit of normal of LSM and age-dependent increase in LSM might be explained by genetic makeup of regional population and environmental factors such as toxic insults which increases with age and it controls the accumulation of extracellular matrix in the liver. We also demonstrated significantly higher median LSM in boys compared with girls in the age group of 12 to 18 years and this finding was similar to that of Engelman et al.'s study [16]. However, a study by Goldschmidt et al. from Germany [17] in 400 healthy children showed no difference in LSM value between adolescent boys and girls. Higher LSM in adolescent boys was supported by animal study showing inhibitory effect of ovarian hormones on extracellular matrix produced by liver stellate cells [28]. The success rate of LSM by TE depends on the age of the patient as younger age poses some difficulties. The reason for 100% success rate of TE in our study is mainly due to the fact that we have recruited children of 5 years or more. The failure rate between 4 and 27% has been documented in various studies [16, 17], when younger children were included. The strength of our study is that we enrolled a homogenous group of healthy controls after excluding the main confounding factors such as food, sedation, obesity, and systemic illness that could have been affected the LSM values [17, 18, 29, 30]. For the first time, we have generated normative TE value for healthy Indian children albeit in a smaller sample. We need further studies with larger number from different parts of the country to substantiate our observation. As the cut-offs derived from this study are based on our population, and no external validation was done, there is a possibility of overestimation of accuracy of the transient elastography. In group A (children with CLD), we have documented the cut-off value for LSM (kPa) by AUROC for significant fibrosis (10.6 kPa) and severe fibrosis (15.05 kPa) with good

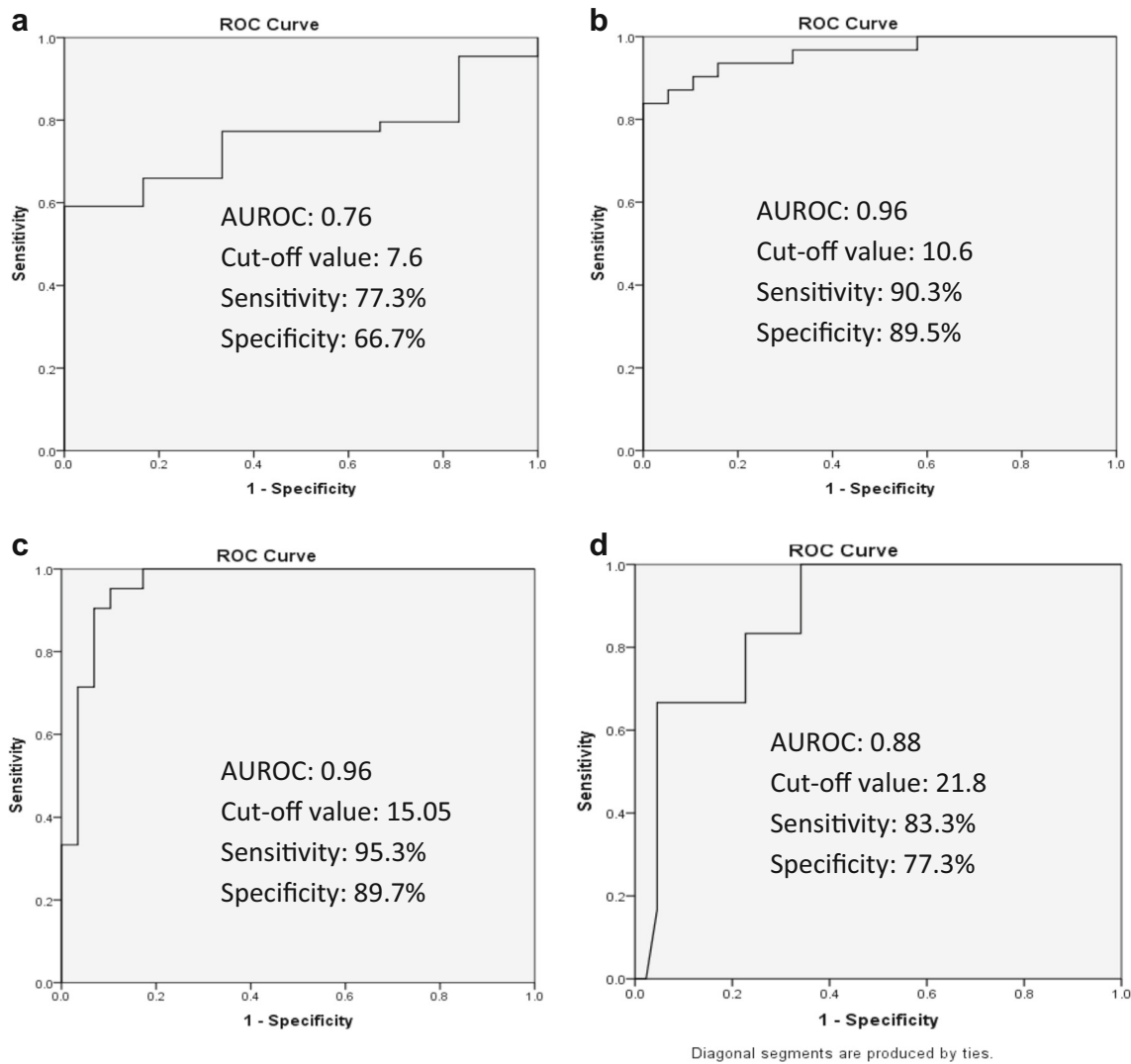


Fig. 1 Receiver operating characteristic (ROC) curve showing the prediction of **a** “any” fibrosis ($\geq F1$), **b** significant fibrosis ($\geq F2$), **c** advanced fibrosis ($\geq F3$), and **d** cirrhosis with TE in group A. The ideal area under the curve is 1.00. TE, transient elastography; AUROC, areas under the ROC

sensitivity and specificity (close to 90%). This result is similar to the study by de Ledinghen et al. [11] who showed the cut-off value of 10.2 kPa for significant fibrosis. On the contrary,

Fitzpatrick et al. [14] showed cut-off value of 6.9 kPa and 7.5 kPa for significant and severe fibrosis respectively in CLD children and in a study of children with NAFLD;

Table 2 Group B ($n = 50$): liver stiffness measurement in kPa (LSM) in healthy children

Age (year)	Number	Sex	Liver stiffness measurement (kPa)				<i>p</i> value	Probe (S2/M)	IQR (kPa)
			Median	SD	5th	95th			
> 5–11	14	M	4.6	1.28	2.6	7.2	0.918	14/0	0.35
	12	F	4.3	1.33				11/1	0.35
> 11–18	17	M	5.6	0.88	3.7	7.2	0.001	6/11	0.40
	7	F	4.3	0.41				7/0	0.30
> 5–18	31	M	5.2	1.14	3	7.2	0.02	20/11	0.40
	19	F	4.3	1.08				18/1	0.30

Nobili et al. [12] showed LSM value of 7.4 kPa and 10.2 kPa for significant and severe fibrosis, respectively. These differences in cut-off value for significant and severe fibrosis could be due to difference in etiology and severity of the liver disease. We have documented a positive correlation between TE and liver fibrosis assessed by histopathology. However, we could not document significant correlation between TE with histologically documented necroinflammation. This may be due to smaller sample size. Raizner et al. showed children with no/minimal hepatic fibrosis but having significant hepatic inflammation as depicted by high ALT values were associated with high LSM values that typically seen in advanced fibrosis but interpretation of serial LSM with serial liver function test in the absence of repeated liver biopsies might be misleading for the assessment of inflammatory effect on TE [15]. There is an unmet need of a larger study with patients having only inflammation and no fibrosis to document the effect of inflammation on LSM. Often it is not feasible to do repeated liver biopsies after first diagnostic biopsy in CLD patients to document progression. In this context, TE is a valuable tool in long-term follow-up and monitoring of CLD children as it detects the fibrosis progression or regression non-invasively. Patients with non-cirrhotic chronic hepatitis B would be benefited from non-invasive TE monitoring by early detection of significant fibrosis in follow-up especially in patients with normal transaminases or low viral load so that timely treatment can be started. Another important role of TE in monitoring of AIH children as they require longer duration of immunosuppression and early detection of progression or regression of fibrosis may be another parameter which might help in modification of immunosuppressive therapy. Though in this study we have done one-time TE, we have a plan to do serial TE especially in AIH group (the largest subset of CLD cases in our study) to document its utility in assessment of progression or regression of the disease. Further prospective studies are required for serial TE in children with chronic liver disease to assess the effect of treatment on TE. The limitations of our study are, firstly, we could not calculate the LSM cut-off for each disease because of small sample size in each etiology. Secondly, we could not completely negate the effect of inflammation in LSM estimation by TE despite the absence of correlation between necroinflammation and LSM, mainly due to small sample size. Third, we could not do serial TE to assess the progression of fibrosis in liver disease patients; lastly, we could not completely rule out the presence of liver disease in the subset of group B children in whom no laboratory tests were done due to ethical issues and the number of children in age- and gender-specific subgroups was small. In conclusion, the normative data of TE generated in this study for healthy Indian children (with 95th centile) can be used to differentiate various stages of fibrosis in chronic liver disease. It has got good sensitivity and specificity except when any fibrosis (\geq F1) is differentiated from no fibrosis (F0) where TE should be

used cautiously because of its average sensitivity and specificity.

Authors' contributions All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Vikas Jain, Ujjal Poddar, Negi TS, Vivek A Saraswat, Narendra Krishnani, Surender K Yachha, and Anshu Srivastav. The first draft of the manuscript was written by Vikas Jain and Ujjal Poddar. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors. The study was conducted after obtaining clearance from the Institutional Ethics Committee (IEC code: 2014-83-DM-77). Informed written consent was obtained from either parent before doing all invasive procedures and TE. Informed consent: Informed written consent was obtained from either parent before doing all invasive procedures and transient elastography.

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