

Original Article

Liver echogenicity by ultrasound to predict liver fibrosis of chronic hepatitis B patients without clear treatment indications

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Abstract: Objective: To evaluate the value of liver echogenicity in predicting liver fibrosis in chronic hepatitis B patients who have no clear indication for antiviral treatment. Methods: One hundred and sixty five chronic hepatitis B (CHB) patients who had no clear indication for antiviral treatment, and who underwent liver biopsies were included in this study. Ultrasound parameters and 11 serum makers were assessed retrospectively. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were assessed. Results: Among the ultrasound parameters and serum makers, only liver echogenicity showed a significant difference ($P < 0.009$) between the mild (Metavir fibrosis stage $F < 2$) and severe groups (Metavir fibrosis stage $F \geq 2$). Increased liver echogenicity ($= 2$) had a sensitivity of 18.3%, a specificity of 94.6%, and a PPV of 72.2%. Coarse liver echogenicity had a sensitivity of 67.6%, a specificity of 47.87% and a NPV of 66.2%. Conclusion: In this study ultrasound was not found to be reliable in evaluating liver fibrosis. Patients with normal ultrasound result need advanced investigations such as MRI, FibroScan and liver biopsy. Increased liver echogenicity may be a potential treatment indication for CHB patients.

Keywords: Chronic hepatitis B, liver fibrosis, liver biopsy, ultrasonography, liver echogenicity

Introduction

There are approximately 240 million chronic Hepatitis B (HBV) carriers worldwide [1], and up to 40% of these are known to develop complications such as hepatic decompensation, cirrhosis or hepatocellular carcinoma (HCC) [2]. A growing body of evidence suggests benefit of long-term antiviral therapy in preventing progression of chronic hepatitis B (CHB) to cirrhosis, HCC and end-stage liver disease, and possibly in ameliorating fibrosis and cirrhotic changes [3]. Several guidelines [4-6] recommend that patients with serum HBV DNA levels $> 20,000$ IU/mL and/or alanine aminotransferase (ALT) level $>$ two-fold of the upper limit of normal (ULN) should receive antiviral treatment. However, increasing evidences also show that patients who are not treatment candidates may still progress to cirrhosis and other end-

stage clinical complications [7-9]. For these patients, all guidelines recommend assessment of liver fibrosis by biopsy. Treatment is indicated in patients with moderate to severe inflammation or fibrosis.

To date, liver biopsy remains the gold standard for staging of liver fibrosis. However, patients are often reluctant to undergo liver biopsy owing to the invasive nature of the procedure and its attendant risks. Therefore, it is important to develop non-invasive strategies for the determination of the stage of liver fibrosis that can be used as a reference in clinical practice.

In recent years, several non-invasive methods have been developed for the prediction of liver fibrosis. These include, FibroTest [10], AST-to-platelet ratio index (APRI) [11], Lok index [12], Forns' index [13], FIB-4 [14] and Zeng score

[15]. However, a non-invasive model for assessment of liver fibrosis in patients who have no clear indication for treatment has not been established. Transient elastography, a new imaging technique, is shown to evaluate the degree of liver fibrosis with high accuracy, and this technology has recently been approved in the US. However, it has limited application for the following reasons: first, it is not as widely applicable as the serum markers (limited by presence of ascites, obesity and limited operator experience); secondly, it may give false positive results in case of acute hepatitis, extra-hepatic cholestasis and liver congestion; thirdly, it is unable to discriminate between intermediate stages of fibrosis. Lastly, it requires a special, dedicated device; and does not allow for the choice of a specific region of interest for assessment of fibrosis [16].

Ultrasound is widely used imaging modality in clinical practice. It can provide useful information about the liver and extra-hepatic changes. Moreover, ultrasound is a non-invasive, inexpensive, safe, and examination may be repeated multiple times. The aim of this study is to apply ultrasound imaging and routine serum markers for staging of liver fibrosis in patients who need biopsy, but in whom there is no clear indication for treatment.

Materials and methods

Patients

One hundred and sixty five CHB patients who had undergone percutaneous liver biopsy, abdominal ultrasound and blood investigations were retrospectively enrolled in the third affiliated Hospital of Wenzhou Medical University, from June 2009 to January 2013. All patients were positive for hepatitis B surface antigen (HBsAg) for at least six months and had HBV DNA levels > 500 IU/mL. Patients with the following characteristics were included: Age > 40 years, serum ALT levels normal or raised more than twice the ULN (normal value < 40 IU/L), abnormal ultrasound features such as increased liver echogenicity, irregular liver surface, widened portal vein and enlarged spleen. Exclusion criteria were: co-infection with Human immunodeficiency virus (HIV) or Hepatitis C virus (HCV), history of alcoholism, non-alcoholic fatty liver diseases (NAFLD), autoimmune liver disease, and other causes of chronic liver dis-

ease, renal insufficiency, insufficient biopsy sample and incomplete clinical data. None of the patients had received antiviral therapy prior to the liver biopsy.

Liver biopsy

Liver biopsies were performed by experienced physicians at the Third Affiliated Hospital of Wenzhou Medical University. Each patient had undergone ultrasonography-guided liver biopsy with a 16 G×15 cm biopsy needle. The biopsy specimens were rapidly fixed with formalin, routinely embedded in paraffin, and stained with hematoxylin and eosin, Masson's trichrome, and reticular fiber staining. Optimum tissue requirement for diagnosis included > 1 cm length, and presence of at least 6 portal tracts. The staging of fibrosis was performed using the Metavir system [17]. Patients without fibrosis were classified as F0; F1 indicates mild fibrosis without septa; F2 indicates moderate fibrosis with a few septa; F3 indicates severe fibrosis with numerous septa without cirrhosis; and F4 indicates hepatic cirrhosis.

Abdominal ultrasonography

Ultrasound examination was performed by a single experienced radiologist using Acuson Sequoia 512 by Siemens, and a curvilinear array probe, 4C1-S (output display at 3.5-5 MHz). Morphological parameters assessed included liver surface, echogenicity, spleen thickness and portal vein diameter. Liver surface was graded using three possible scores: smooth (0), irregular (1), nodular (2); Liver echogenicity was classified as normal (0), coarse (1), and increased (2) [18]. Another two quantitative indicators spleen thickness and portal vein diameter (both in mm) were also documented.

Laboratory test

Serum HBV DNA levels were measured with a fluorescence quantitative polymerase chain reaction (PCR) assay (PG Company, Shenzhen, China). Serum parameters included total bilirubin (TB), serum ALT (reference range: 0-40 IU/L), aspartate aminotransferase (AST), gamma glutamyl transpeptidase (GGT), total cholesterol (TC), pre-albumin (Pre-ALB), albumin (ALB), blood platelets (PLT), triglyceride (TG) and total cholesterol (TC). Blood investigations were performed using an automatic biochemistry ana-

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Table 1. Characteristics of 94 patients in the mild group and 71 patients in the sever group

Characteristics	All patients (n = 165)	Mild group (n = 94)	Sever group (n = 71)	P
Age (yr)	39.30±9.375	40.15±9.314	37.96±9.168	0.183
Male, n (%)	99 (60%)	53 (56.4%)	46 (64.8%)	0.277
ALT (U/L)	36.71±17.168	37.03±16.845	36.28±17.698	0.782
AST (U/L)	31.40±12.055	32.23±13.900	30.24±9.040	0.294
GGT (U/L)	29.53±17.319	31.11±18.536	27.17±15.421	0.149
ALP (U/L)	79.92±26.208	80.20±23.919	79.07±28.857	0.784
TBil (mmol/L)	13.081±5.8123	13.257±5.6629	12.849±6.0374	0.657
Pre-ALB (U/L)	0.1994±0.05811	0.2009±0.06076	0.1970±0.05415	0.677
ALB (g/L)	41.896±4.3640	41.805±3.6198	41.923±5.1826	0.864
HBV DNA (log10 IU/mL)	5.5988±1.90619	5.6732±1.85946	5.4891±1.99215	0.542
PLT (10 ⁹ /mL)	176.67±45.912	172.55±44.669	182.39±46.993	0.173
TG (U/L)	1.339±0.7059	1.304±0.6719	1.383±0.7523	0.483
TC (U/L)	4.6222±0.95606	4.6033±0.83851	4.6586±1.09170	0.717
spleen thickness (mm)	36.38±2.493	36.21±2.130	36.61±2.906	0.318
portal vein diameter (mm)	11.15±1.228	11.15±1.261	11.15±1.191	0.975
Liver surface				0.786
= 0, n (%)	150 (90.9%)	64 (90.1%)	86 (91.5%)	
= 1, n (%)	1 (0.6%)	1 (1.4%)	0 (0%)	
= 2, n (%)	14 (8.5%)	6 (8.5%)	8 (8.5%)	
Liver echogenecity				0.009
= 0, n (%)	68 (41.2%)	23 (32.4%)	45 (47.9%)	
= 1, n (%)	79 (47.9%)	35 (49.3%)	44 (46.8%)	
= 2, n (%)	18 (10.9%)	13 (18.3%)	5 (5.3%)	
Fibrosis stage, n (%)				
F0	37 (22.4%)			
F1	57 (34.5%)			
F2	49 (29.7%)			
F3	17 (10%)			
F4	5 (3%)			

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: γ -glutamyl transpeptidase; ALP: Alkaline phosphatase; TB: Total bilirubin; Pre-ALB: Prealbumin; ALB: Albumin; PLT: Blood platelet; TG: Triglyceride; TC: Total cholesterol.

lyzer. Biochemical and virological parameters were assessed in serum samples collected within one week prior to liver biopsy.

Statistic methods

Quantitative variables are expressed as mean values. Categorical variables are expressed as percentages. SPSS 19.0 software (SPSS Inc, Chicago, IL) was used for statistical analyses. Categorical data were compared by Mann-Whitney U test. Inter-group differences in mean values were assessed using Student's t-test. $P < 0.05$ was considered indicative of a statistically significant difference. Diagnostic accuracy was assessed by calculating sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV).

Result

One hundred and sixty five patients were enrolled in the study (99 men and 66 women; mean age, 39.3 years $\pm$ 9.3 years). According to the histological severity of liver diseases, the enrolled patients were divided into two groups: mild group ($F < 2$; $N = 94$) and severe group ($F \geq 2$; $N = 71$). Baseline characteristics of patients are presented in **Table 1**. According to the practice guidelines [3-5], the severe group should be considered for antiviral treatment.

On univariate analysis, no significant inter-group differences were observed with respect to the following parameters: age, ALT, AST, GGT, TBIL, ALB, Pre-ALB, TCHO, PLT Log10 [HBV DNA], TG and TC (**Table 1**, $P > 0.05$). Among the

Table 2. Diagnostic accuracy of increased liver echogenicity and coarse liver echogenicity

Liver echogenicity	Sensitivity	Specificity	PPV	NPV
Increased (= 2)	18.3%	94.6%	72.2%	60.5%
Coarse (= 1)	67.6%	47.9%	49.5%	66.2%

PPV: Positive predictive value; NPV: Negative predictive value.

ultrasound parameters, a significant inter-group difference was observed only with respect to liver echogenicity ($P = 0.009$).

Use of increased liver echogenicity (increased = 2) as a predictor for moderate to severe fibrosis was associated with a sensitivity of 18.3%, specificity of 94.6%, and a PPV of 72.2%. Thirteen out of 18 patients with increased liver echogenicity were in the severe group on the basis of liver biopsy. Using coarse liver echogenicity (coarse = 1) as a predictor for moderate to severe fibrosis, 48 out of the 71 patients in the severe group were found to have been correctly identified, with an associated sensitivity of 67.6%, and a NPV of 66.2%. Twenty three out of 68 patients with coarse liver echogenicity belonged to the severe group. The diagnostic accuracy of increased liver echogenicity and coarse liver echogenicity was showed in **Table 2**.

Discussion

Patients with chronic Hepatitis B without out-right indication for treatment are also at risk of developing hepatic decompensation, cirrhosis and hepatocellular carcinoma (HCC). In these patients, assessment of liver fibrosis is needed for treatment decision. Liver biopsy, the gold standard for the assessment of fibrosis is associated with several disadvantages. Non-invasive predictors of fibrosis are urgently needed. In this study, we assessed the use of ultrasound and routine serum marker levels for predicting liver fibrosis in patients who are not considered to be candidates for antiviral treatment according to the current guidelines.

In the present study, the 11 serum markers investigated were not found to be good predictors for liver fibrosis in these patients. These markers included ALT, AST and platelet counts, which are the most wide used parameters in non-invasive models for staging of liver fibrosis [11, 19, 20]. These differences may have contributed to the patients that have no clinical symptoms and unclear treatment candidates.

However, we tested the serum maker levels only on one occasion. Repeat tests may have shown significant inter-group differences.

Abdominal ultrasonography plays an important role in the diagnosis, management and follow-up of chronic hepatitis, cirrhosis and hepatocellular carcinoma. Several studies have investigated the role of ultrasound in the diagnosis of compensated cirrhosis. A study by Gaiani et al. in 1991 [21] showed that ultrasonography can provide a non-invasive prediction of liver histology in moderate and severe steatosis and advanced fibrosis, with a high sensitivity and specificity. They performed ultrasound examination on 212 patients with chronic liver disease and formulated a score based on liver surface nodularity and portal flow velocity. This model correctly identified cirrhosis with a high sensitivity (82.2%) and high specificity (79.9%). Enlargement of the caudate lobe, spleen size and echogenicity pattern had a high specificity, but low sensitivity. Zheng et al. [22] reported hepatic parenchymal echo pattern, liver surface and thickness of gallbladder wall as being three independent predictors for liver fibrosis.

In our study, there was a significant difference with respect to liver echogenicity between the mild and severe groups. This is consistent with previous studies [21-23]. These studies demonstrated that liver echogenicity was an independent predictor in patients with cirrhosis. Liver surface, the most widely reported and most consistent ultrasound parameter, has excellent specificity but only moderate sensitivity [24-28]. In our study liver surface was not found to be different between the mild group and severe group, neither were spleen thickness and portal vein diameter. These differences may be due to variability in sample characteristics, selection criteria of participants, the reference standard used, and the level of expertise of the sonographers, interpreting radiologists, and, possibly, to non-standardized equipment.

Although our study showed significant inter-group difference in liver echogenicity make difference between the mild ($F > 2$) and severe groups ($F \geq 2$), we found that the use of liver echogenicity may have poor efficacy in the assessment of the stage of liver fibrosis. The sensitivity of coarse liver echogenicity and increased liver echogenicity are both relatively

low in case of use of only one of these as a predictor for moderate to severe fibrosis. Accordingly, we suggest that patients with normal liver echogenicity should undergo advanced investigations for staging of liver fibrosis, such as, magnetic resonance imaging (MRI), FibroTest, or liver biopsy.

Although liver echogenicity was not reliable in diagnosing the stage of liver fibrosis, increased liver echogenicity showed a relatively high PPV (72.22%); 13 out of 18 patients with increased liver echogenicity had severe fibrosis ($F \geq 2$) on liver biopsy. Liver echogenicity can be a potential indication for treatment of CHB patients who lack clear treatment indications.

Ultrasound, being non-invasive and relatively inexpensive, is one of the most widely used imaging modalities in clinical practice, especially in middle and low income countries. For these reason, we recommend that in patients with CHB, without clear treatment indications, abdominal ultrasound examination should be done before liver biopsy. Findings of increased liver echogenicity should prompt an initiation of antiviral treatment, thereby avoiding liver biopsy.

Our study had several limitations. Firstly, we did not consider other ultrasound parameters, such as portal venous flow velocity, which has been shown to be of value in several studies [21, 23, 29-31]. Secondly, other imaging techniques were not performed, such as FibroScan, MRI and computerized tomography. Strategies combining other imaging techniques and biochemistry may improve diagnostic accuracy for liver fibrosis and are areas for future research.

In conclusion, ultrasound was not found to be reliable in assessment of the stage of liver fibrosis. Patients with normal ultrasound findings may benefit from advanced investigations such as MRI, FibroTest and liver biopsy. However, increased liver echogenicity may be a potential treatment indication for CHB patients in whom there is no outright indication for antiviral treatment. Such a strategy may be particularly relevant in LMICs.

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Disclosure of conflict of interest

None.

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