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Evaluation of Indirect Serum Markers in Prediction of Hepatic Fibrosis in Chronic Hepatitis B Patients

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Abstract

Background: *Liver biopsy is considered the best method for evaluation of hepatic fibrosis. However, it has important adverse events. Therefore, non-invasive markers have been developed to determine the degree of hepatic fibrosis in patients with chronic hepatitis B (CHB).*

Aim of the work: *To assess the ability of alpha fetoprotein (AFP), Lok index, globulin platelets (GP) model and Hui index to predict hepatic fibrosis in CHB patients.*

Patients and Methods: *This prospective cohort study included 88 consecutive adult patients with CHB attending the Viral Hepatitis Outpatient Clinic of Tropical Medicine and Gastroenterology Department, Sohag University Hospital. Patients with CHB for more than 6 months were included. Liver biopsy was done and examined histopathologically for grading and staging of chronic hepatitis according to Metavir scoring system. Laboratory results and abdominal ultrasound reports performed within one month from the date of liver biopsy were used. AFP was measured, Lok index, GP model and Hui index were calculated and compared with liver biopsy.*

Results: *AFP was the most valuable test for detection of significant fibrosis with the best sensitivity (100%), negative predictive value (NPV) (100%) and accuracy (96.5%). It was the most accurate marker in differentiation between insignificant and significant fibrosis. Lok index was found to be a valuable test for the detection of significant fibrosis with sensitivity and NPV less than AFP but higher than GP model and Hui index. GP model had the best specificity (100%), positive predictive value (PPV) (100%) and high accuracy (83.35%). Hui index was found to be less valuable after AFP, Lok index and GP model for detection of significant fibrosis with accuracy 74.9%. All these non-invasive markers could distinguish mild fibrosis from advanced fibrosis with different accuracies and significances. AFP, Lok index and GP model achieved very good performance where the area under the receiver operator characteristic curve (AUROC) was >0.8.*

Conclusion: *Using simple formulae, significant fibrosis can be predicted accurately in CHB patients, potentially avoiding the need for liver biopsies in these patients.*

Keywords: *Chronic hepatitis B, non-invasive marker, alpha fetoprotein, Lok index, globulin platelets model and Hui index.*

1. Introduction

Hepatitis B virus (HBV) infection is a major global health problem with over 240 million people chronically infected worldwide [1]. The spectrum of chronic HBV infection is variable, ranging from inactive carrier state to progressive CHB, which can evolve to cirrhosis in up to 20% of the cases, with hepatic insufficiency and portal hypertension being the most serious consequences [2]. Chronically infected subjects also have a 100 times higher risk to develop hepatocellular carcinoma (HCC) than non-carriers [3]. In untreated patients with CHB, the cumulative incidences of cirrhosis, hepatic decompensation and HCC at 5 years were approximately 20%, 15% and 5%, respectively [4]. Liver fibrosis is part of the structural and functional alterations in most chronic liver diseases. It is one of the main prognostic factors as the amount of fibrosis is correlated with the risk of developing cirrhosis and liver-related complications in viral and non viral chronic liver diseases [5]. The current guidelines for HBV management propose liver biopsy to begin antiviral therapy and to obtain prognostic information. Liver biopsy is an invasive procedure and is not readily repeatable for the assessment of post-treatment liver fibrosis.

It also results in sampling errors and inter and intra-observer variability. For these reasons, numerous non-invasive markers have been derived to assess hepatic fibrosis [6]. The fact that the process of fibrogenesis is a component of the normal healing response hampers the development of disease-specific biomarkers [7]. The ideal marker for liver fibrosis would be

highly sensitive, specific to identify different stages of fibrosis, readily available, safe, inexpensive, reproducible, applicable to monitor disease progression or regression and not susceptible to false positive results. Although no single ideal marker exists, several markers have been identified as possible useful indicators of fibrosis when used in conjunction with each other [8]. A few of them have been validated as diagnostic and prognostic markers of liver disease severity in CHB, as AST-to-Platelets Ratio Index (APRI), Fibro Test and Fibrosis based on 4 (Fib-4) [9]. The non-invasive markers reliably determine the presence or absence of cirrhosis and distinguish significant fibrosis from no/mild fibrosis with different accuracies and significances but could not discriminate between different stages of fibrosis [10]. Therefore, there is a need to test the performance of some simple biomarkers derived from routine blood tests done in our hospital to predict the stage of liver fibrosis in CHB patients.

2. Patients and Methods

This prospective cohort study included 88 consecutive adult patients with CHB attending the Viral Hepatitis Outpatient Clinic of Tropical Medicine and Gastroenterology Department, Sohag University Hospital. Patients with chronic HBV infection diagnosed by hepatitis B surface antigen (HBsAg), polymerase chain reaction (PCR) for more than 6 months and agreed to be liver biopsied were included. Patients with history of alcohol use, co-infection with hepatitis C, other causes of liver disease as nonalcoholic steatohepatitis, autoimmune hepatitis, or Wilson's disease, usage of antiviral drugs, and HCC were excluded.

2.1. All Patients Were Subjected to

Full history taking and clinical examination including history of risk factors of hepatitis B infection and signs of liver diseases.

Laboratory investigations including Alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, albumin, globulin, prothrombine time (PT), prothrombin concentration (PC), international normalized ratio (INR), complete blood count, HBs Ag, hepatitis C virus antibody (HCV Ab), PCR for HBV, hepatitis Be antigen (HBeAg) and AFP.

Abdominal ultrasonography to evaluate liver size, echogenicity and any focal lesion, portal vein diameter and spleen size.

Liver biopsy a core of liver tissue was obtained from all participants under ultrasonographic guidance using true cut needle biopsy. All samples were examined histopathologically for grading and staging of chronic hepatitis according to Metavir scoring system [11].

F0 = no scarring.

F1 = portal fibrosis without septa.

F2 = portal fibrosis with rare septa.

F3 = numerous septa without cirrhosis.

F4 = cirrhosis or advanced scarring of the liver.

We considered Mild fibrosis: stages 0-2, Advanced fibrosis: stages 3-4

Laboratory results and abdominal ultrasound reports performed within one month from the date of the liver biopsy were used.

2.2. The Following Indices were Calculated

Lok index =-5.56 – (0.0089 x platelets (10^3/mm^3)) + (1.26 x AST/ALT ratio) + (5.27 x INR) [12].

GP model = Globulin (mg/dL) x 100 / PLT (10^3/mm^3) [13].

Hui score = 3.148 + (0.167 x BMI) + (0.088 x bilirubin) – (0.151 x albumin) – (0.019 x platelets) [14].

We compared AFP, Lok index, GP model and Hui index with liver biopsy results to evaluate the possibility of using them as non-invasive markers to predict significant fibrosis.

2.3. Statistical Analysis

Data were analyzed using STATA intercooled version 12.1. Quantitative data were represented as mean, standard deviation, median and range. Data were analyzed using student t-test to compare means of two groups and ANOVA with post-hoc for comparison of the means of three groups or more. When the data were not normally distributed Mann-Whitney test was used to compare two groups and Kruskal Wallis test for comparison of three or more groups. Qualitative data were presented as number and percentage and compared using either Chi square test or fisher's exact test. Data were analyzed by sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) derived from the receiver operating characteristic (ROC) curve. The diagnostic accuracy of different variables was expressed as the area under the ROC curve (AUROC). Graphs were produced by using Excel or STATA program. P value was considered statistically significant if it was less than 0.05.

3. Results

Our results showed that the mean age of the 88 patients was 35.26 ±11.98 years. Sixty-five (73.86%) were men. Seventy (79.55%) patients

had no to mild fibrosis (F0-2), while 18 (20.45%) patients had advanced fibrosis (F3-4). Sixty-six (75.00%) patients were HBeAg negative (Table1).

Table1. *Characteristics of the 88 chronic hepatitis B patients*

Variable	Statistics
Age (year)	
Mean ± SD	35.26±11.98
Gender	
Females	23 (26.14%)
Males	65 (73.86%)
Metavir score, n(%):	
No fibrosis: F0	1 (1.14%)
Mild fibrosis: F1	31 (35.23%)
F2	38 (43.18%)
Advanced fibrosis: F3	7 (7.95%)
F4	11 (12.50%)
Body mass index (Kg/m^2)	
Mean ± SD	28.86±4.45
Hepatitis Be antigen	
Negative	66 (75.00%)
Positive	22 (25.00%)

3.1. Comparison between Patients with Mild Versus Advanced Fibrosis

Univariate analysis of our data showed that ALT, AST, bilirubin and globulin levels, INR and AFP were significantly higher in patients with advance fibrosis than those with mild fibrosis. On the other hand, albumin level and platelets count were significantly lower in patients with advanced fibrosis than those with mild fibrosis. We found that HBeAg had no significant association with severity of fibrosis (Table 2).

3.2. Indices Predicting Liver Fibrosis

Because ALT, AST, bilirubin, albumin and globulin levels, INR and platelets count were highly significantly associated with the stage of fibrosis, we used Lok index, GP model and Hui index which depend on these factors to amplify their effects on detection of advanced fibrosis.

3.3. Lok Index

Depends on ALT, AST, INR and platelets count, GP model depends on globulin level and platelets count and Hui score depends on body mass index (BMI), serum bilirubin, albumin and platelets count. We found significant association between the severity of liver fibrosis and the increase in Lok index, GB model and Hui score. By comparison between these indices we found that Lok index and GP model (P= 0.0001) are the best indices in predicting the severity of fibrosis followed by the Hui index (P=0.003) (Figure 1).

Table2. *Comparison between Patients with Mild versus Advanced Fibrosis*

Variable	Mild fibrosis N=70	Advanced fibrosis N=18	P value
Age (year)			
Mean ± SD	34.44±11.14	38.44±14.76	0.21
Gender			
Females	19 (27.14%)	4 (22.22%)	0.77
Males	51 (72.86%)	14 (77.78%)	
Body mass index (kg/m^2)			
Mean ± SD	24.56±4.13	26.06±5.47	0.21
Alanine aminotransferase (IU/l)			
Mean ± SD	29.54±16.86	56.89±32.00	0.0001
Aspartate aminotransferase (IU/l)			
Mean ± SD	32.73±41.50	62.78±37.87	0.0001
Bilirubin (mg/dl)			
Mean ± SD	0.72±0.31	1.09±0.54	0.01
Albumin (gm/dl)			
Mean ± SD	4.04±0.45	3.69±0.88	0.02
Globulin (gm/dl)			
Mean ± SD	3.09±0.45	4.1±0.73	0.0001
International normalized ratio			
Mean ± SD	1.04±0.11	1.17±0.12	0.0001
Platelets (10^3/mm^3)			
Mean ± SD	229.03±67.32	178.22±76.88	0.001
Hepatitis Be antigen			
Negative	53 (75.71%)	13 (72.22%)	
Positive	17 (24.29%)	5 (27.78%)	0.77
Alpha fetoprotein (ng/mL)			
Mean ± SD	3.51±2.80	15.83±11.55	0.0001

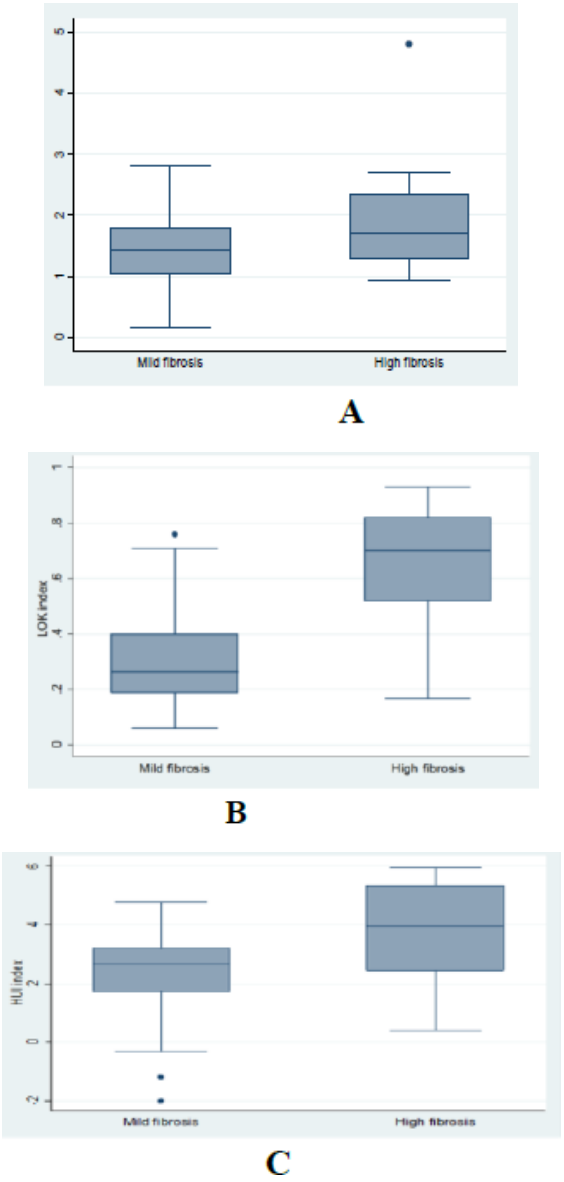

Figure1. *Box plot of (A) Lok index, (B) GP model and (C) Hui score in relation to the metavir fibrosis score. The severity of liver fibrosis is significantly associated with the increase in Lok index, GP model and Hui score. The box represents the interquartile range, the whiskers indicate the highest and lowest values, the line across the box indicates the median value and the circles represent outliers.*

3.4. Diagnostic Performance of Serum Fibrosis Indices

ROC curves were constructed for each model to predict significant fibrosis (F ≥ 3). The AUROC (95% CI) was found to be greatest for AFP, Lok index and GP model followed by Hui index (P=<0.0001, <0.0001, 0.0001 and 0.008 respectively). AFP has the best sensitivity, NPV and accuracy. GP model has the best specificity and PPV (Table 3). AFP, Lok index and GP model achieved very good performance (AUROC >0.8). They are the best indicators of fibrosis in patients with CHB (Figure 2).

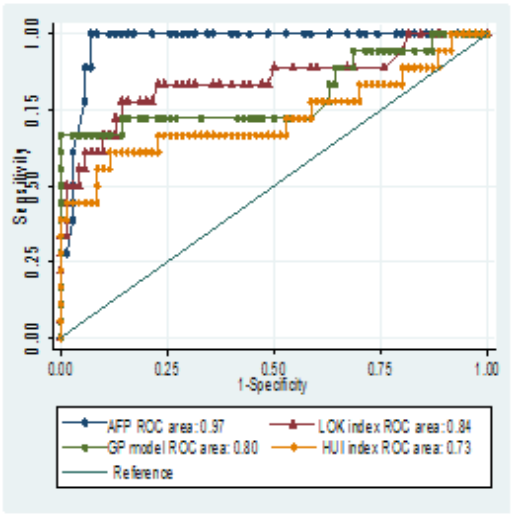

Figure2. *ROC curves of AFP, Lok index, GP model and Hui index to predict significant fibrosis. The AUROC (95% CI) was found to be greatest for AFP, Lok index and GP model followed by Hui index. An AUROC of 1.0 is characteristic of an ideal test, whereas an AUROC of 0.5 or less indicates a test of no diagnostic value.*

Table3. *Optimum diagnostic cut off value, AUROC, sensitivity, specificity, positive and negative predictive values and accuracy of different markers predicting high fibrosis*

Variable	Cutoff	AUROC (95% CI)	Sensitivity (%)	Specificity(%)	PPV (%)	NPV (%)	Accuracy(%)	P value
AFP	>7.7	0.97 (0.91-0.99)	100	92.9	78.3	100	96.5	<0.0001
Lok index	>0.51	0.84 (0.75-0.91)	77.8	85.7	58.3	93.7	81.8	<0.0001
GP model	>2.4	0.80 (0.70-0.88)	66.7	100	100	92.1	83.35	0.0001
Hui index	>3.55	0.73 (0.62-815)	61.1	88.6	57.9	89.9	74.9	0.008

AUROC= Area under receiver operating characteristic curve.NPV= Negative predictive value.CI= Confidence interval. AFP= Alpha fetoprotein, PPV= Positive predictive value. GP model= Globulin platelets model

4. DISCUSSION

Non-invasive methods for assessment of liver disease severity have witnessed major advancements in the last few years. The great interest in developing more and more accurate non-invasive methods for assessment of chronic liver disease can be explained by several reasons: the well-known limitations of liver biopsy, the increasing awareness of its unreliability to predict liver disease severity and development of liver-related complications. In

the setting of chronic HBV-related liver disease, it is extremely important to define novel methods to assess the stage of liver disease and the stage of fibrosis in an acceptable cost-effective safe way.

In our study, we attempted to evaluate the performance of 4 non-invasive indices using routinely available laboratory test results to predict significant fibrosis in a consecutive series of patients with CHB. We found that age, gender and BMI were not predictors for severity of hepatic fibrosis in patients with CHB. Another study done on 228 patients with CHB showed that age had a valuable role in prediction of significant fibrosis while sex had no role [15]. Limin et al. showed that BMI had no value in predicting severity of hepatic fibrosis [16]. In contrast, Hashem et al. showed that age and BMI had a role in predicting severity of fibrosis [17]. In our study, we found that ALT, AST, bilirubin, albumin, globulin, INR levels and platelets count were the independent predictors of significant fibrosis. Our findings agree with many other studies [15,18, 19]. Limin et al. showed that ALT and AST levels had no role in prediction of severity of fibrosis while platelets count had a role[16]. In our study, 66 (75%) patients were HBeAg negative. We as well as others found insignificant correlation between HBeAg status and fibrosis score [20].

In our study, using a cut off >7.7, we found that AFP had 100% sensitivity, 92.9% specificity, 78.3% PPV, 100% NPV and 96.5% accuracy to predict significant fibrosis. It was the most accurate marker in differentiation between insignificant and significant fibrosis. Our findings echoed results from many previous studies [13, 16, 17].

In our study, using a cut off >0.51, the Lok index had 77.8% sensitivity, 85.7% specificity, 58.3% PPV, 93.7% NPV and 81.8% accuracy to predict significant fibrosis. In another study, using a cut off ≥0.5 to confirm cirrhosis, only 14.8% of patients were wrongly classified (98% sensitivity, 99% specificity)[21]. Şirli et al. found that at a cut-off value of 0.5, 91.3% of patients were correctly classified as having or not having significant fibrosis. For values greater than a cut-off of 0.5, Lok index accurately predicted significant fibrosis (20% sensitivity, 98.5% specificity, 91.7% PPV and 60% NPV) [22].

In our study, using a cut off value of >2.4, the GP model had 66.7% sensitivity, 100% specificity, 100% PPV, 92.1% NPV and 83.35% accuracy to predict significant fibrosis. Liu et al. by using a cut off value of < 1.68, the GP model had a sensitivity of 72.4%, a specificity of 69.6%, a PPV of 71.2% and a NPV of 70.8% for prediction of significant fibrosis [13].

In our study, using a cut off >3.55, the Hui index had 61.1% sensitivity, 88.6% specificity, 57.9% PPV, 89.9% NPV and 74.9% accuracy to predict significant fibrosis. Similar results were obtained by Wong et al [23]. Hui et al. by using a cut off ≥ 0.5, the Hui index had a sensitivity of 88%, a specificity of 88% to exclude significant fibrosis [14].

We found all these non-invasive markers could distinguish mild fibrosis from advanced fibrosis with different accuracies and significances. The AUROC (95% CI) was found to be greatest for AFP (0.97), Lok index (0.84), GP model (0.80) followed by Hui index (0.73). AFP was the most valuable test for the detection of significant fibrosis with the best sensitivity, NPV and accuracy. The Lok index was found to be a valuable test for the detection of significant fibrosis with sensitivity and NPV less than AFP but higher than GP model and Hui index. The GP model had the best specificity, PPV and high accuracy. The Hui index was found to be less valuable after AFP, Lok index and GP model for detection of significant fibrosis.

Our study has several unique features. First, we recruited consecutive patients undergoing percutaneous liver biopsies in our hospital who met the inclusion criteria. Many prior studies have recruited only patients enrolled in treatment trials which may have introduced selection bias [18, 19]. Secondly, all the predictive models that we evaluated consisted of objective and readily available laboratory variables as ALT, AST, INR, globulin and platelets count which are routine tests performed in CHB patients in clinical practice, so no additional tests were needed.

5. CONCLUSION

Using simple formulae, significant fibrosis can be predicted accurately in CHB patients, potentially avoiding the need for liver biopsies in these patients.

RECOMMENDATIONS

We recommend further prospective studies to validate AFP, Lok index, GP model and Hui index in comparison with non-invasive radiological methods that measure fibrosis.

ETHICAL CONSIDERATION

We got the acceptance of Sohag Faculty of Medicine Ethical Committee. Patients signed informed written consent before starting data collection.

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