Evaluation of Liver Fibrosis by Ten Noninvasive Methods in Patients with Chronic Hepatitis B: A Comparative Study

Kronik Hepatit B Hastalarında Karaciğer Fibrozisinin On Adet Noninvaziv Metod ile Değerlendirilmesi: Karşılaştırmalı Çalışma

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Received \ Geliş tarihi : 16.05.2017
Accepted \ Kabul tarihi : 04.08.2017

DOI: 10.17954/amj.2018.111

ABSTRACT

Objective: This study aims to evaluate the predictive value of noninvasive serum markers of hepatic fibrosis in patients with chronic hepatitis B.

Material and Methods: This study involved 158 patients with chronic hepatitis B. Noninvasive markers used were as follows; aspartate transaminase (AST) to alanine transaminase (ALT) ratio (AAR), fibrosis-4 (FIB-4) index, age-platelet index (AP), Forns index, cirrhosis discriminant score (CDS), Kings score, Fibro-Q index, PAPAS score, Lok index and red cell distribution width to platelet ratio (RPR). Concurrent liver biopsy specimens were evaluated with the Ishak scoring system. Patients were divided into two groups, those with significant fibrosis (Ishak fibrosis score F ≥ 3) and those without (Ishak fibrosis score F ≤ 2). Receiver operating characteristic (ROC) curve analyses were carried out to compare the results of the noninvasive markers in the two groups.

Results: Of the 158 patients evaluated (mean age 49.39 ± 13.04, 100 males [63.3%], 58 females [36,7%]), 139 (88%) were HBe Ag negative and HBe Ab positive and 19 (%12) were HBe Ag positive and HBe Ab negative.ROC curve analyses with the Ishak fibrosis score had cutoff values as follows: AAR = 0.562, Forns index = 0.604, FIB-4 index = 0.608, PAPAS score = 0.565, AP index = 0.625, Fibro-Q index = 0.589, Kings score = 0.590, CDS = 0.598, RPR = 0.614 and Lok index = 0.631.

Conclusion: The Lok index was found to be the most effective noninvasive method for estimating hepatic fibrosis. The AP index, RPR, Forns index and FIB-4 index were also effective models in our study.

Key Words: Chronic hepatitis B, Fibrosis, Lok index

ÖZ

Amaç: Çalışma kronik hepatit B hastalarında hepatik fibrozisin değerlendirilmesi ve tahmininde noninvaziv metodların değerlendirilmesini amaçlamaktadır.

Gereç ve Yöntemler: Çalışmaya, Akdeniz Üniversitesi Tıp Fakültesi Gastroenteroloji Bölümüne 2009-2014 yılları arasında başvuran, invaziv karaciğer biyopsisi yapılmış, 158 KHB' li hasta dahil edildi. Çalışmamızda, non-invaziv hepatik fibrozis belirteçlerinden literatüre geçmiş Lok indeks, King' s skoru, CDS (Bonacini skoru), Fibro-Q indeks, FİB-4 indeks, HUI modeli, APRI, AAR, Forn's ve PAPAS indeksleri kullanılmış, çalışmaya alınan hastaların verileri retrospektif olarak taranarak, bu veriler ışığında hastaların invaziv biyopsi skorları ile 10 farklı non-invaziv yöntem karşılaştırılmıştır.

Bulgular: Çalışmaya dahil edilen invaziv karaciğer biyopsisi yapılmış158 hastada ortalama yaş 49,39±13,04 saptandı. Hastaların 100'ü erkek(%63,3), 58'ise kadındı(%36,7). 139 (%88) hasta HBe Ag negatif, 19 hasta(%12) HBe Ag pozitif idi. Çalışmada kullanılan 10 adet noninvaziv metodun her biri için ROC eğrileri oluşturulup Ishak fibrozis skorları ile karşılaştırmalı cutt-off değerleri metodlara göre şu şekilde saptandı; AAR = 0,562, Forns indeks = 0,604, FIB-4 indeks = 0,608, PAPAS skoru = 0,565, AP indeks= 0,625, Fibro-Q indeks = 0,589, Kings skoru = 0,590, CDS = 0,598, RPR = 0,614 ve Lok indeks = 0,631.

Sonuç: İnceleme sonucunda KHB hasta grubumuzda Lok indeks fibrozis tesbitinde en etkin noninvaziv yöntem olarak saptandı. Lok indeksi etkinlik açısından sırasıyla AP indeks, RPR, FİB-4 ve Forns indeksin izlediği saptandı. HBV DNA düzeyi ile HAİ ve anlamlı fibrozis arasında pozitif korelasyon saptanırken, anlamlı fibrozis ile cinsiyet, HBe Ag ve Hbe Ab arasında istatistiksel olarak korelasyon saptanmadı.

Anahtar Sözcükler: Kronik hepatit B, Fibrozis, Lok indeks

INTRODUCTION

Chronic hepatitis B virus infection is a serious public health problem because of its wide distribution and potential adverse sequelae, including cirrhosis and hepatocellular carcinoma (HCC) (1). It has been estimated that 400 million people worldwide are infected with hepatitis B virus (HBV). Approximately one million people infected with HBV die of chronic liver disease every year (2). The most important goal in the treatment of the disease is the prevention of liver fibrosis and the complications associated with it (3). Thus, it is important to estimate liver fibrosis early in the course of the disease and to prevent its progression to cirrhosis (2,3). While several clinical parameters, including male gender, older age, higher levels of serum alanine transaminase (ALT), and serum HBV DNA levels have been identified as risk factors for severe liver disease (4-6), liver biopsy is currently the gold standard for determining the severity of necroinflammation and for the staging of fibrosis. Liver biopsy, however, has several disadvantages, including risks in patients with low platelet counts, prolonged prothrombin time, or massive ascites; poor patient compliance; poorly standardised collection of liver tissues; sampling error; limited usefulness for ongoing surveillance; and poor intra- and interobserver agreement. Considering these limitations and the reluctance of patients to undergo liver biopsy, noninvasive predictors of hepatic histology are urgently needed (7-10). Several markers have shown promise for the detection of advanced fibrosis, but their sensitivities are poor. No single serum marker has been able to correctly diagnose and assess the degree and progression of hepatic fibrosis (9-11). Ultrasound is one of the possible alternatives for liver fibrosis assessment. Fibroscan and Acoustic Radiation Force Impulse (ARFI) elastography are two new noninvasive methods based on ultrasound. However, the accuracy of Fibroscan and ARFI is substantially compromised by higher degrees of necroinflammation and more advanced stages of fibrosis or cirrhosis, especially in patients with chronic hepatitis B (CHB) (12-15).

In recent years, interest in identifying and describing liver fibrosis using noninvasive surrogate markers has been on the rise. Serum markers of liver fibrosis offer an attractive, cost effective alternative to liver biopsy for both patients and clinicians. In addition to being substantially less invasive, there are practically no complications, few or no sampling errors, and small observer-related variability. Moreover, measurements may be performed repeatedly, thus allowing for ongoing monitoring of fibrosis (16). The diagnostic value of serum markers of liver fibrosis has been investigated in numerous studies. Based on clinical and research needs, the ideal marker for liver fibrosis would have the following characteristics:

- Be highly sensitive and specific for identifying different stages of fibrosis
- Be readily available, safe, inexpensive and reproducible
- Be applicable to the monitoring of disease progression or regression to assess the natural history of liver disease or the efficacy of treatment regimens
- Not be susceptible to false positive results, for example, in individuals with inflammation related to other diseases.

Although no single ideal marker exists, several have been identified as possibly useful indicators of fibrosis when used in conjunction with each other. Biomarkers of fibrosis are commonly divided into two main categories, direct and indirect. Direct markers are fragments of the liver matrix components produced by hepatic stellate cells during the process of extracellular matrix remodelling. Indirect markers include molecules released into the blood due to liver inflammation; molecules synthesised, regulated, or excreted by the liver; and markers of processes commonly disrupted by impaired liver function, such as insulin resistance. Direct and indirect markers may be used alone or-more commonly-in combination with each other to produce composite scores. The calculation of such scores can be relatively simple or based on complicated formulas.

The most commonly used direct markers are procollagen type I carboxy terminal peptide, procollagen type III amino-terminal peptide metalloproteinases, tissue inhibitors of matrix metalloproteinases, transforming growth factor-b1, hyaluronic acid, YKL-40 (chondrex), laminin, connective tissue growth factor, paraoxonase 1 and microfibril-associated glycoprotein 4 (17). Unfortunately, direct serum fibrosis biomarkers have some limitations.

- They reflect the rate of matrix turnover (not only deposition) and have a tendency to be higher in the presence of marked inflammatory activity. As a consequence, extensive matrix deposition might not be detected in the presence of minimal inflammation.
- They are not liver-specific, and their serum levels may be elevated in the presence of inflammation elsewhere in the body.
- Serum levels of the markers depend on their clearance rates, which are influenced by endothelial cell dysfunction and impairment of biliary excretion or renal function (17,18).

These limitations have stimulated the search for new and simple noninvasive methods to evaluate hepatic fibrosis. Such studies have led to discovery of indirect hepatic fibrosis markers in recent years, with approximately 30 indirect noninvasive hepatic fibrosis estimation methods identified in the last two decades. In this study, we aimed to evaluate

the efficacy of 10 noninvasive liver fibrosis methods and to identify the most valuable method for the prediction of the stage of liver fibrosis in patients with CHB.

MATERIAL and METHODS

Patients

One hundred fifty-eight consecutive eligible patients with CHB who underwent a liver biopsy in January 2009 to February 2014 at Akdeniz University Hospital Antalya, Turkey were included in this study. CHB was diagnosed based on the presence in serum of HBV surface antigens (HbsAg), fluctuating ALT levels, and positive serum levels of HBV DNA. Exclusion criteria included chronic liver disease due to other causes or coinfection with hepatitis D, clinically overt cirrhosis, previous or concomitant anti-HBV therapy, prior interferon therapy, alcohol consumption, diabetes mellitus, autoimmune hepatitis, fatty liver disease, metabolic liver disease, and inadequate laboratory findings. Data were retrospectively analysed. The study protocol was approved by the Institutional Review Board in our hospital. Written informed consent was obtained from each patient.

Liver biopsy and quantification of fibrosis

Liver tissue was obtained by sonoguided percutaneous biopsy (Hepafix, 16G, USA) and stained with hematoxylin-eosin-safran and Masson's trichrome. Fibrosis staging (F) and inflammatory activity (A) were determined according to the Ishak system. Patients were divided into two groups according to the Ishak score, F ≤ 2 (without significant fibrosis) and F ≥3 (with significant fibrosis). Liver biopsy specimens were evaluated by different pathologists.

Serum parameters

An automatic biochemistry analyser was used to measure serum levels of the following parameters: Alanine transaminase (ALT), aspartate transaminase (AST), γ-glutamyl transpeptidase (GGT), alkaline phosphatase, total bilirubin, direct bilirubin, hemoglobin, leucocytes, platelets, mean platelet volume (MPV), red cell distribution width (RDW), prothrombin time, creatinine, cholesterol, sodium, potassium, calcium, phosphorus and alpha fetoprotein. HBV DNA levels were measured by the polymerase chain reaction. All tests were performed after a 12-hour fast. All biochemical tests and calculation of marker scores were evaluated without knowledge of liver biopsy results. We also assessed physical and clinical variables such as age, sex and body mass index.

Comparison of available noninvasive biomarkers between patients with and without significant fibrosis

Data collected from the records, including patient characteristics and blood test results, were used to calculate the following scores: PAPAS index, AAR (AST/ALT ratio), Forns index, FİB-4 index, Lok index, AP (AST/Platelet ratio), Fibro-Q index, Kings score index, CDS (cirrhosis discriminant score or Bonacini index) and RPR (RDW/platelet ratio). The results of the noninvasive fibrosis models were compared between the two groups based on Ishak scores, and receiver operating characteristics (ROC) curves and correlation analysis were employed to assess the diagnostic accuracy of each model for predicting significant fibrosis.

Statistical analysis

All statistical analysis was performed by using SPSS 15.0. Descriptive statistics were calculated as frequencies and percentages for categorical variables and as mean and standard deviation for continuous variables. To compare differences between the two groups, a chi-square test was used for categorical variables and a t test for continuous variables. $P < 0.05$ was considered statistically significant. Sensitivity, specificity, and positive and negative predictive values (NPV and PPV) were calculated by using cutoffs from previously published studies. The overall diagnostic performance of each score was evaluated by area under the ROC curve (AUROC). We used the Youden index method to calculate the new cutoff value for the Lok index.

RESULTS

Patient data

The demographic data, HBe Ag, HBe Ab and Ishak fibrosis scores for the 158 patients with CHB are shown in Tables I and II.

The mean age of those without significant fibrosis was 48.97 ± 13.57, compared with 49.7 ± 12.72 of those with significant fibrosis. The length of time between laboratory tests and liver biopsy was approximately 1–2 weeks. Laboratory test results and clinical characteristics of the patients are summarised in Table III.

Overall diagnostic performance of ten noninvasive models

Ten noninvasive hepatic fibrosis prediction model scores were calculated for each patient in the two main groups. AUROC was used to evaluate the diagnostic performance of the ten scores. Cutoffs were chosen for each model as previously described (Figure 1).

The most sensitive score was the PAPAS index, and the highest specificity was found for the Bonacini score. We compared our AUROC, sensitivities and specificities, with the originally published studies.

The Lok index had the highest AUROC in our study. In published studies, the sensitivity for the Lok index was 37%, and the specificity was 92% (19,20). The results were

lower in our study. Therefore, we calculated a new specific cutoff value for our study using the Youden index method and assessed the effect of the new value on sensitivity and specificity.

The new cutoff value of 0.35 improved the sensitivity and specificity for significant fibrosis in our study, compared with the cutoff value of 0.5 in the original articles (19,20).

DISCUSSION

Many studies have evaluated noninvasive diagnostic models of liver fibrosis in various types of chronic liver disease. However, most of those studies were carried out in patients with chronic hepatitis C. There are only a few studies evaluating noninvasive models predicting liver fibrosis in CHB, and the results have been controversial or inconsistent (20-22). The present study evaluated the validity of ten noninvasive methods to distinguish between Ishak stages F ≤ 2 and F ≥ 3 in 158 patients with CHB.

The major strength of the current study is the evaluation of all ten noninvasive markers in same study. Previous studies of CHB have only assessed a few of the noninvasive markers. Ma et al. evaluated seven markers in 1168 patients with CHB (20), while Shin et al. assessed only APRI in 264 patients (21). Wu et al. evaluated six (22), and Zhou et al. compared seven (23). Wai et al. evaluated both AAR and APRI in 218 patients with CHB (24).

In our study, we assessed ten noninvasive markers for predicting fibrosis stage in 158 patients with CHB. We evaluated the diagnostic performance of PAPAS, AAR, Forns, FIB-4, Lok, AP, Fibro-Q, Kings, CDS and RPR, using the cutoff values in the original studies for the assessment of liver fibrosis in CHB. These models are mainly based on indirect serum markers. Previous reports have suggested that most noninvasive models are not able to determine the exact stage of fibrosis because of overlap among patients with different stages of fibrosis. In contrast,

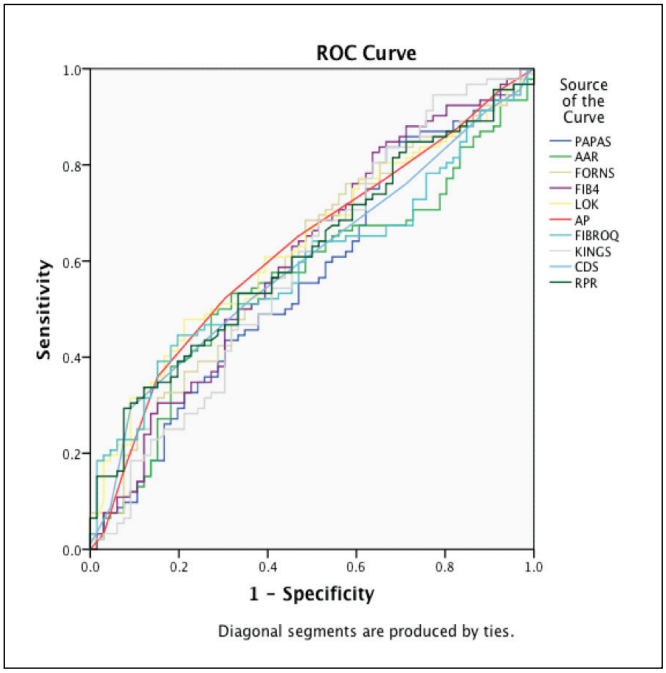

Figure 1: ROC curves for the 10 fibrosis models to discriminate between Ishak fibrosis stage ≤2 and ≥3.

Table I: Gender disribution, HBe Ag, HBe Ab and liver biopsy results of all patients.

GENDER n=158				HBe Ag n=158				ISHAK SCORE n=158			
Male		Female		Negative		Positive		≤2		≥3	
n	%	n	%	n	%	n	%	n	%	n	%
100	63.3	58	36.7	139	88	19	12	66	41.8	92	58.2

Table II: Gender distribution, HBe Ag and HBe Ab according to Ishak score results.

		ISHAK ≤2		ISHAK ≥3		P Value
		n = 66		n = 92		
		n	%	n	%	
GENDER	**Male**	40	60.6	60	65.2	0.55
	Female	26	39.4	32	34.8	
HBe Ag	**Negative**	60	90.9	79	85.9	0.34
	Positive	6	9.1	13	14.1	
HBe Ab	**Negative**	7	10.6	12	13	0.64
	Positive	59	89.4	80	87	

our results showed that the Lok index, RPR, Forns index, FIB-4 and AP were all effective in distinguishing the stage of fibrosis. Lok's model was the most precise marker in our study.

An ideal noninvasive model for effective assessment of liver fibrosis should be based on easily available laboratory tests. The general strength of our study is the ability to use results that are readily available from routine standardised clinical laboratory tests, including such variables as ALT, AST, platelet count, RDW, MPV and serum cholesterol level.

Direct serum markers are useful for assessing the speed of liver fibrogenesis. Hyaluronic acid, a component of the extracellular matrix, is a glycosaminoglycan synthesized by hepatic stellate cells and degraded by liver sinusoidal cells (25). The concentration of A2M, a protease inhibitor, is increased by stellate cell activation and liver fibrosis (26). Studies have demonstrated that hyaluronic acid and A2M levels are correlated with hepatic fibrosis in patients with CHB or chronic hepatitis C (27-29). These markers, however, are not assessed in routine laboratory tests, their serum levels depend on rates of clearance, they are not liver-specific, and they may be elevated by inflammation of other organs. Therefore, these models are not useful for predicting liver fibrosis.

Indirect noninvasive fibrosis prediction models are usually based on routine laboratory tests. Albumin, prothrombin, thrombopoetin and most coagulation factors are produced in the liver. Hypoalbuminemia, prolonged PT and thrombocytopenia are often seen in patients with advanced liver fibrosis. Therefore, abnormal values of ALT, AST, PLT, Alb and PT or INR reflect liver dysfunction and may be associated with liver fibrosis. However, no single laboratory test is specific for liver fibrosis. Although these tests reflect liver damage, they give no information about the aetiology of the liver disease, nor do the noninvasive models, even though they are better at specifically reflecting liver fibrosis.

We found lower AUROC values in our study compared with previous studies. Among these models, AAR had the smallest AUROC (0.562), and Lok's index had the highest AUROC (0.614). Lok's model had a higher AUROC value in patients with significant fibrosis, although less than what was reported in previous studies (19,20). There are some limitations of our study. First, it was retrospective, and the liver biopsy specimens were evaluated by a number of different pathologists. Second, the biopsy specimens and blood samples were not obtained on the same day. There was a time gap of approximately one or two weeks. Another limitation was the small number of patients. If we had been able to analyse data from more patients, we could have obtained higher AUROC values and sensitivities and specificities. Ours was a single centre study, with patients from only one hospital. Thus they may be less representative of the general population compared with patients from multiple centres. The cutoff values are

Table III: Characteristics of study population.

	ISHAK ≤2 (Without significant fibrosis) n = 66				ISHAK ≥3 (Significant fibrosis) n = 92				P value
	Min	**Max**	**Mean**	**SD**	**Min**	**Max**	**Mean**	**SD**	
Age	16	78	48.97	13.57	21	80	49.7	12.72	0.731
BMI	18.4	31.25	24.80	3.26	18.94	38.63	25.39	3.59	0.289
ALT (U/L)	12	325	84.97	62.71	14	855	110.04	126.26	0.103
AST (U/L)	14	222	62.14	47.00	18	570	74.63	74.33	0.231
GGT (U/L)	5	181	36.53	26.24	5	126	40.92	26.23	0.301
ALP (U/L)	43	286	120.73	52.02	43	313	131.11	62.12	0.270
Plt (mm3)	**137**	**346**	**223.55**	**47.14**	**49**	**435**	**202.64**	**60.87**	**0.021**
RDW (%)	11.3	16.4	14.23	1.11	11.6	128	15.31	11.93	0.466
PT (sec)	11.2	15.59	12.60	0.96	10.59	17.7	12.74	1.16	0.444
INR	0.9	1.3	1.08	0.09	0.9	1.64	1.09	0.11	0.300
CHOL (mg/dl)	113	272	182.89	31.72	58	285	182.40	35.86	0.929
ALB (g/dl)	3.4	5.47	4.47	0.35	3.2	5.1	4.38	0.34	0.119
AFP	1	13.2	3.56	2.11	1.28	120	5.13	12.38	0.308
HAI	**6**	**12**	**8.17**	**1.64**	**6**	**15**	**10.71**	**2.09**	**<0.001**

another limitation of this study. We used published cutoff values rather than calculating specific cutoff values for each model in our study. When we calculated a new cutoff value for Lok's model, we found better sensitivity and specificity for predicting fibroses. Finally, we compared only two groups based in Ishak scores above and below 3, rather than calculating AUROCs for each Ishak fibrosis stage.

In summary this study showed that Lok's model is effective in predicting and staging fibrosis in patients with CHB, being the best among indirect noninvasive markers for fibrosis in CHB. We think that prospective studies with larger number of patients and study-specific cutoff values will produce better information about assessing hepatic fibrosis in CHB.

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