Cukurova Medical Journal
ÇUKUROVA ÜNİVERSİTESİ TIP FAKÜLTESİ DERGİSİ

Cukurova Med J 2017;42(2):299-307
DOI: 10.17826/cutf.322924

ARAŞTIRMA / RESEARCH

Assessment of laboratory indices for predicting severity of fibrosis in treatment naive young male patients with non-cirrhotic hepatitis B virus infection

Tedavi görmemiş sirozu olmayan hepatit B virüs enfeksiyonu olan erkek hastalarda fibrozis şiddetini öngörmede laboratuar indekslerinin değerlendirilmesi

Murat Afyon[1], Cumhur Artuk[2], Ercan Yenilmez[3], Berksan Şimşek[4], Ayper Kaya[4]

[1]GATA Haydarpasa Training Hospital Naval Academy Clinic, Istanbul; Turkey
[2]Gülhane Military Medical Academy, Department of Infectious Diseases, Ankara, Turkey
[3]GATA Haydarpasa Training Hospital, Department of Infectious Diseases, Istanbul, Turkey
[4]Kasımpasa Military Hospital, Beyoglu-Istanbul; Kasımpasa Military Hospital, Istanbul, Turkey

Cukurova Medical Journal 2017;42(2):299-307

Abstract

Purpose: We aimed to assess serum levels of AST and ALT, platelet counts, INR or serum HBV DNA levels, especially in score systems such as APRI (AST/platelet ratio), FIB-4 index and King's score, for predicting severity of fibrosis.

Material and Methods: One hundred fifty treatment naive male HBV patients underwent percutaneous liver biopsy were evaluated retrospectively. For evaluating the difference in variables and scores, patients were divided into 2 groups such as no or minimal fibrosis group (fibrosis score<2) and significiant fibrosis group (fibrosis score≥2).

Results: There were 79 patients (52.7%) with minimal fibrosis and 71 patients (42.3%) with significiant fibrosis. We considered that optimal cut-off values defining significant fibrosis for serum HBV DNA level was 4.5 log10 IU/ml (sensivity:71.8%, specificity:70.9%), for APRI was 0.45 (sensivity:63.4%, specificity: 63.3%), for FIB-4 index was 0.45 (sensivity:64.8%, specificity:64.3%) and lastly for King's score was 4.00 (sensivity:67.6%, specificity:60.8%).

Conclusion: We identified that the scoring systems such as APRI, FIB-4 index and ing's score are not superior to each other, but serum HBV DNA level is a little better compared with those to predict significiant fibrosis in treatment naive young male patients with non-cirrhotic HBV infection.

Key words: Hepatitis B virus, fibrosis, HBV DNA.

Öz

Amaç: Bu çalışmada fibrozis ciddiyetini öngörmede, serum aspartat aminotransferaz (AST) ve alanin aminotransferaz (ALT) seviyeleri, platelet sayısı, INR değeri veya serum Hepatit B virüs (HBV) deoksiribonükleik asit (DNA) seviyelerinin, özellikle AST-platelet oranı (APRI), FIB-4 indeks ve King's skoru gibi skorlama sistemleri içinde değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntem: Yüzelli karaciğer iğne biyopsisi olan tedavi almamış erkek HBV hastaları geriye dönük olarak değerlendirildi. Değişkenler arasında farkı değerlendirmek amacıyla, hastalar minimal fibrozisi olanlar (fibrozis skoru<2) ve anlamlı fibrozisi olanlar (fibrozis skoru≥2) olmak üzere iki gruba ayrıldı.

Bulgular: Minimal fibrozisi olan 79 hasta (52.7%) ve anlamlı fibrozisi olan 71 hasta (42.3%) vardı. Anlamlı fibrozisi tanımlayan optimal değeri, HBV DNA seviyesi için 4.5 log10 IU/ml (duyarlılık:71.8%, özgüllük:70.9%), APRI için 0.45 (duyarlılık:63.4%, özgüllük:63.3%), FIB-4 indeks için 0.45 (duyarlılık:64.8%, özgüllük:64.3%) ve son olarak King's skor için 4.00 (duyarlılık: 67.6%, özgüllük:60.8%) olarak belirledik.

Sonuç: Tedavi görmemiş sirozu olmayan HBV enfeksiyonu olan erkek hastalarda fibrozis ciddiyetini öngörmede APRI, FIB-4 index ve King's skorun birbirlerine üstünlüğünün olmadığını, ancak serum HBV DNA seviyelerinin bu skorlara kıyasla biraz daha iyi olduğunu değerlendirmekteyiz.

Anahtar kelimeler: Hepatit B virüsü, fibrozis, HBV DNA

Yazışma Adresi/Address for Correspondence: Dr. Murat Afyon, GATA Haydarpasa Training Hospital, Tuzla-Istanbul; Turkey E-mail: muratafyon2002@yahoo.com
Geliş tarihi/Received: 01.09.2016 Kabul tarihi/Accepted: 16.10.2016

INTRODUCTION

Accurate evaluation and staging of liver fibrosis is substantial for predicting prognosis and selecting treatment options in patients with viral hepatitis[1-4]. Percutaneous liver biopsy is still defined as the gold standard in determining severity of liver disease[1-6]. But, liver biopsy may cause complications such as moderate pain, bleeding, biliary perforation and peritonitis, pneumothorax or death and have drawbacks including patient discomfort, need for hospitalization and repeated biopsies for defining the therapy response or predicting prognosis in the posttreatment follow-up period, sampling error, lack of standardization of staining, inter or intra observer diagnostic variations and its high cost[1,4-9]. Hence, there are non-invasive predictive models increasingly used for determining liver fibrosis degree[1,6-18].

Imaging methods evaluating liver stiffness can accurately assess the degree of liver fibrosis, but access to these technigues and their cost are the limitations for these radiological tests[1,6-8, 12]. Another option for non-invasive tests is based on blood or serum indices. Indirect serum markers, singly or in combination, such as aspartate aminotransferase (AST)-to-alanine aminotransferase (ALT) ratio (AAR), AST-to-platelet ratio index (APRI) score, Forns' index, FIB-4 index (fibrosis index based on four factors), FibroTest, Fibroindex, GUCI index (Göteborg University Cirrhosis Index), King's score or hepatic matrix metabolism markers as direct markers (e.g., type IV collagen, hyaluronic acid, laminin, transforming growth factor beta 1, YKL-40, metalloproteinases or tissue inhibitors of metalloproteinases) have been performed to evaluate liver fibrosis in patients with different liver diseases[6-14]. Direct serum markers and some commercial patented serum tests are not routinely available in clinical practice. However, some scores such as APRI, FIB-4 index or King's score are cheaper, consist of routinely used blood tests, do not require particular expertise, can be performed in an outpatient setting and allow a more widespread use[1,6,8].

In recent years, serum HBV deoxyribonucleic acid (DNA) has been described as an important predictor of the development of HBV-related liver disease and serum HBV DNA levels also defined as a predictor of significiant liver fibrosis in treatment naive patients with hepatitis B envelope antigen (HBeAg) negative or positive HBV infection[15-18].

In this regard, we aimed to assess serum levels of AST and ALT, platelet counts, INR (International Normalized Ratio) in scoring systems and/or serum HBV DNA levels for predicting severity of fibrosis in treatment naive male patients with HBV infection.

MATERIAL AND METHODS

Patients

For this study, ethics commitee approval was obtained from Gulhane Military Medical Academy Haydarpasa Teaching Hospital Non-invasive Clinical Research Ethics Committee (26.02.2016, 1491-45-16/1539). Patients with HBV infection who applied Kasımpasa Military Hospital Infectious Diseases Clinic between 01 January 2012 and 01 June 2015 were evaluated retrospectively. Treatment naive male HBV patients underwent percutaneous liver biopsy were included in the study. Patients with hepatitis C (HCV), hepatitis D (HDV) or human immunodefency virus (HIV) coinfection or with comorbidities such as autoimmune hepatitis, hepatosteatosis, alcohol abuse, chronic inflamatory diseases, malignancies, corticostreoid use, myeloproliferative disorders and other any infectious diseases were excluded.

The data including patients' age, platelet counts, serum AST and ALT levels, INR values, HBeAg and antibodies aganist HBeAg (anti-HBe) status, serum HBV DNA levels, fibrosis scores and histological activity index (HAI) scores were recorded. These laboratory indices were performed with blood samples which had been taken about one week before liver biopsy.

The reference ranges of AST, ALT were 0-40 IU/ml and 5-40 IU/ml respectively. All patients included the study had serum ALT levels persistently higher than upper limit of normal. Architect HBeAg commercial kits (CMIA; chemiluminescence microparticul immunassay) were used. HBV DNA levels were detected by Taqman Real Time PCR assay, Fluorion HBV QNP 2.0 (Istanbul, Turkey) (analytical sensivity: 10 IU/ml, the linear range:20-2×10^9 IU/ml, 1 IU/ml=8 copies/ml). Fibrosis score and HAI score were evaluated via means of modified histological activity index by the same observer. For evaluating the difference in variables and scores, firstly, patients were divided into 2

groups such as no or minimal fibrosis group (fibrosis score<2) and significiant fibrosis group (fibrosis score≥2). Furthermore, patients with significiant fibrosis were also divided into two groups such as moderate fibrosis group (fibrosis score=2) and advanced fibrosis group (fibrosis score≥3).

Scores

APRI, FIB-4 index and King's score were calculated for all patients using laboratory data based on the following formulas:

APRI= [AST(U/l)/(Upper limit of normal)/Platelet count (10^3/mm^3)] × 100 (Note: Upper limit of normal of males 40 U/l).
FIB-4 index= [AST (U/l)×Age (years)]/[Platelet count (10^3/mm^3) × √ALT (U/l))]
King's score= [AST (U/l)×Age (years)×INR]/(Platelet count (10^3/mm^3).

Statistical analysis

Statistical analyses were performed by SPSS 15.0 (SPSS Inc., Chicago, ILL., USA). The variables were investigated using visual (histograms, probability plots) and analytic methods (Kolmogorov-Simirnov/Shapiro-Wilk's test) to determine whether or not they are normally distributed. All continuous variables were summarised as mean±standart deviation or median (interquartile range (Iq)). The Mann-Whitney U test or Student's *t*-test were applied to compare continuous variables, depending on the normality of the data distribution. And also, the differences in the variables were analyzed using analysis of variance (ANOVA) or the Kruskal–Wallis tests. The Chi-square test was used to compare the proportions in different groups. *p* values <0.05 were considered to be statisticaly significant for all analysis.

The capacity of scores in predicting significiant fibrosis were analyzed using ROC (Receiver Operating Characteristics) curve analysis. When a significiant cut-off value was observed, the sensivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) were presented.

RESULTS

Overall, 150 treatment naive male patients with non-cirrhotic HBV infection were included in the study. Age variable was not distributed normally and mean age was 22.32±2.69 years and median age (interquartile range) was 21 (2) with an age range of 19-37 years. Of all patients, 120 patients (80%) were between 19 and 23 years old. Baseline characteristics of subjects are described in Table 1.

There were 15 patients (10%) with fibrosis score 0, 64 patients (42.7%) with fibrosis score 1, 56 patients (37.3%) with fibrosis score 2, 14 patients (9.3%) with fibrosis score 3 and only one patient with fibrosis score 4 (0.7%). According to fibrosis scores, there were 79 patients (52.7%) with no or minimal fibrosis (fibrosis score<2) and 71 patients (42.3%) with significiant fibrosis (fibrosis score≥2). Furthermore, in significiant fibrosis group, there were 56 patients (78.9% of patients with significiant fibrosis) with moderate fibrosis (fibrosis score=2) and 15 patients (21.1% of patients with significiant fibrosis) with advanced fibrosis (fibrosis score≥3).

Of all patients included the study, 64 patients (42.7%) were positive for HBeAg and 86 patients (57.3%) were negative for HBeAg. Serum AST and ALT levels, HBV DNA levels, fibrosis scores and HAI scores were significiantly higher in HBeAg positive group compared with HBeAg negative group. (respectively; p=0.003, p=0.029, p<0.001, p=0.003 and p<0.001).

Table 1. Baseline characteristics of subjects

Variables	Mean±Standart Deviation (Minimum-Maximum)	Median (Interquartile Range)
Age (years)	22.32±2.69 (19-37)	21 (2)
AST (U/l)	49.06±23.00 (24-147)	40 (19)
ALT (U/l)	95.25±58.58 (41-352)	75 (52)
PLT (103/mm3)	241.83±52.87 (140-425)	234 (65)
INR (International Normalized Ratio)	1.04±0.09 (0.72-1.41)	1.04 (0.11)
HBV DNA (log10 IU/ml)	5.27± 3.19 (0 IU/ml-10.13)	4.39 (5.77)
Fibrosis Score	1.48±0.82 (0-4)	1 (1)
HAI score	4.01±2.23 (1-10)	4 (4)

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; PLT: Platelet counts; HBV DNA: Hepatitis B virus deoxyribonucleic acid; HAI: Histological activity index

Table 2. Age, laboratory and histological variables of groups according to HBeAg status.

Variables M±SD (Min.-Max.)	HBeAg Positive Group (n=64, 42.7%)	HBeAg Negative Group (n=86, 57.3%)	p
Age (years)	22.14±2.86 (20-37)	22.45±2.57 (19-30)	0.221
AST (U/l)	54.86±24.04 (24-147)	44.74±19.51 (25-111)	0.003
ALT (U/l)	105.23±63.20 (42-352)	87.83±54.07 (41-316)	0.029
PLT (103/mm3)	243.08±58.00 (149-425)	240.90±49.03 (140-409)	0,983
INR (International Normalized Ratio)	1.03±0.08 (0.87-1.26)	1.04±0.10 (0.72-1.41)	0.341**
HBV DNA (log10 IU/ml)	8.18±1.98 (2.39-10.13)	3.10±1.96 (0 IU/ml -9.16)	<0.001
Fibrosis Score	1.70±0.88 (0-4)	1.31±0.74 (0-3)	0.003
HAI Score	4.83±2.33 (1-10)	3.40±1.95 (1-10)	<0.001

M±SD (Min.-Max.): Mean±Standart Deviation (Minimum-Maximum); AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, PLT: Platelet counts, HBV DNA: Hepatitis B virus deoxyribonucleic acid, HAI: Histological activity index, * Mann-Whitney U test; ** Student t test

However, there were no statistical difference in age variable (p=0.221), platelet counts (p=0.983) and INR values (p=0.341) between two groups. Age, laboratory and histological variables of groups according to HBeAg status are described in Table 2.

Of patients with negative HBeAg, 31 patients (36%) had significiant fibrosis, and 40 patients (62.5%) with HBeAg positivity had also significiant fibrosis. There was a significiantly difference (p=0.001) in significiant fibrosis between groups with respect to HBeAg status. In other words, there was a significiant difference in HBeAg positivity rate between significiant fibrosis group (40/71, 56.3%) and no or minimal fibrosis group (24/79, 30.4%) But, in patients with significiant fibrosis, there was no difference (p=0.364) in HBeAg positivity rate between moderate fibrosis group (30/56, 53.6%) and advanced fibrosis group (10/15, 66.7%).

Results of age, laboratory and histological variables and scores according to the fibrosis groups described in Table 3. While there was no difference in age variable (p=0.199) and INR values (p=0.186) between significiant fibrosis group and no or minimal fibrosis group, serum AST levels, serum ALT levels, HBV DNA levels, APRI, FIB-4 index and King's score were significiantly higher in patients with significiant fibrosis compared with no or minimal fibrosis group (respectively; p=0.001, p<0.001, p<0.001, p<0.001, p=0.017 and p<0.001) and platelet counts were lower in significiant fibrosis group (p=0.001) (Table 3.).

However, in significiant fibrosis group, platelet counts were significiantly lower (p=0.045) in patients with advanced fibrosis. Serum AST and HBV DNA levels, INR values, APRI, FIB-4 index and King's score were higher in patients with advanced fibrosis, but there were no statistical difference in age variable (p=0.136), serum AST levels (p=0.562), serum ALT levels (p=0.927), INR values (p=0.719), serum HBV DNA levels (p=0.324), APRI (p=0.167), FIB-4 index (p=0.172) and King's score (p=0.237) between moderate fibrosis group and advanced fibrosis group. (Table 3.).In the light of these results, we performed ROC curve analysis to evaluate whether significiant fibrosis could be detected efficiently and accurately with serum HBV DNA level, APRI, FIB-4 index or King's score in treatment naive male patients with non-cirrhotic HBV infection, since there was a significiant difference in serum HBV DNA levels, APRI, FIB-4 index or King's score between no or minimal fibrosis group and significiant fibrosis group. As a predictor of significiant fibrosis, area under ROC curve (AUROC) for serum HBV DNA levels 0.683 (p<0.001), AUROC for APRI was 0.700 (p<0.001), AUROC for FIB-4 index was 0.642 (p=0.003) and AUROC for King's score was 0.708 (p<0.001). (Figure 1.)

We considered that optimal cut-off value (due to the highest sensivity + specificity rate) defining significant fibrosis for serum HBV DNA level was 4.5 $\log_{10}$ IU/ml (sensivity: 71.8%, specificity: 70.9%, PPV:68.9% and NPV:73.7%), for APRI was 0.45 (sensivity: 63.4%, specificity: 63.3%, PPV:60.8% and NPV:65.8%), for FIB-4 index was 0.45 (sensivity: 64.8%, specificity: 64.3%, PPV:57.5% and NPV:57.0%) and lastly for King's score was 4.00

(sensivity: 67.6%, specificity: 60.8%, PPV:60.8% and NPV:67.6%). Afterwards, we assessed the cut-off for serum HBV DNA level to predict significiant fibrosis in groups according to HBeAg status. 4.5 log_{10} IU/ml as a cut-off for serum HBV DNA level had sensivity of 97.5%, specificity of 20.8%, PPV of 67.2% and NPV of 83.3% in patients with positive HBeAg (n=64), and had sensivity of 38.7%, specificity of 92.7% of a specificity, PPV of 75% and NPV of 72.9% in patients with negative HBeAg (n=86)

DISCUSSION

The patients included in the study were young adult and male, because our hospital is a military hospital and our patients are usually young adult male patients and very small number women have been seen in our clinic. Hereby, as a drawback, our findings might not be generalizable to women.

Classically, it is accepted that HBeAg positivity corraletes with viral replication in the liver and high serum HBV DNA levels, and the seroconversion of HBeAg is usually accompanied by decrease in viral replication and hepatic disease remission except from precore or basal core promoter mutants [18-20]. Accordingly, in our cohort, patients with positive HBeAg had higher serum AST and ALT levels, HBV DNA levels, fibrosis scores and HAI scores than patients with negative HBeAg. Furthermore, between significiant fibrosis group and no or minimal fibrosis group, there were significiant difference in HBeAg positivity rate (56.3% vs. 30.4%) and also serum HBV DNA levels (6.38±3.19 log_{10} IU/ml vs. 4.27±2.87 log_{10} IU/ml). However, HBeAg positivity rate and serum HBV DNA levels were lower in moderate fibrosis group compared with advanced fibrosis group, whereas there were no statistically difference in those between two groups (53.6% vs. 66.7% and 6.24±3.16 log_{10} IU/ml vs. 6.89±3.36 log_{10} IU/ml).

Table 3. Results of variables and scores according to the fibrosis groups.

Variables	No or Minimal fibrosis (Fibrosis score≤ 1)	Significiant fibrosis (Fibrosis score ≥ 2)	p Value	Moderate fibrosis (Fibrosis score=2)	Advanced fibrosis (Fibrosis score≥3)	p Value
	n=79 (52.7%)	**n=71 (42.3%)**		**n=56 (37.3%)**	**n=15 (10%)**	
	M±SD (Min.-Max.)			M±SD (Min.-Max.)		
Age (years)	22.51±2.88 (19-37)	22.11±2.47 (20-30)	0.199	22,29±2.48 (20-30)	21.47±2.46 (20-30)	0.136
AST (U/l)	42.67±15.88 (26-112)	56.17±27.34 (24-147)	**0.001**	55.04±27.04 (25-147)	60.40±29.02 (24-120)	0.562
ALT (U/l)	93.28±39.33 (41-268)	114.14±69.95 (43-352)	**<0.001**	115.12±72.05 (43-352)	110.47±63.67 (43-278)	0.927
PLT (10^3/mm^3)	257.05±56.35 (172-425)	224.89±43.11 (140-342)	**0.001**	230.52±42.57 (162-342)	203.87±39.64 (140-284)	**0.045**
INR	1.03±0.08 (0.72-1.25)	1.05±0.10 (0.89-1.41)	0.186	1.05±0.10 (0.89-1.41)	1.06±0.10 (0.95-1.26)	0.719
HBV DNA (log_{10} IU/ml)	4.27±2.87 (0 IU/ml - 10.04)	6.38±3.19 (0 IU/ml - 10.13)	**<0.001**	6.24±3.16 (0 IU/ml -10.13)	6.89±3.36 (0 IU/ml - 10.04)	0.324
APRI	0.43±0.20 (0.19-1.30)	0.66±0.20 (0.23-1.89)	**<0.001**	0.62±0.33 (0.23-1.57)	0.80±0.51 (0.24-1.89)	0.167
FIB-4 index	0.44±0.15 (0.18-0.96)	0.54±1.96 (0.25-1.32)	**0.017**	0.51±0.16 (0.25-0.97)	0.63±0.28 (0.30-1.32)	0.172
King's score	4.08±2.04 (2.03-12.58)	6.01±3.19 (1.98-15.47)	**<0.001**	5.72±2.87 (1.98-14.30)	7.11±4.10 (2.25-15.47)	0.237

M±SD (Min.-Max.): Mean±Standart Deviation (Minimum-Maximum); AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, PLT: Platelet counts, INR: International Normalized Ratio, APRI: Aspartate aminotransferase to Platelet Ratio Index: [(AST/Upper limit of normal) / PLT] × 100; FIB-4 index (fibrosis index based on four factors) = (AST×Age)/(PLT ×√ALT); King's score= (AST×Age×INR)/PLT

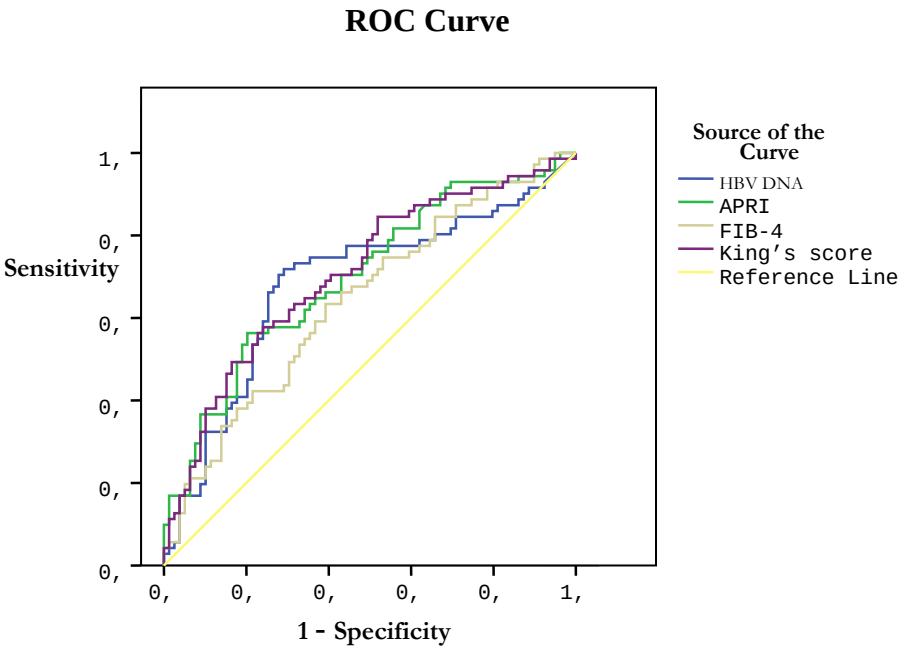

Figure 1. ROC curve analysis for serum HBV DNA level, APRI, FIB-4 index and King's score to predict significiant fibrosis.

In line with universal knowledge and as a result of our study, it can be said that HBeAg positivity is a risk factor for severity of HBV infection and also HBeAg positivity or more specificially serum HBV DNA level may be a predictor for the presence of significiant liver fibrosis in treatment naive male patients with persistant ALT elevation. In our study, we considered that optimal cut-off values (due to the highest sensivity + specificity rate) defining significant fibrosis for serum HBV DNA levels was 4.5 $\log_{10}$ IU/ml (sensivity: 71.8%, specificity: 70.9%, PPV:68.9% and NPV:73.7%).

In the literature, 5.5 $\log_{10}$ IU/ml with sensivity of 71.4%, specificity of 93.3%, PPV of 83.3 % and NPV of 87.5% to predict Metavir fibrosis score ≥ 2 in HBeAg negative patients, 4.91 $\log_{10}$ IU/ml with sensivity of 74% and specificity of 80% to predict Ishak stage≥3 in patients with HBeAg negativity and 7.3 $\log_{10}$ IU/ml with sensivity of 71.1% and specificity of 73.4% in patients with HBeAg positivity have been defined as cut-off values for serum HBV DNA level [15-17]. The difference between cut-off values in studies including our study may result from HBeAg status in patients included the trial, methods used to evaluate fibrosis degree, the definition of significiant fibrosis in the study and the inclusion of cirrhotic patients in the study. Also, the authors' choice of high sensivity or high specificity may be important when selecting cut-off values. For example, in our our study, while 4.5 $\log_{10}$ IU/ml as a cut-off for serum HBV DNA level could determined significiant fibrosis with sensivity of 97.5% and specificity of 20.8% in patients with HBeAg positivity, using this cut-off, significiant fibrosis could be excluded with sensivity of 38.7% and specificity of 92.7% in HBeAg negative patients. Similarly, in another study, 4.71 $\log_{10}$ IU/ml has been identified as a low cut-off to exclude significiant fibrosis with NPV of 99% in patients with negative HBeAg[17].

Distincly, historic or current HBV DNA was not defined as a independent predictor of the presence of cirrhosis, while AST and protrombin time were found to be associated with the development of HBV related cirrhosis in non-Asian women with chronic HBV [20]. Particularly in patients with cirrhosis, serum AST levels, INR values may increase and platelet counts may decrease depending on severity of liver fibrosis, and there may be a negative correlation between patients' age and liver

fibrosis degree[14-18, 21-30]. Hence, there are several score systems, using these parameters to predict fibrosis degree, such as AAR, APRI score, FIB-4 index, GUCI index and King's score [6-18, 22-30]. Our findings showed that serum AST levels, serum ALT levels, APRI, FIB-4 index and King's score were significiantly higher in patients with significiant fibrosis compared with no or minimal fibrosis group and platelet counts were lower in significiant fibrosis group, although there were no difference in age variable and INR values between two groups.

However, in significiant fibrosis group, aside from platelet count, there was no difference in variables or scores between moderate fibrosis group and advanced fibrosis group. In our cohort, the number of patients with advanced fibrosis was limited (n=15) and a great majority of those (n=14, 93.3%). had fibrosis score of 3. And also, when defining fibrosis score of 2 or 3, intra observer variations may have occured. No statisticallly difference in variables between moderate fibrosis group and advanced fibrosis group may be due to these reasons mentioned above, although serum AST levels, INR values, APRI, FIB-4 index and King's score were higher in patients with advanced fibrosis. Morever, there may be really no marked difference in variables or scores between moderate fibrosis group and advanced fibrosis group. Ultimately, the use of these scores seems not eligible to seperate advanced fibrosis (but not cirrhosis) from moderate fibrosis according to our findings. Anyway, the scores are generally have been considered to be more important and useful to distinguish between significiant or severe fibrosis and no or minimal fibrosis[1, 6-11, 22-30].

The cut-off values of the scores for defining severity of fibrosis are still questionable with a wide range and varied sensivity and specificity rates[6-11, 22-30]. The difference between cut-off values in these studies including our study may result from patients included the study (infected with HBV, HCV and HIV, or liver transplant recipients etc.), the methods used to evaluate fibrosis degree, predicted fibrosis degree (significiant, advanced or cirrhosis), the definition of significiant or severe fibrosis in the study, the inclusion of cirrhotic patients in the study, the authors' choice of high sensivity or high specificity, the time of the calculation (pre or posttreatment) [6-11, 22-30].

Also, while our cut-off value for APRI to predict significiant fibrosis was almost similiar to those in the other studies, values and the cut-off values for FIB-4 index (0.45) and for King's score (4.00) in our study were apparently lower than those in the other trials[6-11, 22-30]. Different from APRI, age variable is a component of FIB-4 index and King's score. Patients included our study were young with a mean age of 22.32±2.69 years, 80% (n=120) of all patients were between 19 and 23 years old, and also there was no difference in age variable between significiant fibrosis group and no or minimal fibrosis group in our cohort. These conditions may be one of the reasons for lower values and cut-off values of FIB-4 index and King's score in our cohort.

In some trials, the score systems such as APRI have been reported to be not associated with significiant fibrosis and to have beter accuracy in diagnosis of cirrhosis than significiant fibrosis and to have acceptable accuracy for assessment of liver fibrosis in patients with HCV infection, but not in those HBV infection[9,15, 26, 27]. Majority of the trials evaluating the scores were based on patients with HCV infection, rather than HBV patients[10, 11, 22, 25, 28-30]. Furthermore, different from HCV infection, HBeAg status and serum HBV DNA level are substantial in predicting severity of infection and liver disease[1,9,18]. In patients with HBV infection, scoring systems including APRI and FIB-4 index which are derived from HCV patients have been described as not adequate replacement liver biopsy in clinical practise and HBeAg status and HBV DNA have been considered as more accurate to predict fibrosis degree than the models in light of the complex natural history of chronic HBV infection in contrast to HCV infection[9]. World Health Organization (WHO) recommends that treatment can be deferred in adults in resource-limited settings without clinical features of cirrhosis (or based on APRI ≤ 2), who also have persistenly normal serum ALT levels and serum HBV DNA level lower than 2000 IU/ml, and who can be re-evaluated at subsequent visits[1]. Also, age, ALT, HBV DNA and precore/core promoter HBV variants were identified as strong independent risk factor predicting significiant fibrosis (Metavir score≥2) independently of HBeAg status in a recent study[18].

There are several limitations in this study. First of all, the retrospective design is the most important one of these limitations. In addition, the number of study patients was limited. That the study was a single-center study may be another drawback and a

cause of the limited number of study patients. Furthermore, as mentioned above, since the patients included in the study were young adult and male, our findings might not be generalizable to women.

As a conclusion, we think that new score systems have to contain HBeAg status or especially serum HBV DNA level combine with particularly serum AST level and platelet count. We identified that the scoring systems such as APRI, FIB-4 index and King's score are not superior to each other, but serum HBV DNA level is a little better compared with those to predict significiant fibrosis in treatment naive young male patients with non-cirrhotic HBV infection. Although they do not substitute liver biopsy, serum HBV DNA level combine with APRI, FIB-4 index or King's score can be used to predict significiant fibrosis in treatment naive patients with HBV infection. However, it should be noted that HBV DNA levels can be very high in patients without fibrosis (immunotolerant phase) or can be low/undetectable in patients with advanced fibrosis. Hence, in order to highlight the role and importance of non-invasive scoring systems containing serum AST level, platelet count, HBeAg status and serum HBV DNA level to predict fibrosis degree in patients with HBV infection, randomized large-scale studies are required.

REFERENCES

1. World Health Organization. Guidelines for the Prevention, Care and Treatment of Persons With Chronic Hepatitis B Infection. Geneva, World Health Organization, 2015.
2. Afyon M. Letter: nucleos(t)ide analogues are good, but not sufficient for hepatitis B virus clearance. Aliment Pharmacol Ther. 2016;43:654-5.
3. Yenilmez E, Afyon M, Ulcay A, Ulus S, Kaya A, Erdem H. Evaluation of 135 liver biopsy results between 2011 and 2014, according to hepatitis B virus DNA and liver transaminase levels in naive young patients with HBeAg positive and negative chronic hepatitis B infection. J Clin Virol. 2015;69:235.
4. Kose S, Ersan G, Tatar B, Adar P, Sengel BE. Evaluation of percutaneous liver biopsy complications in patients with chronic viral hepatitis. Eurasian J Med. 2015;47:161-4.
5. Afyon M, Artuk C. Noninvasive indirect serum markers of liver fibrosis in patients with chronic viral hepatitis. Turk J Gastroenterol. 2016;27:397-8.
6. Stasi C, Milani S. Non-invasive assessment of liver fibrosis: Between prediction/prevention of outcomes and cost-effectiveness. World J Gastroenterol. 2016;22:1711-20.
7. Abdollahi M, Pouri A, Ghojazadeh M, Estakhri R, Somi M. Non-invasive serum fibrosis markers: A study in chronic hepatitis. Bioimpacts. 2015;5:17-23.
8. Schmid P, Bregenzer A, Huber M, Rauch A, Jochum W, Müllhaupt B et al. Progression of liver fibrosis in HIV/HCV co-infection: a comparison between non-invasive assessment methods and liver biopsy. PLoS One. 2015;10:e0138838
9. Kim WR, Berg T, Asselah T, Flisiak R, Fung S, Gordon SC et al. Evaluation of APRI and FIB-4 scoring systems for non-invasive assessment of hepatic fibrosis in chronic hepatitis B patients. J Hepatol. 2016;64:773-80.
10. Vergara M, Bejarano G, Dalmau B, Gil M, Miquel M, Sanchez-Delgado J et al. Usefulness of indirect noninvasive methods in predicting progression to cirrhosis in chronic hepatitis C. Eur J Gastroenterol Hepatol. 2015;27:826-33.
11. Tachi Y, Hirai T, Toyoda H, Tada T, Hayashi K, Honda T et al. Predictive ability of laboratory indices for liver fibrosis in patients with chronic hepatitis c after the eradication of hepatitis c virus. PLoS One. 2015;10:e0133515.
12. Li SM, Li GX, Fu DM, Wang Y, Dang LQ. Liver fibrosis evaluation by ARFI and APRI in chronic hepatitis C. World J Gastroenterol. 2014;20:9528-33.
13. Kliemann DA, Wolff FH, Tovo CV, Alencastro PR, Ikeda ML, Brandão AB et al. Biochemical non-invasive assessment of liver fibrosis cannot replace biopsy in HIV-HCV coinfected patients. Ann Hepatol. 2016;15:27-32.
14. Kandemir O, Polat G, Saracoglu G, Tasdelen B. The predictive role of AST level, protrombin time, and platelet count in the detection of liver fibrosis in patients with chronic hepatitis C. Turk J Med Sci. 2009;39:857-62.
15. Praneenararat S, Chamroonkul N, Sripongpun P, Kanngurn S, Jarumanokul R, Piratvisuth T. HBV DNA level could predict significant liver fibrosis in HBeAg negative chronic hepatitis B patients with biopsy indication. BMC Gastroenterol. 2014;14:218.
16. Gao Y, Zou G, Ye J, Pan G, Rao J, Li F et al. Relationship between hepatitis B surface antigen, HBV DNA quantity and liver fibrosis severity Zhonghua Gan Zang Bing Za Zhi. 2015;23:254-7.
17. Mohamadnejad M, Montazeri G, Fazlollahi A, Zamani F, Nasiri J, Nobakht H et al. Noninvasive markers of liver fibrosis and inflammation in chronic hepatitis B-virus related liver disease. Am J Gastroenterol. 2006;101:2537-45.
18. Lapalus M, Laouenan C, Cardoso AC, Estrabaud E, Carvalho-Filho RJ, Zhang Q et al. Precore/core promoter variants to predict significant fibrosis in both HBeAg positive and negative chronic hepatitis B. Liver Int. 2015;35:2082-9.
19. Koziel MR, Thio CL. Hepatitis B virus and Hepatitis

D virus. In Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases, 7th ed (Eds GL Mandell, JE Bennett, R Dolin):2059-87. Philadelphia, PA, USA, Churchill-Livingstone, 2010.
20. Pondé RA. Atypical serological profiles in hepatitis B virus infection. Eur J Clin Microbiol Infect Dis. 2013;32:461-6.
21. Harkisoen S, Arends JE, van den Hoek JA, Whelan J, van Erpecum KJ, Boland GJ et al. Historic and current hepatitis B viral DNA and quantitative HBsAg level are not associated with cirrhosis in non-Asian women with chronic hepatitis B. Int J Infect Dis. 2014;29:133-8.
22. Gökcan H, Kuzu UB, Öztaş E, Saygılı F, Öztuna D, Suna N et al. The predictive value of noninvasive serum markers of liver fibrosis in patients with chronic hepatitis C. Turk J Gastroenterol. 2016;27:156-64.
23. D'Souza RS, Neves Souza L, Isted A, Fitzpatrick E, Vimalesvaran S, Cotoi C et al. AST-to-platelet ratio index in non-invasive assessment of long-term graft fibrosis following pediatric liver transplantation. Pediatr Transplant. 2016;20:222-6.
24. Orasan OH, Iancu M, Sava M, Saplontai-Pop A, Cozma A, Sarlea ST et al. Non-invasive assessment of liver fibrosis in chronic viral hepatitis. Eur J Clin Invest. 2015;45:1243-51.
25. Derbala M, Elbadri ME, Amer AM, AlKaabi S, Sultan KH, Kamel YM et al. Aspartate transaminase to platelet ratio index in hepatitis C virus and Schistosomiasis coinfection. World J Gastroenterol. 2015;21:13132-9.
26. Degos F, Perez P, Roche B, Mahmoudi A, Asselineau J, Voitot H et al. Diagnostic accuracy of FibroScan and comparison to liver fibrosis biomarkers in chronic viral hepatitis: a multicenter prospective study (the FIBROSTIC study). J Hepatol. 2010;53:1013-21.
27. Yilmaz Y, Yonal O, Kurt R, Bayrak M, Aktas B, Ozdogan O. Noninvasive assessment of liver fibrosis with the aspartate transaminase to platelet ratio index (APRI): Usefulness in patients with chronic liver disease: APRI in chronic liver disease. Hepat Mon. 2011;11:103-6.
28. Wai CT, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS, Lok AS. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. Hepatology. 2003;38:518-26.
29. Vallet-Pichard A, Mallet V, Nalpas B, Verkarre V, Nalpas A, Dhalluin-Venier V et al. FIB-4: an inexpensive and accurate marker of fibrosis in HCV infection. comparison with liver biopsy and fibrotest. Hepatology. 2007;46:32-6.
30. Cross TJ, Rizzi P, Berry PA, Bruce M, Portmann B, Harrison PM. King's Score: an accurate marker of cirrhosis in chronic hepatitis C. Eur J Gastroenterol Hepatol. 2009;21:730-8.