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Original Article

# Are noninvasive biochemical parameters an alternative to liver biopsy in patients with chronic hepatitis B?

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## Abstract

**Aim:** Although there is a gold standard liver biopsy in showing the histological activity of the disease, there are contradictory results in the studies conducted for using some non-invasive methods that are alternatively biochemically calculated due to being an invasive procedure. We aimed to investigate the relationship of FIB-4, APRI, API and AAR values with histopathology in patients undergoing liver biopsy due to chronic hepatitis B virus (HBV) infection.

**Materials and Method:** Patients with follow-up between November/2016-October/2019 with the diagnosis of chronic HBV were included. Demographic data and histopathological data were documented. Accompanying comorbid diseases, medications and previous operations were questioned. Patients were grouped as mild, moderate and advanced fibrosis according to fibrosis scoring. Sensitivity, specificity, cutt-of, AUC values of biochemical parameters were calculated between the groups.

**Results:** A total of 151 patients, 64(42.3%) women, were included in the study. As a result of the liver biopsy; mild fibrosis was found in 73(48.3%) patients, moderate fibrosis in 33(21.8%) patients and advanced fibrosis in 45(29.9%) patients. There was a significant correlation between fibrosis level and age, liver function tests, bilirubin and albumin ($p<0.05$). While there was no relationship between fibrosis stage and HBV-DNA, there was a significant relationship between groups with AAR, API, APRI and FIB4 ($p<0.05$).

**Conclusions:** Although FIB-4, APRI, AAR and API values are important in determining the level of hepatic fibrosis, the effect of biochemical parameters on various factors negatively affects the specificity and sensitivity of these tests. For this reason, liver biopsy is still seen as the gold standard.

**Keywords:** APRI; chronic hepatitis B; FIB-4; fibrosis

## INTRODUCTION

Hepatitis B virus (HBV) is an enveloped virus with partial double helix circular DNA. It can only infect humans and hepatocytes (1). HBV is still the most common cause of chronic hepatitis, cirrhosis, and hepatocellular cancer (HCC), and about 350 million people worldwide are infected (2). Approximately 20-30% of chronic HBV patients develop cirrhosis with end-stage liver failure (3). This reveals the importance of HBV diagnosis and treatment. Although HBV diagnosis is made by laboratory tests, the treatment decision is made based on the result of liver biopsy, which is mostly an invasive procedure. Non-invasive methods are not as clear as liver biopsy. Today, the gold standard is still liver biopsy in demonstrating the disease's fibrosis stage and histological activity (4,5).

Transient elastography is a non-invasive method and can provide information about the level of fibrosis (6). However, the fact that the examination is expensive makes accessibility difficult. In addition, some causes such as obesity, bilirubin height and presence of necroinflammatory activity restrict transient elastography (7,8). In studies conducted on non-invasive tests, fibrosis and some values of calculated values such as Fibrosis-4 (FIB4), Aspartate Amino Transferase-Platelet Ratio Index (APRI), Aspartate Amino Transferase-Alanine Amino Transferase Ratio (AAR), Age-Platelet Index (API) It is thought to be related to the histological stage. Following the studies, the use of APRI was added to the diagnosis and treatment guideline in 2015, where the examination was limited by the World Health Organization (WHO) (7). Although there are some differences in recent studies, it has been observed that FIB-4, APRI, AAR and API values increase in patients with chronic viral hepatitis (8-11).

In our study, we aimed to examine the relationship of FIB-4, APRI, API and AAR values with histopathologically

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determined fibrosis in patients undergoing liver biopsy due to chronic hepatitis B.

## MATERIALS and METHODS

### Study Design

The study included 151 patients with the diagnosis of chronic HBV from our Hospital gastroenterology and hepatology outpatient clinic between November 2016 and October 2019. Our study was designed retrospectively from single center. Patients who had HBsAg positivity and / or liver function test levels with a high course of six months and who had HBV-DNA positive (HBV DNA>2.000 IU/mL) in the serum sample studied with polymerase chain reaction (Roboscreen, Germany) were evaluated as chronic HBV and liver biopsy was performed. Patients with chronic HBV and volunteers are included in the study.

### Exclusion Criteria

Patients with alcohol and cigarette use, with antibiotics and anti-inflammatory drugs use, with hepatitis D virus (HDV), hepatitis C virus (HCV), human immundeficiency virus (HIV), patients with other etiology that may lead to chronic liver disease such as autoimmune hepatitis, metabolic liver disease, alcohol, patients with decompensated liver disease, patients with hepatocellular carcinoma and other malignancies, patients had liver surgery, liver and other organ transplantation, chronic cardiovascular, respiratory, endocrine, hematological and renal problems, and pregnant women were excluded in the study. Also patients who received blood transfusions were excluded from the study because it may affect the biochemical parameters used in the formulation, which are related to the severity of liver disease.

### Data Evaluation

Demographic data (age, gender) of all patients were recorded. Histopathological data of the patients were documented. Accompanying comorbid diseases, medications and previous operations were questioned. Comparison was made in terms of biochemical parameters in chronic HBV patient groups. In addition, according to fibrosis scoring, the sensitivity, specificity, cutt-of and AUC values of biochemical parameters were calculated among patients with mild fibrosis, pronounced fibrosis and advanced fibrosis (cirrhosis).

### Biochemical and Hematological Measurements

Biochemical parameters taken before liver biopsy were analyzed. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyl transpeptidase (GGT), Total bilirubin, Direct bilirubin, Total protein, albumin, prothrombin time (PT), INR and alpha fetoprotein (AFP) ) level views. Some formulas were calculated from the biochemical parameters.

1. AAR: It was obtained by dividing AST by ALT (12).

2. API: It is the sum of the points from the age and PLT group. Age (years): <30 = 0; 30-39 = 1; 40-49 = 2; 50-59 = 3; 60-69 = 4; > 70 = 5. PLT count (109 / L): ≥ 225 = 0; 200-224 = 1; 175-199 = 2; 150-174 = 3; 125-149 = 4; ≤ 125 = 5 (13).

3. APRI: Calculated using the formula [(AST / ULN) / PLT (109 / L)] × 100, a score of ≤ 0.5 was considered to be indicative of the absence of fibrosis and a> 1.5 significant fibrosis (14).

4. FIB4: The FIB-4 score = (age x AST) / platelet value x (ALT) 1/2 was calculated using the formula, ≤ 1.5 was considered to be indicative of the absence of fibrosis and ≥ 3.25 as an indication of the presence of pronounced fibrosis (15).

### Liver Biopsy and Histopathological Evaluation

Liver biopsy was performed using ultrasonography guided 16G biopsy needle. The liver biopsies taken were sent to the pathology laboratory in 10% formaldehyde. After routine tissue follow-up, tissue samples embedded in paraffin were cut into 5 micron thickness and stained with routine Hematoxylin-eosin (H-E) and Masson trichrome and evaluated under the light microscope. Numerical biopsy material length of less than 1.5 cm and the number of portal areas insufficient (less than 11 portal areas) for evaluation were excluded. The materials were evaluated by three experienced pathologists without clinical information. Knodell histological activity score was used to perform grade and staging (16). The presence of fibrosis in liver biopsies was made according to the Scheuer score (17). Those with a fibrosis score of 1 and 2 were considered to be mild, those with a score of 3 and 4 were moderate, and those with a score of 5 and 6 had advanced fibrosis (cirrhosis).

### Ethical Statement

Ethical approval for this study was obtained from the Ethics Committee of our hospital (Date: 07.03.2019 Decision number: 2019/05). All procedures were in accordance with the ethical standards of our institution's human experiment committee and the Helsinki Declaration. Written informed consent forms were obtained from all participants in the study.

### Statistical Analysis

The results of our study were analyzed with the program "The Statistical Package for the Social Sciences 19.0 (SPSS Armonk, NY: IBM Corp.)". Data with continuous values were given as mean (± standard deviation), categorical data as frequency and percentage (n,%). The data were tested for compliance with the normal distribution using the kolmogorov-simirnov test, histogram and ± sd. Parametric data of the groups were compared using one-anova test and comparisons between the binary groups were made using the post-hoc test. Chi-square test was used to test categorical data. Cases with $p < 0.05$ were considered statistically significant.

## RESULTS

A total of 151 patients, 64 (42.3%) women and 87 (57.7%) men, were included in the study. As a result of the liver biopsy; Mild fibrosis (steage 1-2) in 73 (48.3%) patients, moderate fibrosis (steage 3-4) in 33 (21.8%) patients, and advanced fibrosis (cirrhosis: stage 5-6) in 45 (29.9%) patients. In the demographic comparison between three

separate groups made according to the fibrosis stages; While there was no significant difference between sex, the age was found to be significantly higher in moderate and advanced fibrosis (p <0.05). n the comparison of liver function tests and bilirubin; It was observed that the bilirubin, Protrombin time and INR values increased significantly as the level of fibrosis increased. It was also observed that albumin level decreased significantly (p <0.05). There was no significant relationship between HBV DNA level and fibrosis stage (p> 0.05). In the comparison between some formulas made using biochemical parameters and fibrosis levels; AAR, API, APRI and FIB4 were significantly associated with the groups (p <0.05) (Table 1).

In our ROC analysis to distinguish mild fibrosis from moderate fibrosis, AUC for FIB 4 was found to be 0.799, and when 0.965 was taken as cut-off, sensitivity and specificity were 75.8% -74%, respectively. In our ROC analysis to distinguish mild fibrosis from moderate fibrosis, AUC was detected as 0.731 for API, and when taken as 2,500 cut-off, sensitivity and specificity were 78.8% to 67.5%, respectively. In our ROC analysis to distinguish mild fibrosis from moderate fibrosis, AUC for APRI was 0.648, and when taken as 0.2544 cut-off, sensitivity and specificity were 72.7% to 57.5%, respectively (Table 2).

In our ROC analysis to distinguish moderate fibrosis from advanced fibrosis, AUC for FIB 4 was found to be 0.944, and when taken as 2,265 cut-off, sensitivity and specificity were 84.4% to 90.9%, respectively. In our ROC analysis to distinguish moderate fibrosis from advanced fibrosis, AUC was detected as 0.93 for API, and sensitivity and specificity were found as 84.4% -87.9%, respectively, when it was taken as 5.5 cut-off. In our ROC analysis to distinguish moderate fibrosis from advanced fibrosis, AUC was detected as 0.839 for APRI, and when taken as 0.6294 cut-off, sensitivity and specificity were found to be 77.8% to 81.8%, respectively. In our ROC analysis to distinguish moderate fibrosis from advanced fibrosis, AUC for AAR was found 0.705, and when 1.1266 was taken as cut-off, sensitivity and specificity were 62.2% - 72.7%, respectively (Table 3).

**Table 1. Comparison of fibrosis stage with demographic and laboratory parameters in chronic HBV patients**

| | Mild fibrosis (Stage 1-2) (n=73) | Moderate fibrosis (Stage 3-4) (n=33) | Advanced fibrosis (Stage 5-6) (n=45) | p value |
|---|---|---|---|---|
| **Sex (Male/Female)** | 34/39 | 20/13 | 33/12 | **0.016** |
| **Age (mean, SD)** | 41.8±12.6 | 51.7±9.9 | 56.2±8.8 | **<0.001** |
| **AST (U/L)** | 26.18±15.0 | 43.3±47.8 | 42.8±29.8 | **0.003** |
| **ALT (U/L)** | 35.3±32.5 | 52.7±67.3 | 35.9±30.5 | 0.118 |
| **ALP (U/L)** | 81.6±27.3 | 81.8±33.1 | 113.6±57.0 | **<0.001** |
| **GGT (U/L)** | 17.2±10.5 | 34.3±33.4 | 81.8±96.6 | **<0.001** |
| **Albumin (gr/dL)** | 4.35±0.46 | 4.30±0.39 | 3.40±0.67 | **<0.001** |
| **Total bilirubin (mg/dL)** | 0.55±0.26 | 0.83±0.44 | 2.18±3.17 | **<0.001** |
| **Direct bilirubin (mg/dL)** | 0.59±3.37 | 0.28±0.44 | 1.09±1.98 | 0.393 |
| **INR** | 1.04±0.07 | 1.06±0.08 | 1.15±0.09 | **<0.001** |
| **Protrombin time (second)** | 12.4±0.9 | 12.7±1.1 | 13.5±0.7 | **<0.001** |
| **HBV DNA($10^6$)** | 195.57±991.3 | 123.0±429.7 | 4.1±18.5 | 0.407 |
| **AAR** | 0.93±0.33 | 1.05±0.42 | 1.31±0.42 | **<0.001** |
| **API** | 2.46±1.24 | 3.72±1.56 | 7.35±1.67 | **<0.001** |
| **APRI** | 0.31±0.21 | 0.56±0.67 | 1.25±0.83 | **<0.001** |
| **FIB4** | 0.82±0.3 | 1.41±0.63 | 4.45±2.14 | **<0.001** |

AST:Aspartate transaminase, ALT:Alanine transaminase, GGT:Gamma-glutamyltransferase, ALP: Alkaline phosphatease, SD: Standart deviation

**Table 2. Biochemical parameters showing distinction between the mild fibrosis stage and moderate fibrosis stage in chronic HBV patients**

| | AUC | Cut-off | p | CI 95% | Sensitivity | Specificity |
|---|---|---|---|---|---|---|
| **FIB4** | 0.799 | 0.965 | **<0.001** | 0.699-0.898 | %75.8 | %74 |
| **API** | 0.731 | 2.500 | **<0.001** | 0.624-0.837 | %78.8 | %67.5 |
| **APRI** | 0.648 | 0.2544 | **0.015** | 0.527-0.769 | %72.7 | %57.5 |

**Table 3. Biochemical parameters showing distinction between the moderate fibrosis stage and advanced fibrosis stage (cirrhosis) in chronic HBV patients**

| | AUC | Cut-off | p | CI 95% | Sensitivity | Specificity |
|---|---|---|---|---|---|---|
| **FIB4** | 0.944 | 2.265 | **<0.001** | 0.899-0.990 | %84.4 | %90.9 |
| **API** | 0.93 | 5.5 | **<0.001** | 0.876-0.984 | %84.4 | %87.9 |
| **APRI** | 0.839 | 0.6294 | **<0.001** | 0.744-0.934 | %77.8 | %81.8 |
| **AAR** | 0.705 | 1.266 | **0.002** | 0.579-0.832 | %62.2 | %72.7 |

## DISCUSSION

HBV infection still continues as a health problem in the world. Most noninvasive examinations used in diagnosis and follow-up are not sufficient to make treatment decisions. Therefore, gold standard is liver biopsy in showing hepatic tissue damage (4,5). Although the biopsy procedure seems simple, it is an interventional procedure that may have complications (18). In recent studies, it has been concluded that some parameters have close sensitivity and specificity with biopsy in showing hepatic fibrosis (19-21). Since these values are calculated according to the results of the tests studied from the blood samples taken, they do not generate costs and are advantageous in terms of accessibility. In recent studies, it has been concluded that FIB-4 and APRI values can be extremely good indicators for hepatic fibrosis, although the data differ. However, doubts remain that the calculated parameters could replace liver biopsy (19-24). Wang et al. stated that FIB-4, APRI, AAR values have high sensitivity and specificity in determining hepatic fibrosis, but cannot be used instead of biopsy (11). Fazley et al. stated that AAR value is similar in patient and control groups, therefore it is not sufficient to differentiate fibrosis, and FIB-4 and APRI values have more significant results than control group (21). In our study, FIB-4 and APRI values were found to be significant both in distinguishing mild fibrosis from moderate fibrosis and in distinguishing moderate fibrosis from advanced fibrosis. In our analysis for AAR values, it was shown that there was no significant difference between mild fibrosis and moderate fibrosis, but significant in distinction between moderate fibrosis and advanced fibrosis.

Similarly, it was concluded that API value was correlated with fibrosis stage in many studies conducted on different populations (22-24). The relationship between API value and fibrosis stage was investigated by Poynard et al. for the first time in Chronic Hepatitis C patients and it was reported that there was a significant relationship between them (13). Korkmaz et al. also found that the value of API is significant in the distinction of mild and advanced fibrosis in HBV (22). In parallel with the current studies, in our study, there was a significant relationship between API and fibrosis level. It was seen that it is an important parameter in differentiating mild fibrosis from moderate fibrosis and also distinguishing moderate fibrosis from advanced fibrosis.

Although our study is an important study examining the relationship between biochemical parameters and fibrosis, it contains some limitations. First of all, since our study was designed retrospectively, the accuracy of all results may not be clear. In addition, even if all the reasons that biochemical parameters may be affected are excluded, some situations such as medication and infection that may affect the level of these parameters may not be detected, which may lead to different results. In addition, the presence of all stages of fibrosis in our patient group, and the comparison of fibrosis stages in this context, and a large sample number are among the strengths of our study.

## CONCLUSION

In conclusion, FIB-4, APRI, AAR and API values were shown to be important in determining the level of hepatic fibrosis. However, the changes in the biochemical values of the patients with different factors, at different times and being affected by non-liver causes negatively affect the specificity and sensitivity of these tests. For this reason, liver biopsy is still seen as the gold standard. More comprehensive studies are needed for non-invasive examinations to replace liver biopsy.

*Competing Interests: The authors declare that they have no competing interest.*

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*Ethical Approval: Ethical approval for this study was obtained from the Ethics Committee of our hospital (Date: 07.03.2019 Decision number: 2019/05).*

## REFERENCES


1. Akarca SU. Structure, Molecular Biology, Natural Mutants and Variants of Hepatitis B Virus. J Gastroenterohepatol Special Topics 2010;3:16-23.
2. Perz JF, Armstrong GL, Farrington LA, et al. The contributions of hepatitis B virüs and hepatitis C virüs infections to cirrhosis and primary liver cancer worldwide. J Hepatol 2006;4:529-38.
3. Fattovich G, Brollo L, Giustina G, et al. Natural history and prognostic factors for chronic hepatitis type B. Gut 1991;32:294-8.
4. Terrault NA, Bzowej NH, Chang KM, et al. American Association for the Study of Liver Diseases. AASLD guidelines for treatment of chronic hepatitis B. Hepatology 2016;63:261-83.
5. Sarin SK, Kumar M, Lau GK, et al. Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update. Hepatol Int 2016;10:1-98.
6. Seo YS, Kim MY, Kim SU, et al. Accuracy of transient elastography in assessing liver fibrosis in chronic viral hepatitis: a multicentre, retrospective study. Liver Int 2015;35:2246-55.
7. World Health Organization. Guidelines for the prevention, care and treatment of persons with chronic hepatitis B infection. Geneva: World Health Organization; 2015. Geneva, Switzerland; 2015. pp.15-8.
8. Kim DY, Kim SU, Ahn SH, et al. Usefulness of FibroScan for detection of early compensated liver cirrhosis in chronic hepatitis B. Dig Dis Sci 2009;54:1758-63.
9. Kekilli M, Tanoglu A, Sakin YS, et al. Is the neutrophil to lymphocyte ratio associated with liver fibrosis in patients with chronic hepatitis B? World J Gastroenterol 2015;21:5575-81.
10. Ozel Coskun BD, Dizdar OS, Baspinar O, et al. Usefulness of the Neutrophil-to-lymphocyte Ratio and Platelet Morphologic Parameters in Predicting Hepatic Fibrosis in Chronic Hepatitis C Patients Ann Clin Lab Sci 2016;46:4-8.
11. Wang J, Yan X, Yang Y, et al. A novel predictive model using routinely clinical parameters to predict liver fibrosis in patients with chronic hepatitis B. Oncotarget 2017;8:59257-67.
12. Sheeth SG, Flamm SL, Gordon FD, et al. AST/ALT ratio predicts cirrhosis in patients with chronic hepatitis C virus infection. Am J Gastroenterol 1998;93:44-8.
13. Poynard T, Bedossa P. Age and platelet count: A simple index for predicting the presence of histological lesions in patients with antibodies to hepatitis C virus. METAVIR and CLINIVIR Cooperative Study Groups. J Viral Hepat 1997;4:199-208.
14. Chrysantos NV, Papatheodoritis GV, Savvas S, et al. Aspartate aminotrasferase to platelet ratio index for fibrosis evaluation in chronic viral hepatitis. Eur J Gastroenterol Hepatol 2006;18:389-96.
15. Sterling RK, Lissen E, Clumerk N, et al. Development of a simple noninvazive index to predict significant fibrosis in patients with HIV/HCV coinfection. Hepatology 2006;43:1317-25.
16. Knodell RG, Ishak KG, Black WC, et al. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. Hepatology 1981;1:431-5.
17. Desmet VJ, Gerber M, Hoofnagle JH, et al. Classification of chronic hepatitis: Diagnosis, grading and staging. Hepatology 1994;19:1513-20.
18. Regev A, Berho M, Jeffers LJ, et al. Sampling error and intraobserver variation in liver biopsy in patients with chronic HCV infection. Am J Gastroenterol 2002;97:2614-8.
19. Ucar F, Sezer S, Gunes Z, et al. APRI, the FIB-4 score, and Forn's index have noninvasive diagnostic value for liver fibrosis in patients with chronic hepatitis B. Eur J Gastroenterol Hepatol 2013;25:1076-81.
20. Akin MS, Yeniova AO, Demir O. Non-invasive prediction of fibrosis in chronic hepatitis B. Ann Med Res 2019;26:1588-93.
21. Sha FR, Uddin M, Abuelezz NZ, et al. Investigating the Efficiency of APRI, FIB-4, AAR and AARPRI as Noninvasive Markers for Predicting Hepatic Fibrosis in Chronic Hepatitis B Patients in Bangladesh. Open Microbiol J 2019;13:34-40.
22. Korkmaz P, Demirturk N, Batirel A, et al. Noninvasive Models to Predict Liver Fibrosis in Patients with Chronic Hepatitis B: A Study from Turkey. Hepat Mon 2017;17:60266.
23. Eminler AT, Ayyildiz T, Irak K, et al. AST/ALT ratio is not useful in predicting the degree of fibrosis in chronic viral hepatitis patients. Eur J Gastroenterol Hepatol 2015; 27:1361-6.
24. Xiao G, Yang J, Yan L. Comparison of diagnostic accuracy of aspartate aminotransferase to platelet ratio index and fibrosis-4 indexfor detecting liver fibrosis in adult patients with chronic hepatitis B virus infection: a systemic review and meta-analysis. Hepatology 2015; 61:292-302.