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Türk Hijyen ve Deneysel Biyoloji Dergisi

# The effect of HBeAg positivity on susceptibility in noninvasive methods used to detect liver injury in chronic hepatitis B patients

## Kronik hepatit B hastalarında karaciğer hasarını tespit etmek için kullanılan noninvaziv yöntemlerde HBeAg pozitifliğinin duyarlılığa etkisi

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### ABSTRACT

**Objective:** Hepatitis B is a common health problem and deaths from acute or chronic hepatitis B are more than 600,000 per year. Liver damage that develops throughout the course of the disease forms the basis of mortality and morbidity. The most accurate and most difficult method to detect the extent of liver damage is liver biopsy. There are conflicting data in the literature regarding noninvasive methods.In HBeAg+ and HBeAg- patients, liver cell damage develops by different physiopathological mechanisms. We aimed to find more sensitive results by evaluating HBeAg + and HBeAg - patients in separate groups, which are biomarkers used as an alternative to liver biopsy.

**Methods:** Patients over the age of 18, who were followed by the Infectious Diseases Clinics of Dışkapı Yıldırım Beyazıt Training and Research Hospital and Yıldırım Beyazıt University Yenimahalle Training and Research Hospital between 2010 and 2020, with HBs ag positivity for more than six months and with HBV DNA >2000 IU were evaluated. The patients were divided into two groups according to the presence of HBeAg.

### ÖZET

**Amaç:** Hepatit B yaygın bir sağlık sorunudur ve akut veya kronik hepatit B'den ölümler yılda 600.000'den fazladır. Hastalığın seyri boyunca gelişen karaciğer hasarı, mortalite ve morbiditenin temelini oluşturur. Karaciğer hasarının boyutunu tespit edebilmenin en doğru ve en zor uygulanan metodu karaciğer biyopsisidir. Biyobelirteçlerle geliştirilen noninvaziv metodlar ile ilgili ise literatürde çelişkili veriler mevcuttur. HBeAg+ ve HBeAg- hastalarda karaciğer hücre hasarı farklı fizyopatolojik mekanizmalarla gelişir. Karaciğer biyopsisine alternatif olarak kullanılan biyobelirteçleri HBeAg+ ve HBeAg- hasta gruplarında ayrı ayrı değerlendirerek daha duyarlı sonuçlara ulaşmayı amaçladık.

**Yöntem:** 2010-2020 yılları arasında Dışkapı Yıldırım Beyazıt Eğitim ve Araştırma Hastanesi ve Yıldırım Beyazıt Üniversitesi Yenimahalle Eğitim ve Araştırma Hastanesi Enfeksiyon Hastalıkları Kliniği'nde altı aydan uzun süredir HBsAg pozitifliği ile takip edilen 18 yaş üstü ve HBV DNA >2000 IU olan hastalar değerlendirildi. Hastalar HBeAg varlığına göre iki gruba ayrıldı. Daha

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Afterwards, each group was divided into mild fibrosis or non-fibrosis group with a fibrosis score of less than 3, and patients with a score of 3 and above in the advanced fibrosis group, according to the histologically determined treatment indication. Indirect fibrosis indicators were evaluated separately in all patients and in all subgroups.

**Results:** 191 CHB patients were included in the study. 89 male and 102 female patients comprised the entire cohort. Among the patients, there were 89 patients with fibrosis 3 or more, and 102 patients with fibrosis below 3. There were 48 HBeAg positive patients in the whole patient group and the patients were equally distributed regardless of fibrosis. No noninvasive marker was found to detect fibrosis in the entire cohort, only the histological activity index was associated with fibrosis. In the HBeAg-positive patient group, the API score, which increased with aging and low platelet counts, was associated with fibrosis, while in the HBeAg-negative patient group, total protein was associated with fibrosis.

**Conclusion:** Determining fibrosis by non-invasive methods in chronic hepatitis B patients is important as it can be an alternative to biopsy for patient follow-up and treatment. When evaluating noninvasive methods that can detect liver damage, we emphasize the importance of evaluating patients by dividing them into appropriate subgroups. More studies are needed to determine the appropriate biomarkers to detect the severity of fibrosis in chronic hepatitis B patients.

**Key Words:** Chronic hepatitis B, biomarker, fibrosis, HBeAg

sonra her grup histolojik olarak Modifiye İshak Skoruna göre fibrozis skoru 3'ün altında olan ve fibrozis skoru 3 ve üzeri olan hastalar olmak üzere ikiye ayrıldı. Fibrozis göstergesi olabilecek biyobelirteçler tüm hastalarda ve tüm alt gruplarda ayrı ayrı değerlendirildi.

**Bulgular:** Çalışmaya 191 KHB hastası dahil edildi. Tüm kohortu 89 erkek ve 102 kadın hasta oluşturdu. Hastalar arasında fibrozis skoru 3 veya daha fazla 89 hasta ve fibrozis skoru 3 den az 102 hasta değerlendirildi. Tüm hasta grubunda 48 HBeAg pozitif hasta vardı ve hastalar fibrozis skorundan bağımsız eşit olarak dağılmıştı. Tüm kohortta gruplara ayrılmadan yapılan incelemede fibrozis şiddetini saptayacak invaziv olmayan bir belirteç bulunmadı, sadece histolojik aktivite indeksi fibrozis ile ilişkilendirildi. Gruplara ayrıldıktan sonra HBeAg pozitif hasta grubunda API skoru fibrozis ile ilişkilendirilirken, HBeAg negatif hasta grubunda total protein fibrozis ile ilişkilendirildi.

**Sonuç:** Kronik Hepatit B hastalarında fibrozis şiddetini invaziv olmayan metodlarla belirlemek hasta takip ve tedavisi için biopsiye alternatif olabileceğinden önemlidir. Bu çalışmada karaciğer hasarını tespit edebilecek noninvaziv metodlar değerlendirilirken hastaları uygun alt gruplara ayırarak değerlendirmenin önemine değiniyoruz. Kronik Hepatit B hastalarında fibrozisin şiddetini etkin şekilde saptayacak biyobelirteçlerin tespiti için daha fazla çalışmaya ihtiyaç vardır.

**Anahtar Kelimeler:** Kronik hepatit B, biyobelirteç, fibrozis, HBeAg

## INTRODUCTION

Hepatitis B virus (HBV) infection is a significant health problem worldwide. More than 600 thousand patients die annually due to acute or chronic HBV infections and related complications (1). Currently, chronic HBV infection, which is moderately endemic in Turkey, is present in more than 350 million people worldwide (2). Although chronic HBV infection can usually cause cirrhosis or hepatocellular carcinoma, it may also present with a clinical picture including persistent viremia with normal aminotransferase

levels and liver histology. (3). While HBV continues to multiply in liver cells during the chronic infection process, liver damage develops mainly secondary to the body's immune response through T cells (4). The Hepatitis B e antigen (HBeAg) is an antigenic component of HBV that indicates viral replication and cannot be continuously detected in chronic HBV infection (5). It has been shown that HBeAg suppresses T cells, and the immune mechanism leading to its clearance has not been clarified yet. This antigen suppresses cellular immunity, allowing viral particles to multiply and accumulate in hepatocytes, developing direct cytopathic effects (6, 7). Otherwise cellular immune response in CHB patients mostly develops after HBeAg clearance, with decreased suppressive effects of this antigen on T cells and liver cell cytopathy of cellular immunity.

It was previously suggested that non-invasive markers such as aspartate aminotransferase (AST)/ thrombocyte (PLT), red cell distribution width (RDW)/PLT, AST/alanine aminotransferase (ALT) and neutrophil/lymphocyte ratio could predict liver damage (2,8,9). These studies investigated the efficacy of noninvasive biomarkers in predicting liver fibrosis, but did not compare them between HbeAg positive and negative patients. In addition, since different pathophysiological processes can lead to liver damage, the sensitivities of these non-invasive indicators may differ in various settings.

It is widely accepted that the introduction of non-invasive markers with high sensitivity can negate the need for liver biopsy and facilitate the treatment and follow-up of patients with liver damage. Therefore, this study aimed to compare the efficacy of the non-invasive markers in predicting liver fibrosis between HBeAg positive and HBeAg negative patients.

## MATERIAL and METHOD

Data of the adult (age>18) patients who were followed-up at the Infectious Diseases and Clinical Microbiology departments of the Ankara Diskapi Yildirim Beyazit Training and Research Hospital and Yildirim Beyazit University Yenimahalle Training and Research Hospital were retrospectively reviewed. Patients who were followed-up between January 2010 and December 2020 with the diagnosis of HBsAg positive chronic HBV infection for more than six months constituted the target population of this study. All patients had an HBV DNA level of higher than 2000 IU. All study participants were naiv chronic HBV (CHB) patients. Patients who consume alcohol regularly, are on or anti-inflammatory drugs, and had a history of antiviral use, kidney failure, chronic inflammatory disease, diabetes mellitus, malignancy, or other liver disease were excluded.

Demographic, laboratory and radiological data of the study participants were retrieved from computerized patient databases of the hospitals and the Türkiye Ministry of Health.

All study participants had undergone a liver biopsy, and experienced histopathologists assessed the specimens by histological activity index (HAI) and fibrosis scoring via the modified Ishak scoring system (10,11). First, the patients were divided into two groups as per the presence or absence of HbeAg. Subsequently, each group was divided into "mildly fibrous" or "non-fibrous" (i.e., fibrosis score <3) and "advanced fibrosis" (i.e., fibrosis score ≥3) subgroups based on the fibrosis scores.

Laboratory data at the time of biopsy included the measurements of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, total protein, platelet, globulin, international normalization ratio (INR), leukocyte, lymphocyte, neutrophil, alpha-fetoprotein (AFP), HBV DNA levels, red cell distribution volume (RDW) and platelet distribution volume (PDW).

Indirect fibrosis indicators were calculated using the following formulas: neutrophil/lymphocyte ratio, AST-platelet ratio index (APRI) [(AST/AST upper limit)/PLT (109/L)×100], and age-platelet index (API) [Age< 30:0, age 30-39:1, 40-49:2, 50-59:3, 60-69:4, age>70:5, PLT count (109/L): ≥225:0, 200-224:1,

175-199:2, 150-174:3, 125-149:4, PLT count≤125:5. The index is calculated by summing the scores of regarding age and PLT counts] (12-18).

Anti HBe were analyzed by chemiluminescent microparticle immunoassay kits (ARCHITECT i2000 system; ARCHITECT, Abbott Park, Wiesbaden-Delkenheim, Germany), and HBV-DNA levels were analyzed by LightCycler real-time polymerase chain reaction (PCR) System (Corbett Research Rotor Gene 6000 and HBV QS-RGQ Kit, Qiagen, Germany). HBV DNA PCR results were reported as international units (IU).

All statistical analyzes were performed using the Statistical Package for Social Sciences (SPSS) software (IBM SPSS Statistics v24). Frequency tables and descriptive statistics were used for the interpretation of the data. The independent samples t-test (t-table value) was used to compare two independent groups. The Analysis of Variance (ANOVA) test (F-table value) method was used to compare three or more independent groups. The Tukey test was performed for pairwise comparisons of the variables with significant differences between three or more groups. Two independent groups were compared using the Mann-Whitney U test (Z-table value) as per non-parametric methods. A comparison of three or more independent groups was made by performing the Kruskal-Wallis H test ($x^2$-table value). Bonferroni correction was used for pairwise comparisons of three or more groups with significantly different variables. Pearson-$x^2$ cross tables were used to analyze the relationships between qualitative variables. Backward LR model was used for binary logistic regression analysis to determine the factors affecting the treatment requirement and cirrhosis status (i.e., fibrosis scores). Receiver operating characteristic (ROC) curves were used to determine the variables' diagnostic values.

The study was approved by the Ankara Yildirim Beyazit Training and Research Hospital Clinic Researches Ethics Committee (Date: 05.04.2021 and Number: 108/04).

## RESULTS

After applying the inclusion and exclusion criteria, 191 patients were included in the study. Among these patients, 89 were male, and 102 were female. The mean age of all patients was 45,57. Histopathological examinations revealed that 89 patients had fibrosis scores of three or higher, while 102 patients had scores of less than three. Demographic, histopathological, and laboratory data of these patients are displayed in Table 1. In the multiple regression performed between two groups, no biomarkers that could indicate fibrosis were detected (Table 2).

There were 48 patients in the HbeAg positive and 143 in the HBeAg negative patient groups. The rate of the patients with fibrosis scores of 3 or above was 52.1% (n=25) and 44,8% (n=64) in the HBeAg positive and HbeAg negative patient groups, respectively (p>0,05) (Table 3).

Fibrosis-related parameters were compared between HBeAg positive and negative patient groups. A statistically significant difference was found in the HBeAg negative patient group in terms of AST, ALT, globulin, total protein, AFP, HBV DNA, HAI, and APRI scores (p<0.05) (Table 4).

The logistic regression analysis revealed that HAI and serum total protein levels were significantly associated with fibrosis scores, and the cut-off value was 5.5 as per ROC analysis (p<0.05, OR=2.123, p<0.05, OR=10.516) (Table 5, Figure 1).

Comparison of demographic and laboratory parameters according to fibrosis scores in HbeAg positive patients revealed that the two groups differed significantly in terms of age, AST, ALT, HAI, API and APRI (p<0.05) (Table 6).

The logistic regression analysis elucidated that HAI and API scores were significantly associated with fibrosis; the cut-off value was 5.5 as per ROC analysis (p<0.05, OR=2.944, p<0.05, OR=5.512) (Table 7) (Figure 2).

**Table 1.** Comparison of the demographic and laboratory parameters as per fibrosis scores

| | Fibrosis<3 (n=102) | | Fibrosis≥ 3 (n=89) | | Statistical Analysis * |
|---|---|---|---|---|---|
| | $\bar{X} \pm S.S.$ | Median [IQR] | $\bar{X} \pm S.S.$ | Median [IQR] | |
| Age (year) | 44.17±12.17 | 44.0 [20.0] | 47.19±14.81 | 48.0 [23.0] | t=-1.549 p=0.123 |
| AST | 32.60±34.70 | 24.5 [12.3] | 62.80±70.52 | 36.0 [46.5] | Z=-5.23 **p=0.000** |
| ALT | 38.34±75.72 | 23.7 [18.1] | 68.94±72.10 | 43.0 [53.0] | Z=-5.224 **p=0.000** |
| PLT | 236.59±57.73 | 235.0 [76.8] | 231.18±63.68 | 218.0 [89.0] | Z=-0.586 p=0.558 |
| Neutrophil | 4638.33±1446.77 | 4505.0 [1945.0] | 4911.12±1504.76 | 4900.0 [2045.0] | t=-1.276 p=0.204 |
| Leukocyte | 7147.06±1685.01 | 7110.0 [2345.0] | 7494.72±1991.73 | 7210.0 [2760.0] | Z=-0.903 p=0.367 |
| Lymphocyte | 2248.13±655.79 | 2120.0 [800.0] | 2395.76±861.86 | 2190.0 [1000.0] | Z=-1.056 p=0.291 |
| INR | 1.04±0.09 | 1.0 [0.1] | 1.18±1.19 | 1.0 [0.1] | Z=-0.200 p=0.842 |
| RDW | 13.69±1.66 | 13.4 [1.7] | 14.34±4.66 | 13.5 [1.6] | Z=-0.706 p=0.480 |
| PDW | 22.10±14.55 | 16.3 [2.1] | 20.45±11.10 | 16.4 [1.5] | Z=-0.952 p=0.341 |
| Albumin | 4.34±0.34 | 4.4 [0.4] | 5.09±5.55 | 4.3 [0.5] | Z=-1.227 p=0.220 |
| Globulin | 3.00±0.40 | 3.1 [0.6] | 3.22±0.42 | 3.2 [0.4] | t=-2.861 **p=0.005** |
| Total protein | 7.41±0.49 | 7.4 [0.7] | 7.57±0.54 | 7.6 [0.6] | t=-1.625 p=0.107 |
| AFP | 2.77±1.65 | 2.4 [2.1] | 3.34±2.03 | 3.0 [2.1] | Z=-2.109 **p=0.035** |
| HBV DNA | 5.56±1.79 | 4.8 [1.6] | 6.42±1.99 | 6.0 [3.2] | Z=-3.568 **p=0.000** |
| HAI | 4.70±2.05 | 4.0 [3.0] | 7.73±2.56 | 7.0 [3.0] | Z=-7.938 **p=0.000** |
| API score | 2.81±1.81 | 3.0 [2.3] | 3.45±2.12 | 3.0 [3.0] | Z=-2.061 **p=0.039** |
| APRI | 0.39±0.48 | 0.3 [0.2] | 0.76±0.94 | 0.4 [0.7] | Z=-4.652 **p=0.000** |
| Neutrophil/ Lymphocyte | 2.20±0.89 | 2.0 [1.1] | 2.22±0.95 | 2.1 [0.9] | Z=-0.164 p=0.870 |
| Platelet/ Lymphocyte | 0.113±0.040 | 0.110 [0.004] | 0.107±0.045 | 0.099 [0.050] | Z=-1.519 p=0.129 |
| AST/ALT | 1.06±0.42 | 1.0 [0.5] | 1.02±0.49 | 0.9 [0.5] | Z=-1.299 p=0.194 |
| RDW/Platelet | 0.061±0.015 | 0.059 [0.020] | 0.066±0.028 | 0.061 [0.020] | Z=-0.991 p=0.322 |
| INR/Platelet | 0.004±0.001 | 0.005 [0.000] | 0.006±0.005 | 0.005 [0.000] | Z=-0.883 p=0.377 |

***** "Independent Sample-t" test (t-table value) statistics were used to compare two independent groups with normal distribution. "Mann-Whitney U" test (Z-table value) statistics were used to compare two independent groups with non-normal distribution. AST: aspartate aminotransferase, ALT: alanine aminotransferase, PTL: platelet, RDW: Red Cell Distribution Width, PDW: Platelet Distribution Width, AFP: alpha fetoprotein, HAI: histological activity index, API: age-platelet index, APRI: spartate aminotransferase platelet ratio index.

**Table 2.** Results of the logistic regression model based on fibrosis scores

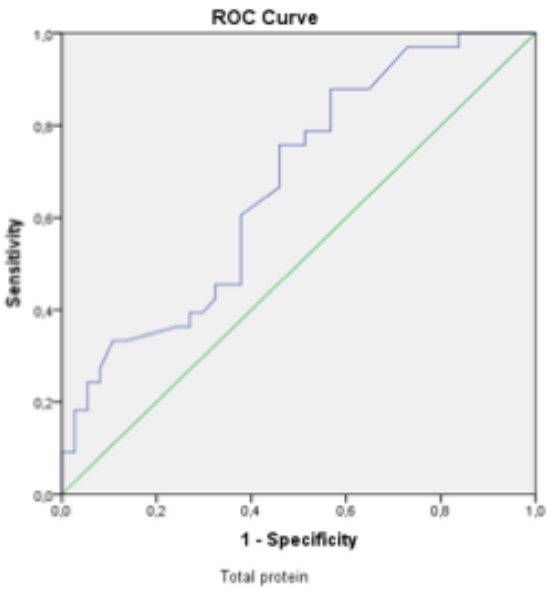
| Variable | B | S.H. | Wald | sd | p | OR | 95% Confidence Interval (OR) | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Lower limit | Upper limit |
| AST | 0.028 | 0.017 | 2.834 | 1 | 0.092 | 1.029 | 0.995 | 1.063 |
| ALT | -0.011 | 0.007 | 2.532 | 1 | 0.112 | 0.989 | 0.975 | 1.003 |
| Globulin | 1.294 | 0.671 | 3.723 | 1 | 0.054 | 3.648 | 0.980 | 13.582 |
| HAI | 0.675 | 0.151 | 19.847 | 1 | **0.000** | 1.963 | 1.459 | 2.642 |
| | -8.599 | 2.326 | 13.663 | 1 | 0.000 | | | |
| $x^2_{(8)}$=3.525; p=0.897 | | | | | | | | |

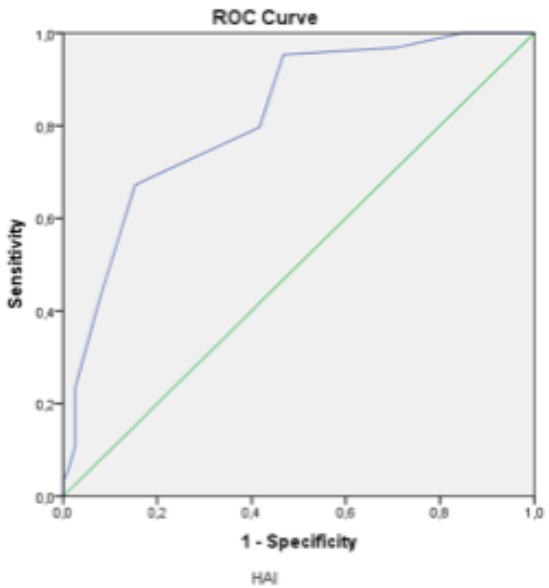
AST: aspartate aminotransferase, ALT: alanine aminotransferase, HAI: histological activity index

**Table 3.** Analysis of the relationships between HbeAg status and fibrosis scores

| Hbe Ag | Negative (n=143) | | Positive (n=48) | | Statistical Analysis * |
|---|---|---|---|---|---|
| | n | % | n | % | |
| Fibrosis<3 | 79 | 55.2 | 23 | 47.9 | $x^2$=0.509 |
| Fibrosis≥3 | 64 | 44.8 | 25 | 52.1 | p=0.476 |

* "Pearson-x2" crosstabs were used to examine the relationships between two qualitative variables.




| Variable | Area | Standard error | p | AUC %95 G.A. | | Cut-off value |
|---|---|---|---|---|---|---|
| | | | | Down | Up | |
| **Total protein** | 0.673 | 0.064 | **0.013** | 0.548 | 0.798 | 7.5 |
| **HAI score** | 0.819 | 0.035 | **0.000** | 0.751 | 0.888 | 5.5 |

HAI: histological activity index

**Figure 1.** Determination of the serum total protein level and HAI score cut-off values by ROC analysis and comparison of parameters based on fibrosis scores in HbeAg negative patients

**Table 4.** Comparison of the demographic and laboratory parameters as per fibrosis scores in HbeAg negative patients

| Variable | Fibrosis<3 (n=79) | | Fibrosis≥ 3 (n=64) | | Statistical Analysis * |
|---|---|---|---|---|---|
| | $\bar{X} \pm S.S.$ | Median [IQR] | $\bar{X} \pm S.S.$ | Median [IQR] | |
| Age (year) | 44.09±11.88 | 45.0 [20.0] | 44.97±15.66 | 45.5 [26.0] | t=-0.371 p=0.711 |
| AST | 31.54±26.14 | 25.0 [13.0] | 64.70±73.65 | 38.5 [54.3] | Z=-4.847 **p=0.000** |
| ALT | 32.28±25.07 | 24.0 [17.8] | 67.35±63.54 | 46.0 [51.8] | Z=-4.737 **p=0.000** |
| PLT | 234.34±58.82 | 235.0 [82.0] | 233.97±62.55 | 216.0 [93.8] | Z=-0.146 p=0.884 |
| Neutrophil | 4571.77±1427.62 | 4500.0 [1910.0] | 4920.94±1536.98 | 4900.0 [2047.5] | t=-1.405 p=0.162 |
| Leukocyte | 7161.14±1598.29 | 7120.0 [2010.0] | 7630.78±20.73 | 7300.0 [2867.5] | t=-1.489 p=0.139 |
| Lymphocyte | 2291.27±604.45 | 2160.0 [850.0] | 2448.64±915.34 | 2240.0 [1020.0] | Z=-0.808 p=0.419 |
| INR | 1.04±0.10 | 1.0 [0.1] | 1.23±1.40 | 1.1 [0.1] | Z=-0.325 p=0.745 |
| RDW | 13.63±1.63 | 13.3 [1.8] | 14.34±5.26 | 13.5 [1.6] | Z=-0.674 p=0.500 |
| PDW | 23.56±15.88 | 16.3 [3.8] | 22.15±12.76 | 16.6 [3.6] | Z=-0.862 p=0.388 |
| Albumin | 4.33±0.35 | 4.4 [0.4] | 5.41±6.53 | 4.3 [0.5] | Z=-1.027 p=0.305 |
| Globulin | 2.96±0.40 | 3.0 [0.6] | 3.27±0.39 | 3.2 [0.5] | t=-3.191 **p=0.002** |
| Total protein | 7.36±0.51 | 7.4 [0.9] | 7.68±0.43 | 7.6 [0.7] | t=-2.804 **p=0.007** |
| AFP | 2.59±1.61 | 2.2 [1.9] | 3.29±2.04 | 2.9 [2.2] | Z=-2.380 **p=0.017** |
| HBV DNA | 5.72±1.82 | 4.8 [1.7] | 6.86±1.88 | 6.9 [3.3] | Z=-3.952 **p=0.000** |
| HAI | 4.80±2.13 | 4.0 [3.0] | 7.67±2.46 | 7.0 [3.0] | Z=-6.616 **p=0.000** |
| API score | 2.91±1.88 | 3.0 [2.0] | 3.16±2.22 | 3.0 [4.0] | Z=-0.446 p=0.656 |
| APRI | 0.39±0.41 | 0.3 [0.2] | 0.76±0.96 | 0.4 [0.1] | Z=-4.089 **p=0.000** |
| Neutrophil/Lymphocyte | 2.09±0.76 | 1.9 [1.0] | 2.17±0.95 | 2.0 [0.8] | Z=-0.386 p=0.700 |
| Platelet/Lymphocyte | 0.109±0.036 | 0.107 [0.050] | 0.105±0.038 | 0.099 [0.040] | Z=-1.009 p=0.313 |
| AST/ALT | 1.07±0.44 | 1.0 [0.6] | 1.05±0.55 | 0.9 [0.6] | Z=-1.039 p=0.299 |
| RDW/Platelet | 0.062±0.017 | 0.060 [0.020] | 0.064±0.023 | 0.060 [0.030] | Z=-0.451 p=0.652 |
| INR/Platelet | 0.005±0.001 | 0.005 [0.000] | 0.006±0.005 | 0.005 [0.000] | Z=-0.583 p=0.560 |

***** "Independent Sample-t" test (t-table value) statistics were used to compare two independent groups with normal distribution. "Mann-Whitney U" test (Z-table value) statistics were used to compare two independent groups with non-normal distribution. AST: aspartate aminotransferase, ALT: alanine aminotransferase, PTL: platelet, RDW: Red Cell Distribution Width, PDW: Platelet Distribution Width, AFP: alpha fetoprotein, HAI: histological activity index, API: age-platelet index, APRI: spartate aminotransferase platelet ratio index.

**Table 5.** Results of the logistic regression model in HbeAg negative patients based fibrosis scores

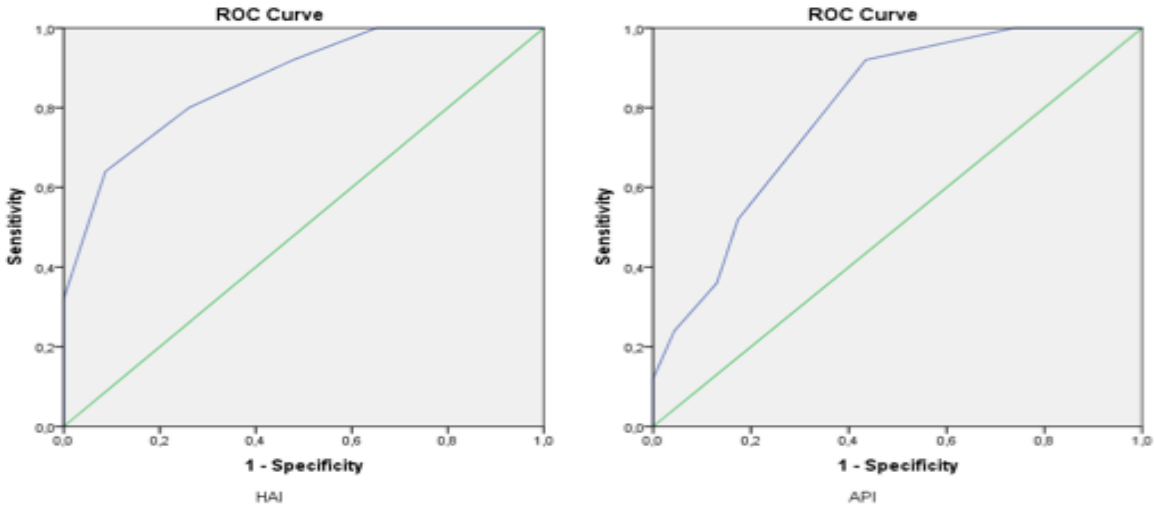
| Variable | B | S.H. | Wald | sd | p | OR | 95% Confidence Interval (OR) | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Lower limit | Upper limit |
| AST | 0.050 | 0.037 | 1.766 | 1 | 0.184 | 1.051 | 0.977 | 1.131 |
| Total protein | 2.353 | 0.943 | 6.226 | 1 | **0.013** | 10.516 | 1.656 | 36.764 |
| HAI | 0.753 | 0.214 | 12.392 | 1 | **0.000** | 2.123 | 1.396 | 3.229 |
| APRI | -3.055 | 2.961 | 1.064 | 1 | 0.302 | 0.047 | 0.000 | 15.624 |
| | -23.108 | 7.786 | 8.808 | 1 | 0.003 | 0.00 | | |
| $x^2_{(8)}$=8.307; p=0.404 | | | | | | | | |

AST: aspartate aminotransferase, HAI: histological activity index, APRI: spartate aminotransferase platelet ratio index.

**Table 7.** Results of the logistic regression model in HbeAg positive patients based on treatment risk status and fibrosis scores

| | B | S.H. | Wald | sd | p | OR | 95% Confidence Interval (OR) | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Lower limit | Upper limit |
| Age(year) | -0.119 | 0.072 | 2.733 | 1 | 0.098 | 0.888 | 0.771 | 1.022 |
| HAI | 1.080 | 0.373 | 8.395 | 1 | **0.004** | 2.944 | 1.418 | 6.113 |
| API score | 1.707 | 0.772 | 4.887 | 1 | **0.027** | 5.512 | 1.214 | 25.033 |
| | -5.504 | 2.134 | 6.654 | 1 | 0.010 | 0.004 | | |
| $x^2_{(8)}$=3.615; p=0.890 | | | | | | | | |

HAI: histological activity index, APRI: spartate aminotransferase platelet ratio index.




| Varibale | Area | Standard error | p | AUC %95 G.A. | | Cut-off value |
|---|---|---|---|---|---|---|
| | | | | Down | Up | |
| **HAI score** | 0.869 | 0.050 | **0.000** | 0.771 | 0.966 | 5.5 |
| **API score** | 0.794 | 0.065 | **0.000** | 0.666 | 0.922 | 2.5 |

HAI: histological activity index, APRI: spartate aminotransferase platelet ratio index.

**Figure 2.** Determination of the HAI and API score cut-off values by ROC analysis based on fibrosis scores in HbeAg positive patients

**Table 6.** Comparison of the demographic and laboratory parameters as per fibrosis scores in HbeAg positive patients

| Category-1 Variable | Fibrosis<3 (n=23) | | Firosis≥ 3 (n=25) | | Statistical analysis* Probability |
|---|---|---|---|---|---|
| | $\bar{X} \pm S.S.$ | Medyan [IQR] | $\bar{X} \pm S.S.$ | Medyan [IQR] | |
| Age (year) | 44.43±13.41 | 43.0 [21.0] | 52.88±10.66 | 52.0 [15.0] | t=-2.426 **p=0.019** |
| AST | 36.25±55.56 | 24.0 [10.0] | 57.96±62.97 | 31.0 [39.5] | Z=-2.426 **p=0.015** |
| ALT | 59.17±153.33 | 23.0 [19.0] | 72.99±91.87 | 34.0 [62.5] | Z=-2.291 **p=0.022** |
| PLT | 244.35±54.36 | 234.0 [64.0] | 224.04±67.28 | 218.0 [83.5] | t=1.144 p=0.259 |
| Neutrophil | 4866.96±1520.83 | 4800.0 [3010.0] | 4886.00±1449.31 | 4700.0 [2080.0] | Z=-0.175 p=0.861 |
| Leukocyte | 7098.70±1993.65 | 7100.0 [3110.0] | 7146.40±1755.52 | 7100.0 [1650.0] | t=-0.088 p=0.930 |
| Lymphocyte | 2100.00±805.86 | 2000.0 [830.0] | 2260.40±705.46 | 2100.0 [1035.0] | Z=-0.951 p=0.342 |
| INR | 1.05±0.08 | 1.0 [0.1] | 1.07±0.13 | 1.0 [0.1] | Z=-0.010 p=0.992 |
| RDW | 13.92±1.79 | 13.5 [1.5] | 14.35±2.72 | 13.5 [1.4] | Z=-0.093 p=0.926 |
| PDW | 17.14±6.79 | 16.5 [1.0] | 16.23±1.35 | 16.4 [0.7] | Z=-0.031 p=0.975 |
| Albumin | 4.38±0.30 | 4.5 [0.4] | 4.26±0.43 | 4.3 [0.5] | Z=-0.671 p=0.502 |
| Globulin | 3.06±0.41 | 3.1 [0.7] | 3.16±0.45 | 3.1 [0.5] | Z=-0.407 p=0.684 |
| Total protein | 7.50±0.46 | 7.5 [0.6] | 7.41±0.64 | 7.5 [0.5] | Z=-0.275 p=0.783 |
| AFP | 3.39±1.68 | 3.0 [2.5] | 3.47±2.05 | 3.1 [1.9] | Z=-0.268 p=0.788 |
| HBV DNA | 5.03±1.65 | 4.8 [1.9] | 5.32±1.85 | 5.3 [3.0] | t=-0.558 p=0.579 |
| HAI | 4.35±1.72 | 4.0 [3.0] | 7.88±2.85 | 7.0 [3.5] | Z=-4.407 **p=0.000** |
| API score | 2.48±1.47 | 2.0 [2.0] | 4.20±1.66 | 4.0 [2.5] | Z=-3.573 **p=0.000** |
| APRI | 0.42±0.68 | 0.3 [0.2] | 0.77±0.90 | 0.4 [0.6] | Z=-2.035 **p=0.042** |
| Neutrophil/ Lymphocyte | 2.56±1.19 | 2.2 [1.4] | 2.34±0.95 | 2.3 [1.1] | Z=-0.599 p=0.550 |
| Platelet/ Lymphocyte | 0.130±0.051 | 0.114 [0.040] | 0.112±0.057 | 0.096 [0.060] | Z=-1.372 p=0.170 |
| AST/ALT | 1.02±0.34 | 1.0 [0.6] | 0.95±0.28 | 0.9 [0.4] | t=0.806 p=0.425 |
| RDW/Platelet | 0.059±0.011 | 0.061 [0.020] | 0.072±0.037 | 0.062 [0.020] | Z=-1.073 p=0.283 |
| INR/Platelet | 0.005±0.001 | 0.004 [0.000] | 0.006±0.003 | 0.004 [0.000] | Z=-0.836 p=0.403 |

***** "Independent Sample-t" test (t-table value) statistics were used to compare two independent groups with normal distribution. "Mann-Whitney U" test (Z-table value) statistics were used to compare two independent groups with non-normal distribution. AST: aspartate aminotransferase, ALT: alanine aminotransferase, PTL: platelet, RDW: Red Cell Distribution Width, PDW: Platelet Distribution Width, AFP: alpha fetoprotein, HAI: histological activity index, API: age-platelet index, APRI: spartate aminotransferase platelet ratio index.

## DISCUSSION

This study aimed to evaluate the sensitivity of the biomarkers predicting the severity of liver fibrosis between HbeAg positive and HbeAg negative patients. Analysis of the entire cohort -without grouping as per HBeAg positivity- revealed that none of the biomarkers assessed was found to be related to the severity of fibrosis. However, after dividing the entire group into two according to the presence or absence of the HbeAg antigen, our analysis revealed that the API scores were associated with the fibrosis scores in HbeAg positive patients. On the other hand, serum total protein levels were associated with the fibrosis scores in the HbeAg negative patient group. Our results show that noninvasive fibrosis assessment methods should be performed in separate groups in HbeAg positive and HbeAg negative patients.They also imply that different biomarkers should be used to assess fibrosis severity in these two patient populations.

Detection and accurate assessment of the severity of liver fibrosis in CHB patients is essential for initiation of therapy (19). Although these assessments are conventionally performed by liver biopsies, the use of non-invasive markers predicting the presence and severity of fibrosis can help avoid unnecessary biopsies and related complications (20). It was postulated that HBV was not cytopathic and HBV-related liver damage developed via cytotoxic T cells (21). The resultant inflammation induces liver fibrosis in the chronic period (22). It is known that elevated serum total protein levels can be associated with acute and chronic infections (23). In line with this, we found a significant relationship between elevated serum total protein levels and liver fibrosis in our HbeAg negative patients in our study. The high total protein level is indicated in the literature that can be seen in chronic infections such as viral hepatitis (24). It was stated that serum total protein increased even in hepatotoxicity, which is known to develop severe inflammation in the liver, except for infectious factors (25,26). It was previously reported that in patients with HBV, the serum profile of both globulins and proteins other than globulins differed according to the degree of liver fibrosis (27-29). However, these studies did not compare the serum total protein values between HbeAg positive and HbeAg negative patients. In line with this, we found a significant correlation between high serum total protein, which can be a sign of the severity of liver inflammation, and liver fibrosis in our HbeAg-negative patients in our study.

It is known that the immune processes are different between HBeAg positive and HBeAg negative patients (30). This antigen has immunomodulatory effects, suppresses T cell-mediated immunity, and helps in developing tolerance in the host immune response against HBV (30). It also attenuates the CD4+ T helper cell response by several mechanisms, including clonal deletion of HBV-specific T cells and immune depletion (6). Since HBeAg suppresses HBV-specific immunity, it facilitates viral replication and is considered an indicator of viral replication (5). As replication continues, the accumulation of the envelope particles in the hepatocytes leads to the formation of ground glass appearance in the hepatocytes histopathologically (31). Since the extent of this damage will increase over time, not surprisingly, age is a significant risk factor for liver fibrosis during CHB (27). The fact that advanced patient age was considered one of the main criteria for determining the optimal treatment option in the international guidelines is consistent with our findings (32). We found a significant correlation between API and fibrosis scores, and the ROC analysis revealed a cut-off value of 2,5 in the diagnosis of advanced fibrosis. In a study conducted using Fibroscan, age was an independent risk factor for fibrosis in HbeAg positive patients (33). Erdoğan et al. (34) reported that the API score was insufficient to detect fibrosis in CHB patients. However, it should be considered that only 15% of their study participants were HbeAg positive. Kim et al. worked on patients 55% of whom were HbeAg positive, and noted that API was significantly associated with

fibrosis (35). In our study, HbeAg positive patients constituted 25% of the entire cohortHowever, analysis of the 48 HbeAg positive patients elucidated that API could be used to detect liver fibrosis.

Some studies suggested that low platelet counts were associated with advanced liver fibrosis (36). For example, Kekilli et al. (8) found that the mean platelet count in the advanced fibrosis group was significantly lower than counts in the mild fibrosis and non-fibrosis group. On the contrary, some studies suggested that platelet count was not related to the degree of fibrosis (14, 20). Although the exact relationship between low platelet count and significant liver inflammation has not yet been clarified, it has been reported that platelets could attract the inflammatory cells to the liver parenchyma in the setting of HBV infection (37). In our study, platelet count was not associated with liver fibrosis.

Some studies worked on RDW and reported that RDW and RDW/PLT ratios were independent predictors of fibrosis scores in CHB patients (37, 38). Nevertheless, we did not find an association between these parameters and fibrosis scores in our study.

World Health Organization (WHO) guidelines recommended using APRI to predict liver fibrosis severity in patients with CHB (39). It was also stated that APRI was particularly useful in hepatitis C disease, and hepatitis B and hepatitis C were different diseases regarding prognosis, histopathological findings, and the manifestation of fibrosis (40). However, there are conflicting results in the literature for the APRI score, and it was stated that this biomarker was not cost-effective for predicting advanced fibrosis (41). The APRI was associated with fibrosis severity in univariate analysis in all groups in our study. However, in multivariate analysis, no relationship was found between fibrosis severity and APRI in any patient group.

The N/L ratio is an inexpensive indicator that provides clues about the immune response to CHB and about disease progression (13). Alkhouri et al. (12) reported a significant association between N/L ratio and fibrosis severity in nonalcoholic steatohepatitis patients. However, the literature reported conflicting results regarding the relationship between N/L ratio and fibrosis severity in CHB patients (42). Yilmaz et al. (42) stated that the N/L ratio could be used as a sensitive marker to predict the severity of fibrosis in CHB. On the other hand, some other studies did not find a significant relationship between the N/L ratio and fibrosis severity (43,44). In line with these findings, we did not find an association between the N/L ratio and fibrosis.

Our study has some limitations which need to be considered while evaluating its findings. First, it is a single-centered study with a retrospective design. Second, it should be considered that, since different HBV strains have different HBV integration patterns, fibrogenic processes may also be different among these strains (3, 45).

Despite these limitations, our findings showed that API score predicted liver fibrosis with high sensitivity and specificity in HbeAg positive patients. While analysis of our entire cohort without grouping revealed that none of the biomarkers could predict liver fibrosis, assessments performed after grouping as per HbeAg positivity revealed that different biomarkers could be used for predicting liver fibrosis in these patient groups. While API could predict liver fibrosis with high sensitivity and specificity in HbeAg positive patients, high serum total protein levels were associated with fibrosis in HbeAg negative patients. Therefore, we suggest that patients with CHB should be grouped based on HbeAg status before performing a liver biopsy, and different biomarkers should be used for predicting the severity of liver fibrosis in these patient groups.

## ETHICS COMMITTEE APPROVAL

* The study was approved by the Ankara Yildirim Beyazit Training and Research Hospital Clinic Researches Ethics Committee (Date: 05.04.2021 and Number: 108/04).

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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