Histological Findings in Antigen E Positive and Negative in Chronic Hepatitis B

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ABSTRACT

Objective: To determine the histopathological findings in patients with HBeAg-positive chronic HBV infection (immunotolerant phase in old terminology) and HBeAg-negative chronic HBV infection (inactive carrier phase in old terminology).

Study Design: Observational study.

Place and Duration of the Study: Department of Gastroenterology, University of Health Sciences, Diyarbakir Gazi Yasargil Education and Research Hospital, Diyarbakir, Turkiye and Diyarbakir and Mersin University School of Medicine, Diyarbakir, Turkiye, from May 2014 to August 2022.

Methodology: The difference between fibrosis and histological activity indices of 289 patients in the immunotolerant and inactive carrier phase who had liver biopsy was examined statistically. Additionally, the relationship of these data with age and gender was investigated.

Results: While 236 (81.7%) of the patients were in the inactive carrier phase, 53 (18.3%) patients were in the immunotolerant phase. The mean fibrosis score of patients in the immunotolerant stage was 2.0 ± 1.2, while it was 2.0 ± 1.0 in inactive carriers (p = 0.753). The number of patients with a fibrosis score of two and above was 21 (39.6%) in immunotolerant patients and 52 (22.0%) in inactive carrier patients (p = 0.004). In patients under 30 years of age, the mean fibrosis score was 1.7 ± 1.0. It was 2.0 ± 1.1 in those over 30 years of age (p = 0.016).

Conclusion: Biochemical parameters or viral load cannot clearly reflect cellular damage in the liver. In the future, HBV DNA positivity alone may be the only criterion for the treatment.

Key Words: *Chronic viral hepatitis B, Fibrosis, Immune tolerance phase, Inactive carrier phase.*

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INTRODUCTION

Chronic hepatitis B virus (HBV) infection is a major public health problem that infects more than 250 million people in the world and causes approximately 600,000 deaths annually.[1] The course of chronic HBV infection depends on many factors, especially virological factors and the host's immune response. Age, gender, and family history of hepatocellular cancer are important risk factors.[2]

In the current literature, it is stated that antiviral treatment should be started in patients with high HBV DNA, cirrhosis or hepatocellular carcinoma, and extrahepatic manifestations.[3-5]

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Since liver disease and / or HBV replication may become active later in patients who are not treatment candidates at the time of admission and treatment is not initiated, liver biochemical tests, HBV DNA and HBeAg antigens, and liver parenchymal structure should be monitored periodically.[6] In particular, it is important to determine whether the liver parenchyma of this patient group, whose liver function tests are constantly normal but whose HBV DNA level is variable and which constitutes the majority of the population with chronic HBV infection, is really normal as biochemical tests.[7] Because these patients may deserve to receive antiviral treatment. In these patients, fibrosis that progresses silently from the bottom may result in an inevitable picture of cirrhosis or HCC in the long-term.

The aim of this study was to determine the histological changes in the liver parenchyma of HBeAg positive (immuneotolerant phase in old terminology) and HBe negative (inactive carrier phase in old terminology) patients with chronic HBV infection who were followed periodically without antiviral treatment. This may determine whether these patients were candidates for antiviral treatment. In addition, it may provide evidence that some patient groups should use antiviral drugs outside the antiviral treatment indications recommended in the guidelines.

METHODOLOGY

This study was conducted at the Department of Gastroenterology, University of Health Sciences, Diyarbakir Gazi Yasargil Education and Research Hospital, Diyarbakir, Turkiye. It included 289 patients who underwent liver biopsy between May 2014 and August 2022. The data of patients diagnosed with chronic HBV infection who were treated at the study centre during the specified dates were obtained from the hospital's information management system. Among these patients, those who had been under regular follow-up for at least five years and had a liver biopsy were included in the study. Before liver biopsy was performed, care was taken to ensure that the patients' laboratory data and outpatient clinic follow-ups were regular.

In Turkiye, antiviral treatment is started according to the fibrosis score and histological activity score in the liver biopsy result.[8] Patients with chronic hepatitis B for whom liver biopsy is recommended are explained in detail about the reason for the biopsy and written consent is obtained from these patients. Biopsy for patients in the inactive carrier and immuneotolerant phase is performed according to the patient's age, gender, HBeAg status, HBV DNA level, job, patient's wishes and plans to start a family in women of reproductive age, family history of liver cirrhosis and hepatocellular cancer, and a desire to receive antiviral treatment. Patients who do not want to have liver biopsy continue to be kept under routine follow-up. Approval was received for this study from the Health Sciences University, Gazi Yasargil Training and Research Hospital Clinical Research Ethics Committee (Decision Number: 720, Dated: 26.03.2021).

Patients who were older than 18 years of age, had a liver biopsy, and had HbeAg-positive chronic HBV infection and HbeAg-negative chronic HBV infection were included in the study. In addition to patients younger than 18 years of age, those with chronic HBV infection with chronic HDV infection or chronic HCV infection, HIV infection, patients with liver cirrhosis, patients with hepatocellular carcinoma, patients using immunosuppressive medicines, metabolic liver diseases such as haemachromatosis or Wilson's disease, autoimmune liver diseases such as hepatitis or primary biliary cholangitis, and those with heavy alcohol use were excluded from the study. Patients with ascites, oesophageal varices, encephalopathy, thrombocytopenia, hypoalbuminaemia, elevated INR, portal hypertensive gastropathy, and patients with radiological findings compatible with cirrhosis were considered to have liver cirrhosis and were also excluded from the study.[9]

The date of the liver biopsy was used as the reference point, and the data belonged to the dates before the biopsy. Patient age was also defined as biopsy age. Grading of necroinflammatory activity and staging of fibrosis were performed using the Ishak hepatitis activity index (HAI) scoring system histologically.[10] The histological activity index scales necroinflammatory activity in the liver between 0 and 18 points.[11] HBsAg, antiHBs, HBeAg, antiHBe, and HBV DNA levels of the patients were measured.

In addition to these tests, ALT, AST, GGT, albumin, total bilirubin, complete blood count, and INR levels of all patients were checked. Normal ALT was defined as equal to or less than 35 IU/L in males and 25 IU/L in females.[12] In addition, hepatobiliary ultrasonography was performed on all patients to evaluate the liver parenchyma, dimensions of the spleen, and the diameter of portal and splenic veins.

Chronic HBV patients were divided into two groups for analysis; those who were in the HbeAg-positive chronic HBV infection and those with HbeAg-negative chronic HBV infection. The HbeAg-positive chronic HBV infection stage was defined as very high HBV DNA levels (>20,000 international units/mL or >105-7) and the presence of HBeAg with normal ALT in 5 controls at least 3-6 months apart but no evidence of active liver disease.[13] HBeAg negative chronic HBV infection was defined as normal serum ALT level, low HBV DNA levels (≤2,000 international units/mL or ≤105 copies/mL) in 5 controls at least 3-6 months apart with the absence of HBeAg antigen. It was examined whether there was a histopathological difference between these two patient groups in terms of fibrosis and histological activation. In addition, the effects of age and gender on fibrosis between both patient groups were examined.

Normal distribution of patient data was checked with Kolmogorov-Smirnov and Shapiro-Wilk's tests. While mean and standard deviation values were stated for continuous variables, categorical variables were expressed as n (%). Independent Samples t-test with parameters with normal distribution to determine the differences between age, laboratory data and HBV DNA level in patients with chronic HBV infection who are HBe-positive and HBe-negative; Mann-Whitney U test was used for the parameters that did not have normal distribution. Independent Samples t-test was used to determine the difference between histological activity and fibrosis scores between HBe-positive and HBe-negative patients. Two-way analysis of variance was used to measure the joint effect of age and gender on fibrosis and HAI. ROC analysis was used for the effect of age and gender in predicting fibrosis and histological activity. All tests were bilateral and p-value <0.05 was considered statistically significant. Statistical analyses were performed using the SPSS 24.0 for Windows (SPSSInc.Chicago, IL, USA) package programme.

RESULTS

A total of 289 patients were included in the study. While 236 (81.7%) of the patients were in the HBe-negative phase, 53 (18.3%) patients were in the HBe-positive phase. 50.9% of the patients were male (n = 147), 49.1% (n = 142) were female. The mean age was 42.0 ± 11.8 years, and there was no significant difference between the HBe-positive and HBe-negative groups (p = 0.879). Albumin level and INR level were similar in both groups. While no significant difference was found between HBe-positive and HBe-negative patients in terms of biochemical parameters such as ALT, AST, GGT and total bilirubin levels; HBV DNA level was higher in HBe-positive patients (3.6 × 104 *vs*. 1.3 × 103; p <0.001, Table I).

Table I: Demographic and laboratory data of patients with chronic HBV infection (HBe-positive and HBe-negative).

	Study population (n = 289)	HBe-positive (n = 53)	HBe-negative (n = 236)	p-value
Age	42.0 ± 11.8	31.3 ± 12.0	44.4 ± 10.4	<0.001
Gender				
Female	142 (49.1%)	27 (50.9%)	115 (48.7%)	0.879
Male	147 (50.9%)	26 (49.1%)	121 (51.3%)	
Albumin	4.5 ± 0.3	4.4 ± 0.3	4.5 ± 0.3	0.990
ALT	23.2 ± 10.9	21.1 ± 8.1	23.7 ± 11.4	0.112
AST	20.0 ± 9.2	19.5 ± 6.7	20.2 ± 10.0	0.416
GGT	44.7 ± 17.3	47.8 ± 16.7	44.1 ± 15.5	0.172
Total bilirubin	0.4 ± 0.3	0.4 ± 0.2	0.4 ± 0.2	0.996
INR	0.9 ± 0.3	1.0 ± 0.3	0.9 ± 0.2	0.901
Platelet (10^3 cell/ml)	260 (177-503)	263 (202-398)	254 (177-503)	0.315
HBV DNA ($\log_{10}$ IU/mL)	1.6×10^3 (0.88×10^2-1.7×10^8)	3.6×10^4 (2.3×10^4-1.7×10^8)	1.3×10^3 (0.88×10^2-1.9×10^3)	<0.001

Chi-square was used for gender; t-test was used for age; Mann-Whitney U test was used for platelet counts. Independent t-test was used for other parameters.

Table II: Difference between histological activity and fibrosis scores of patients with HBeAg-positive and HBeAg-negative, chronic HBV infection.

	HBeAg-positive stage (n = 53)	HBe-negative stage (n = 236)	p-value
HAI mean	4.5 ± 2.4	4.8 ± 2.1	0.354
HAI (≥6) (n)	11 (20.7%)	37 (15.6%)	0.239
Fibrosis (mean)	2.0 ± 1.2	2.0 ± 1.0	0.753
Fibrosis (≥2) (n)	21 (39.6%)	52 (22.0%)	0.004

Independent samples t-test and Chi-Square test were used.

Table III: Variation of histological activity index and fibrosis score according to gender in patients with chronic HBV infection.

	Male (n = 147)	Female (n = 142)	p-value
HAI mean	5.0 ± 2.1	4.4 ± 1.9	0.016
HAI (≥6)	24 (16.3%)	24 (16.9%)	0.329
Fibrosis (mean)	2.2 ± 1.1	1.8 ± 0.8	<0.001
Fibrosis (≥2)	43 (29.2%)	30 (21.1%)	0.010

Independent samples t-test and Chi-Square test were used.

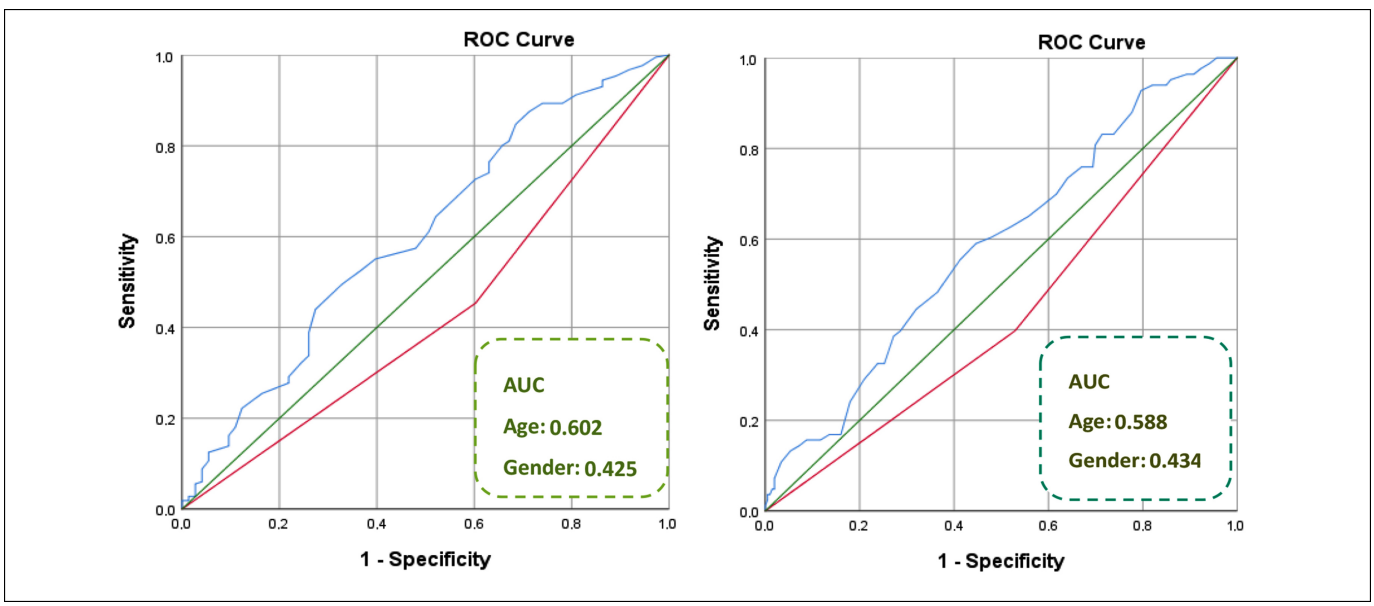

Figure 1: The role of age and gender in predicting fibrosis and histological activity.

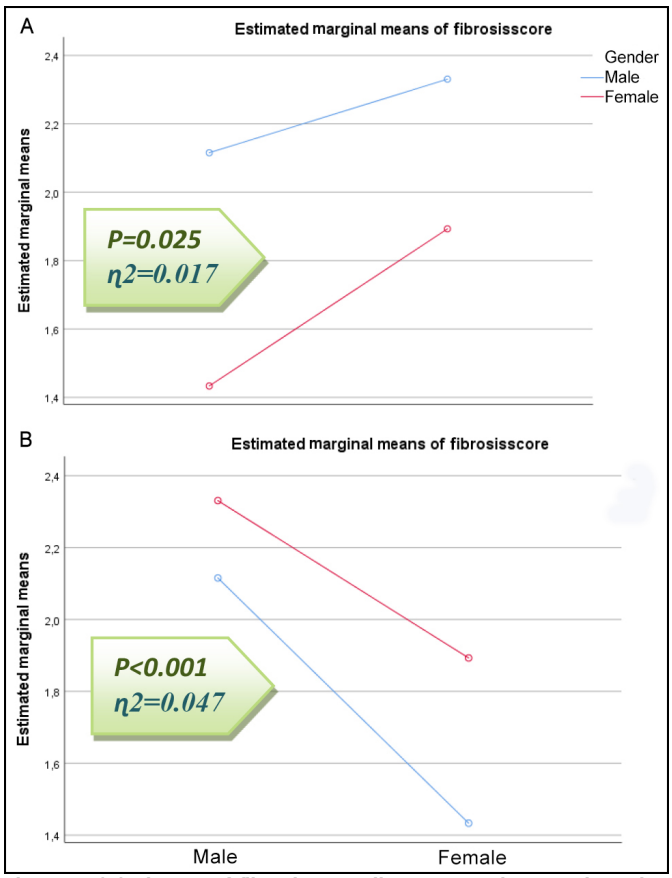

Figure 2: (A) Change of fibrosis according to age when gender value is kept constant. (B) Change of fibrosis according to gender when age value is kept constant.

The mean HAI score was 4.5 ± 2.4 in patients in the HBe-positive stage and 4.8 ± 2.1 in patients in the HBe-negative stage (p = 0.354). While the number of patients with a HAI score of 6 and above was 11 (n = 20.7%) in HBe-positive patients; 37 (15.6%) in HBe-negative patients (p = 0.239).

The mean fibrosis score of patients in the HBe-positive phase was 2.0 ± 1.2, while it was 2.0 ± 1.0 in HBe-negative (p = 0.753). The number of patients with a fibrosis score of 2 and above was 21 (39.6%) in HBe-positive patients and 52 (22.0%) in HBe-negative patients (p = 0.004, Table II).

When patients were separated according to gender, the mean HAI score of male patients with chronic HBV infection was 5.0 ± 2.1, while it was 4.4 ± 1.9 in female patients (p = 0.016). The HAI score was 6 and above in 16.3% of men (n = 24) and 16.9% of women (n = 24, p = 0.329). The mean fibrosis score of male patients was also higher than female patients (2.2 *vs.* 1.8; p <0.001). On the other hand, 21.1% (n = 30) of female patients had a fibrosis score of 2 or higher, while this rate was 29.2% (n = 43) in males (p = 0.010, Table III).

When the patients with chronic HBV infection were grouped and analysed as under 30 years old and over 30 years old, the mean HAI score of patients under 30 years of age was 4.1 ± 2.1; while it was 4.9 ± 2.2 in patients over 30 years of age (p = 0.008). In 17.8% (n = 10) of young patients, the HAI score was 6 and above; Over the age of 30 years, this rate was 31.3% (n = 73, p = 0.030). In patients under 30 years of age, the mean fibrosis score was 1.7 ± 1.0; It was 2.0 ± 1.1 in those over 30 years of age (p = 0.016). While there were 10 patients (17.8%) under the age of 30 years with a fibrosis score of 2 and above, there were 50 patients (27.0%) over the age of 30 years (p = 0.006).

ROC analysis showed that age is a more important parameter than gender in predicting both fibrosis and HAI in chronic HBV infection (Figure 1, AUC: 0.602 and 0.588). One-way analysis of variance showed that when the gender parameter was kept constant in patients with chronic HBV infection; age was an important parameter in predicting fibrosis for both genders. Similarly, the male gender seems to be more disadvantageous in terms of fibrosis among patients in the same age group (Figure 2A and B).

DISCUSSION

This research has shown that a substantial proportion of patients with both HBe-positive and HBe-negative, chronic HBV have fibrosis at a level that requires initiation of antiviral therapy. Approximately one out of every four patients (25.2%) had fibrosis of 2 or more histopathologically according to the ISHAK score. Especially the high number of patients over 30 years of age with high fibrosis scores was remarkable. This showed that age was a strong independent predictor of fibrosis. Although not as strong as age, it can be said that gender is also effective on fibrosis. In this regard, the male gender seems to be somewhat more disadvantaged. The analysis made by keeping the age and gender parameters constant, especially the male gender in the same age group; showed that fibrosis was significantly higher among same-gender patients, especially in older patients.

In a large-scale study on this subject, more than 13,00 HBeAg-negative chronic HBV patients were examined and as a result, it was stated that normal serum ALT level alone could not predict histological findings.[14] Similarly, a study specifically examining patients older than 40 years found that 37% of patients with consistently normal ALT and low HBV DNA levels (approximately <2000 international units/mL) had significant fibrosis and inflammation on liver biopsy. This study emphasises that the loss of tolerance decreases after the age of 30 in chronic HBV patients and that the transition to the chronic fibrotic process begins in patients.[15]

According to the AASLD (2017) guideline, ALT elevation stands out as an important factor to initiate antiviral treatment in chronic HBV infection. In patients with cirrhosis, HBVDNA positivity is sufficient to initiate antiviral treatment. Patients in the HBe-positive phase and HBe-negative phase

can be followed without treatment.[16] Patients who have not started antiviral treatment should be followed periodically. Studies have shown that starting antiviral treatment in patients with inactive HBV infection and in the immune-tolerant period reduces the risk of cirrhosis and HCC.[17]

Current AASLD guidelines recommend evaluating liver biopsy and therapy, especially if the patient is over 40 years of age, even if he or she has a normal ALT level.[16] In addition, guidelines recommend liver biopsy to detect significant histological disease in elderly patients with HBeAg positive or negative high HBV DNA levels and normal ALT levels.[18] Age is an indicator of the duration of the disease. It may be a cause of fibrosis, as advanced age causes more inflammation and active viremia. Most of them did not know how long they had chronic HBV. The duration of the disease and gender should be taken into account in the clinical evaluation of HBV. Because a disease that continues with viremia carries the risk of developing cirrhosis and HCC regardless of ALT levels.[19]

Similarly, it has been shown that some patients in the immunotolerant stage and with normal ALT levels may have significant fibrosis in liver biopsy. In the USA, 29% of patients with immune tolerance stage and ALT level below 40 had fibrosis 3 or higher.[20] Similar results were obtained in another study conducted in China.[21] Consideration of extending treatment indications in patients in the HBe-positive phase is supported by a study conducted in Korea. The study revealed a higher incidence of HCC, cirrhosis, and the need for liver transplantation at a mean follow-up of 6.3 years.[22] In these studies, there is evidence showing that HBV DNA level in the immune tolerance phase is associated with the risk of developing cirrhosis and HCC, especially in relation to age.

The data obtained from biopsy results show that both HBe-positive and HBe-negative patients may need antiviral treatment even if they have lower HBV DNA levels. Normal ALT levels are not accurate in determining histological damage and making treatment decisions, even in patients in the HBe negative stage.

Even if ALT is periodic over time in the HBV inactive carrier phase, may not always be possible to determine whether ALT is normal over the years or whether exacerbations occur over time. In addition, it is a known fact that even though these patients are willing to come to follow-ups at first, they delay their follow-up and prolong the period because they are not symptomatic in time. In this regard, developing non-invasive techniques can help us in determining the need for biopsy, as well as ALT level and HBV DNA level.

The most important limitation of this study is that it is a retrospective study. To determine which patients with chronic HBV infection will benefit more from treatment, the results of a randomised controlled trial that includes a control group and has a follow-up period of at least 10 years will support our results more strongly. Another, limitation of the study was that fibroscan elastography and new-generation fibrosis markers were not used instead of liver biopsy.

CONCLUSION

Biochemical parameters or viral load cannot clearly reflect cellular damage in the liver. Therefore, it is not possible to predict whether a patient will be a candidate for antiviral treatment with these values alone.

ETHICAL APPROVAL:
Approval was received for this study from the Health Sciences University, Gazi Yasargil Training and Research Hospital Clinical Research Ethics Committee (Decision No: 720, Dated: 26.03.2021).

PATIENTS' CONSENT:
Informed consent was obtained from all the patients before conducting the study.

COMPETING INTEREST:
The authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:
MZA: Data collection, introduction and discussion, literature search, and reference settings.
BE: Conception and study design, critical evaluation, and conclusion.
FB: Discussion section.
AU: Material and methods section, interpretation of the data, and statistical analysis.
AY: Critical evaluation of the work.
UK: Conception and design of the work.
All authors approved the final version of the manuscript to be published.

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