THE RELATIONSHIP BETWEEN SERUM DNA LEVELS AND SEROLOGICAL MARKERS, ALT AND AST WITH LIVER HISTOLOGY IN CHRONIC HEPATITIS B PATIENTS


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ABSTRACT

Introduction: Determination of Hepatitis B virus (HBV) by molecular methods is increasingly common. Together with biochemical and serological diagnostic methods and a histopathological assessment is important to detect necrosis, inflammation, and fibrosis in the liver. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) values are tests often used to predict the histological stages of chronic hepatitis.

Materials and methods: One hundred seventy-three patients who were referred to the Kahramanmaras Necip Fazil City Hospital Microbiology Laboratory with prediagnosis of HBV infection between September 2012-January 2016, were evaluated with necroinflammatory activity levels/histological activity index (HAI) and fibrosis scores according to the ISHAK classification criteria, and the results were compared with alanine aminotransferase (ALT), aspartate aminotransferase (AST), and HBV-DNA serum levels of the time of biopsy.

Results: The study included 63 female and 109 male patients. The mean age was 39.39±13.4 years. There was statistically significant difference in age, ALT, AST, and HBV DNA levels between patients in the high fibrosis group and the low fibrosis group (p: 0.034, 0.002, 0.0001, and 0.007, respectively). There was significant difference between patients from severe HAI and mild to moderate HAI groups in terms of AST values (p: 0.045). AST was found to be a risk factor for fibrosis, and ALT and AST were found to be risk factors for HAI. HBV DNA, ALT, and AST levels of HBe-Ag positive patients were significantly higher compared to HBe-Ag negative patients (p:0.0001, 0.027, 0.008).

Conclusions: In our study, advanced age, high HBV DNA, ALT and AST levels were found to be associated with fibrosis. Evaluation of these parameters in chronic hepatitis B patients as indicator parameters for advanced stages of fibrosis and necroinflammation would be a right approach.

Keywords: Hepatitis B virus, fibrosis, histological activity index, alanine aminotransferase.

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Introduction

Approximately 2 billion people worldwide are infected with human hepatitis B virus (HBV), and 350 million of them have chronic HBV infections. Prognosis of the disease differs from asymptomatic carriers to cirrhosis and hepatocellular carcinoma, and causes death of 600,000 people a year(1). Despite neonatal immunization, there are still 400 million HBV carriers around the world(2). Turkey is one of the moderate endemic countries with HBsAg sero-prevalence values between 3.9-12.5%(3). These rates illustrate the importance of early diagnosis of HBV infection, a serious health problem in our country, in
terms of determining viral replication and referring to treatment in the early stages. Nowadays, the determination of HBV DNA by molecular methods is increasingly common. This method is important in terms of determining viral replication in the best possible way, verification of serologic markers, and clarification of complications caused by mutant virus infections.

Progressive disease and end-stage liver failure emerges in 15-40% of patients with chronic HBV infections. Chronic and progressive liver disease results in developing widespread damage to the liver parenchyma and replacement by collagenous scar tissue. The treatment response is more effective in cases with low-stage fibrosis. Therefore, together with biochemical and serological diagnostic methods, a histopathological assessment is important to detect necrosis, inflammation, and fibrosis, especially in liver tissue. According to the current clinical practice, liver biopsy is required for diagnosis and treatment of chronic liver disease. Liver biopsy provides important information on the histological activity and fibrosis stage of the disease, and allows for predictions about the course of the disease and treatment outcomes.

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) values are tests often used to predict the histological stages of chronic hepatitis.

In the study, age, gender, serum ALT, and AST values of patients with a known serum HBV-DNA level were investigated to determine whether there was an association between histological index score (HAI) and fibrosis score. The correlation between the various parameters of the progression of chronic HBV infection, and in cases of high parameters, clinical importance of differences between groups were investigated.

Materials and methods

**Patient selection**

Serum HBV-DNA, ALT, AST levels, and liver biopsy results of 173 patients, who were referred to the Kahramanmaras Necip Fazıl City Hospital Microbiology Laboratory with positive Hepatitis B surface antigen (HbsAg) for at least six weeks between September 2012 and January 2016 were retrospectively evaluated. HBV-DNA, serum ALT, and AST values were investigated before the liver biopsies. Inclusion criteria were as follows: treatment-naïve patients for chronic hepatitis B older than 18 years of age, and no history of co-infections.

**Pathological examination of liver biopsy**

Biopsy samples with a preliminary diagnosis of chronic Hepatitis B were sent in formaldehyde-fixing solution to the Pathology Laboratory. All samples were needle biopsy material, and their lengths were 1-3 cm. The Sections obtained from samples were stained with haematoxylin-eosin, reticulin and Masson’s trichrome and assessed using the modified Ishak scoring system. HAI scores (A0-A18) obtained from liver biopsies were evaluated as mild (A0-A6), moderate (A7-A12), and severe (A13-A18). Fibrosis scores were evaluated as mild (F0-F2) and severe (F3-F6).

**Microbiological and biochemical assays**

The normal range of serum ALT values were <33 U/L for women and <41 U/L for men. The normal range of serum AST values were <32 U/L for women and <40 U/L for men (Roche Diagnostics, Germany). From blood samples sent to our laboratory, viral DNA were obtained using commercial extraction kit (QIAGEN, QIAsymphony DSP Virus/Pathogen Midi Kit, Germany) and were studied the same day using PCR kit (artus HBV QS-RGQ Kit, Germany) and the HBV DNA amplification was studied using Rotor-Gene RG-Q (Qiagen, Germany) device (Sensitivity 10 IU/ml).

**Ethics committee approval**

The study protocol was approved by Kahramanmaras Sutcu Imam University, Faculty of Medicine, Ethics Committee.

**Statistical analysis**

Data were analyzed with the Statistical Package for the Social Sciences (SPSS) for Windows version 21.0 software (SPSS Inc., Chicago, IL, USA). Continuous variables were expressed as mean ± standard deviation and categorical variables were expressed as number and percentage. Mann-Whitney U-test was used in comparing differences of independent groups. In addition, relations between continuous variables were analyzed using the Spearman correlation analysis and differences between the categorical variables were analyzed using the chi-square analysis. To determine risk factors for high HAI and high Fibrosis conditions, a logistic regression analysis was used.
Results

Sixty-three patients (36.6%) were women, 109 (63.4%) patients were male, and the mean age was 39.39 ± 13.4 years. According to the liver biopsy results 122 (70.9%) patients had low fibrosis score (F0-2), and 50 (29.1%) patients had a higher fibrosis score (F3-6). Patients’ age, serum ALT, AST, and HBV DNA levels were significantly higher in the high fibrosis group compared to the low fibrosis group (p = 0.034, p=0.002, p=0.0001, and p = 0.007, respectively). AST values were significantly higher (p=0.045) in the severe HAI group compared to the mild HAI group. Results liver biopsy are given in Table 1.

Among the patients with HBV-DNA levels above 10,000 IU/mL, serum ALT and AST levels were significantly higher. There was no statistically significant difference between serum ALT and AST levels with regard to HBV-DNA groups (p=0.01, p=0.019, respectively). Demographic and clinical characteristics of patients according to HBV-DNA status are shown in Table 2.

HBV-DNA, ALT and AST levels of HBe-Ag positive patients were significantly higher (respectively p=0.0001, p=0.027, and p=0.008) compared to HBe-Ag negative patients. There was no statistically significant difference in HAI and fibrosis scores in terms of HBe-Ag status. A comparison of the variables according to HBe-Ag status of patients is given in Table 3.

In the logistic regression analysis to evaluate factors associated with high fibrosis; AST was found to have significant effect on having high fibrosis risk. It is seen that only AST increases the risk of high fibrosis. A one unit increase in AST increases the risk of high fibrosis by 1.032-fold. Risk factors for high fibrosis are given in Table 4.

In the logistic regression analysis to evaluate factors associated with high HAI, ALT and AST had a significant effect on having High HAI risk.

When examining the relationship between fibrosis scores and other variables, a moderately significant positive relationship was found with HAI and AST, and ALT and AST. There was a moderately significant positive association with HAI scores and AST, and ALT and AST.

Table 1: The relationship between biopsy results and age, gender, HBV DNA, ALT and AST in patients with positive Hepatitis B.

<table>
<thead>
<tr>
<th>HAI index score</th>
<th>Fibrosis score</th>
<th>P</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>Mild HAI</td>
<td>Severe HAI</td>
<td>Low fibrosis score</td>
</tr>
<tr>
<td>Number of patients (δ)</td>
<td>172</td>
<td>161 (94.8%)</td>
<td>9 (5.2%)</td>
</tr>
<tr>
<td>Age (§)</td>
<td>39.39±13.4</td>
<td>39.2±13.5</td>
<td>44.56±11.36</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>109 / 63</td>
<td>104 / 59</td>
<td>5/4</td>
</tr>
<tr>
<td>HBV DNA IU/mL(§)</td>
<td>78634442±</td>
<td>60529565±</td>
<td>62526811±</td>
</tr>
<tr>
<td>ALT IU/mL(§)</td>
<td>78.36±115.9</td>
<td>72.52±94.63</td>
<td>200.78±294.73</td>
</tr>
<tr>
<td>AST IU/ml(§)</td>
<td>48.88±57.8</td>
<td>43.82±38.07</td>
<td>139.4±178.5</td>
</tr>
</tbody>
</table>

*statistically significant, (§): mean ± standard deviation, median (min - max values); (δ) : frequency (percentages %); HAI: Histological activity index; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; HBV DNA : Hepatitis B virus deoxyribonucleic acid.
as well (p = 0.0001; r = 0.407). However, a weak positive association was found between the age, HBV-DNA, and ALT.

<table>
<thead>
<tr>
<th></th>
<th>HBV-DNA IU/ml</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>≥10&lt;sup&gt;6&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gender female</td>
<td>12 (37.5%)</td>
<td>51 (36.4%)</td>
</tr>
<tr>
<td>Gender male</td>
<td>20 (62.5%)</td>
<td>89 (63.6%)</td>
</tr>
<tr>
<td>Age (n=172)</td>
<td>39.75±13.07</td>
<td>39.31±13.53</td>
</tr>
<tr>
<td>ALT (n=170)</td>
<td>53.5±55.4</td>
<td>83.9±124.9</td>
</tr>
<tr>
<td>AST (n=170)</td>
<td>37.84±28.47</td>
<td>51.35±62.32</td>
</tr>
<tr>
<td>HAI Mild (n=163)</td>
<td>31 (96.9%)</td>
<td>132 (94.3%)</td>
</tr>
<tr>
<td>HAI Severe (n=9)</td>
<td>1 (3.1%)</td>
<td>8 (5.7%)</td>
</tr>
<tr>
<td>Fibrosis Mild (n=122)</td>
<td>24 (75%)</td>
<td>98 (70%)</td>
</tr>
<tr>
<td>Fibrosis Severe (n=50)</td>
<td>8 (25%)</td>
<td>42 (30%)</td>
</tr>
</tbody>
</table>

Table 2: The relationship between HBV-DNA status and gender, age, ALT, AST, HAI and fibrosis in patients with positive Hepatitis B.

*statistically significant, (§): mean ± standard deviation, median (min - max values); (δ): frequency (percentages %); HAI: Histological activity index; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; HBV DNA: Hepatitis B virus deoxyribonucleic acid.

Discussion

HBV infection is a very important cause of acute and chronic liver disease. Contact with HBV in early stages of society causes quite diverse cases of “chronic HBV infections”. Accordingly, a significant number of carriers, chronic hepatitis, and liver cirrhosis cases are seen. In this rich spectrum of patients, early diagnosis and treatment is becoming very important in the prognosis<sup>(10)</sup>. In the diagnosis of chronic hepatitis B, elevated serum ALT-AST levels as biochemical parameters, positive HBe-Ag, and HBV-DNA as serological parameters, and HAI and fibrosis as histopathological parameters constitute the main criteria. These criteria are of great importance in determining patient follow-up, treatment, and prognosis<sup>(11)</sup>.

In the study, the mean age of patients was 39.39 years. In a similar study by Yağcı et al. with 179 patients, a mean age of 26.9 years was reported<sup>(10)</sup>. Demir et al.<sup>(8)</sup> reported the mean age of 456 patients included in his study as 36.7 years. When most of the patients being in the productive stages of their lives is taken together with the incidence of the HBV infection, it is manifest that this infection is an important health issue<sup>(12)</sup>.

In some studies on gender predisposition in HBV infections, the male gender was accepted as an independent risk factor in chronic HBV infections and it has been reported that abnormal serum ALT levels are more common in men than women<sup>(13,14)</sup>. Sixty-three point four percent of our patient group were male and 36.6% were female. In the study age and gender, serum HBV-DNA and serum ALT and AST levels and liver biopsy results showed no statistically significant difference (p< 0.05).

HBe-Ag can be either positive or negative in chronic hepatitis B. Positive HBe-Ag in the early stages of chronic hepatitis becomes negative in the advanced stages. During the follow-up, biochemical, serological, and clinical findings must be assessed separately according to the HBe-Ag status<sup>(19)</sup>. Kaya et al.<sup>(12)</sup> found no statistically significant relationship between HBe-Ag positivity and age and serum HBV-DNA levels. Inci et al.<sup>(19)</sup>, Oratlı et al.<sup>(15)</sup>, and Yalçın et al.<sup>(16)</sup> found HBV-DNA levels to be higher in HBe-Ag positive patients than HBe-Ag-negative patients.

In our study, HBV-DNA, ALT and AST values were found statistically higher in HBe-Ag positive patients compared to HBe-Ag negative patients while HBe-Ag negative patients were found to have higher age values. There was no statistical relationship between gender, HAI and fibrosis with HBe-Ag.
higher in patients with necrosis
ship, the reliability of ALT levels was significantly
must be performed. As an indication of this relation
levels, if treatment will be planned, a liver biopsy
follow-up of these patients, in cases of elevated ALT
ing it is a simple and affordable method
positive patient ALT and AST monitoring has great
mining the hepatocellular damage in a HBV-DNA
In this regard, in the chronic HBV infection, in deter
risk factor for fibrosis, and ALT and AST were found
to be risk factors for HAI in the regression analysis
In this study, we identified a positive, weak relationship between HBV DNA and HAI
(p=0.0001; r=0.268), and a positive, weak relation
ship between HBV-DNA and fibrosis
(p=0.03; r=0.166). These findings made us think that
necroinflammation and fibrosis in the liver
does not always have a positive relationship with
viral replication. Host immune mechanisms seem
to have a dominant role in this
ALT is a liver specific enzyme and is mostly
found only in the liver. High serum ALT levels are
associated with reversible and non-reversible hepatocyte membrane injury. High serum ALT
levels have high sensitivity for inflammation, necrosis, primer neoplasia and vacuolar hepatopa
thery (80-100%). AST is more sensitive but less
specific compared to ALT(21). It is possible that
a gradual increase in AST release occurs only in
hepatic injury with severe fibrosis(22-24). AST is an
indirect marker for hepatocellular injury and
fibrosis(25). There have been numerous studies in
the literature examining the relationship between
ALT and AST, with liver necroinflammation and
the degree of fibrosis. Ozkara et al.(25) detected a rela
ship between AST values and HAI and fibrosis, and a positive correlation between serum ALT levels and HAI, although there was no correlation between ALT lev
els and the degree of hepatic fibrosis. Demir et al.(4)
claimed that high ALT and AST levels could be an
indicator for advanced fibrosis in a study of 456
patients(25). Mohamadnejad et al.(20) detected lower
ALT levels in severe fibrosis in a study of 276
patients. In the current study, age, ALT, AST and
HBV DNA levels were higher in patients with severe
fibrosis compared to patients with mild fibrosis.
There was a statistically significant correlation
between fibrosis and age, ALT, AST, and HBV
DNA. Also there was statistically significant correla
tion between HAI with age, ALT, AST and HBV
DNA. According to the results of the regression
analysis AST was for a risk factor for fibrosis
(sig:0.035), and ALT and AST were risk factors for
Knodell HAI (sig: 0.049 and 0.009, respectively)

**Table 4:** Results of binary logistic regression analysis for the presence of high HAI.

<table>
<thead>
<tr>
<th>B</th>
<th>S.E.</th>
<th>Wald</th>
<th>Sig.</th>
<th>OR Lower</th>
<th>OR Upper</th>
<th>95% C.I. for OR Lower</th>
<th>95% C.I. for OR Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender(1)</td>
<td>-0.46</td>
<td>0.392</td>
<td>1.451</td>
<td>0.228</td>
<td>0.631</td>
<td>0.298</td>
<td>1.335</td>
</tr>
<tr>
<td>Age</td>
<td>0.026</td>
<td>0.014</td>
<td>3.592</td>
<td>0.058</td>
<td>1.027</td>
<td>0.999</td>
<td>1.055</td>
</tr>
<tr>
<td>ALT</td>
<td>-0.009</td>
<td>0.006</td>
<td>2.214</td>
<td>0.137</td>
<td>0.991</td>
<td>0.979</td>
<td>1.003</td>
</tr>
<tr>
<td>AST</td>
<td>0.022</td>
<td>0.015</td>
<td>4.43</td>
<td>0.035*</td>
<td>1.032</td>
<td>1.002</td>
<td>1.063</td>
</tr>
<tr>
<td>HBV-DNA</td>
<td>0</td>
<td>0</td>
<td>0.196</td>
<td>0.658</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

**Table 5:** Results of binary logistic regression analysis for the presence of high HAI.

<table>
<thead>
<tr>
<th>B</th>
<th>S.E.</th>
<th>Wald</th>
<th>Sig.</th>
<th>OR Lower</th>
<th>OR Upper</th>
<th>95% C.I. for OR Lower</th>
<th>95% C.I. for OR Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender(1)</td>
<td>-0.059</td>
<td>0.852</td>
<td>0.005</td>
<td>0.845</td>
<td>0.943</td>
<td>0.177</td>
<td>5.008</td>
</tr>
<tr>
<td>Age</td>
<td>0.014</td>
<td>0.03</td>
<td>0.212</td>
<td>0.646</td>
<td>1.014</td>
<td>0.956</td>
<td>1.076</td>
</tr>
<tr>
<td>ALT</td>
<td>-0.033</td>
<td>0.017</td>
<td>3.864</td>
<td>0.049*</td>
<td>0.967</td>
<td>0.956</td>
<td>1</td>
</tr>
<tr>
<td>AST</td>
<td>0.072</td>
<td>0.027</td>
<td>6.86</td>
<td>0.009*</td>
<td>1.074</td>
<td>1.018</td>
<td>1.133</td>
</tr>
<tr>
<td>HBV DNA</td>
<td>0</td>
<td>0</td>
<td>0.35</td>
<td>0.554</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Yalcin et al.(10) reported that all HBV-DNA posi
tive patients who had high ALT activity, had a histo
logical evident of necroinflammation findings on the
liver biopsies, whether they were HBe-Ag positive or
negative. In this study, the patients with a HBV-DNA
level of ≥10,000 IU / mL and high ALT and AST
activity, and those with significant necroinflamma
tion and high ALT activity had increased fibrosis
activity in the liver biopsies. AST was found to be a
risk factor for fibrosis, and ALT and AST were found
to be risk factors for HAI in the regression analysis
In this regard, in the chronic HBV infection, in deter
mining the hepatocellular damage in a HBV-DNA
positive patient ALT and AST monitoring has great
importance compared to other parameters, consider
ing it is a simple and affordable method(15-17). In the
follow-up of these patients, in cases of elevated ALT
levels, if treatment will be planned, a liver biopsy
must be performed. As an indication of this relation
ship, the reliability of ALT levels was significantly
higher in patients with necrosis(18).

Inci et al.(19) found a positive correlation between
HBV DNA, and HAI and fibrosis in HBeAg negative patients. Demir et al.(4) found higher HBV DNA levels in patients with high
fibrosis. Yalcin et al. (10) found in their study that
although serum HBV DNA levels are significantly
lower in HBeAg negative patients compared to HBeAg negative patients, there was no difference
between the two groups in terms of histological
activity. In the study, we identified a positive,
weak relationship between HBV DNA and HAI
(p=0.0001; r=0.268), and a positive, weak rela
relationship between HBV-DNA and fibrosis
(p=0.03; r=0.166). These findings made us think that
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analysis AST was for a risk factor for fibrosis
(sig:0.035), and ALT and AST were risk factors for
Knodell HAI (sig: 0.049 and 0.009, respectively)
Advanced age, high HBV DNA, ALT and AST levels were found to be associated with fibrosis, and high AST levels were found to be associated with liver necroinflammation. It is thought that serum ALT and AST levels could be useful parameters for liver necroinflammation and fibrosis. Therefore, we think that evaluation of these parameters in chronic hepatitis B patients as indicator parameters for advanced stages of fibrosis and necroinflammation would be a right approach. When HBV DNA levels are evaluated with ALT, AST levels and biopsy results in patients with chronic hepatitis B, it is concluded that this can guide us determine clinical course and disease activity.

References

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Authors’ Contributions
Study design, data collection and writing paper were undertaken by Ozlem Kirisci, Esra Ozkaya and Ahmet Caliskan; Tugba Paksoy, Asiye Analan, Beyhan Kirmaci, Rana Citil, Sule Agirbas, Zeki Guzel evaluated biopsies and interpreting findings; Hande Senol made statistical evaluations; Ozlem Kirisci, Esra Ozkaya, Seray Tumer participated collecting data and interpreting findings.

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