Association between serum levels of hepatitis B e-antigen and severity of liver fibrosis

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Abstract: The association between serum levels of hepatitis B e-antigen (HBeAg) and the extent of liver fibrosis remains unclear. We investigated this association and sought to identify quantitative biomarkers that might serve as tools for evaluation of liver fibrosis in a total of 249 treatment-naïve chronic hepatitis B (CHB) patients. In all, 163 patients (65%) were HBeAg-positive (HBeAg(+)) and 86 (35%) were HBeAg-negative (HBeAg(-)). The mean levels of hepatitis B virus (HBV) DNA and HBsAg (hepatitis B surface antigen) in HBeAg(+) patients were significantly higher than in HBeAg(-) patients. Serum levels of HBsAg, HBeAg, and HBV DNA were significantly intercorrelated in HBeAg(+) patients. In HBeAg(+) patients, significant fibrosis (stages S2-4) was associated with significantly lower median levels of HBsAg, HBeAg, and HBV DNA than was insignificant fibrosis (stages S0-1). Binary logistic regression analyses showed that the level of qHBeAg (quantitative Hepatitis B e antigen) and the prothrombin time were independent predictors of significant liver fibrosis in HBeAg(+) patients. The area under the receiver operating characteristic (AUROC) curve of levels qHBeAg for predicting significant fibrosis was 0.732 (95% CI 0.651-0.812). The cut-off value was 2.23 log10 PEIU/mL. For prediction of significant fibrosis, the AUROC curves of HBsAg and HBV DNA levels were 0.755 (95% CI 0.674-0.835) and 0.708 (95% CI 0.626-0.791), respectively. We found a strong inverse correlation between quantitative HBeAg level and severity of fibrosis in HBeAg(+) patients. HBeAg(+) chronic hepatitis B patients with moderate to severe fibrosis had lower levels of HBeAg, HBsAg, and HBV DNA than those with no or mild fibrosis.

Keywords: Hepatitis B e antigen, liver fibrosis, HBsAg, HBV DNA

Introduction

Hepatitis B virus (HBV) infection is a serious global public health problem. It is estimated that at least 2 billion people are infected with HBV. Of these, ~240 million have chronic hepatitis B (CHB) [1]. Each year, ~650,000 HBV infected persons die of liver failure, cirrhosis, and hepatocellular carcinoma (HCC) caused by HBV infection [2]. HBV does not directly kill liver cells; a pathological immune response is the principal mechanism of liver injury and inflammation. Repair of liver necroinflammation is associated with liver fibrosis [3]. Recurrence of inflammation in CHB patients is important in the development of liver cirrhosis and HCC. It is thus necessary to identify fibrosis early.

Liver biopsy is the reference standard for histological evaluation of liver disease [4], but it is invasive and associated with rare but potentially serious complications [5, 6]. Several non-invasive models, including as the fibrosis score-4, the aspartate transaminase-to-platelet ratio index (APRI), and transient elastography, have been used to predict liver fibrosis. However, such noninvasive tests detect only advanced liver disease [7, 8].

Several researchers have found inverse correlations between serum levels of HBsAg and liver fibrosis stage in hepatitis B e-antigen positive (HBeAg(+)) patients [9-11]. In hepatitis B e-antigen negative (HBeAg(-)) patients, levels of HBV DNA may also correlate with fibrosis severity [12, 13]. Wang found that the serum level of hepatitis B e-antigen served as a novel biomarker for predicting liver fibrosis [14]. A positive correlation was evident between levels of
Hepatitis B e antigen titers and liver fibrosis

HBeAg and HBsAg and a strong correlation between levels of HBeAg and HBV DNA [14-16]. Lower extents of immune-mediated liver injury were associated with higher levels of HBeAg [17]. However, the relationship between levels of HBeAg and liver fibrosis remains unclear. Therefore, we investigated the association between HBeAg levels and liver fibrosis and sought quantitative biomarkers that might serve as tools for the evaluation of liver fibrosis in treatment-naïve CHB patients.

Materials and methods

Patients

A total of 249 asymptomatic treatment-naïve patients with chronic HBV infections who attended the Department of Infectious Diseases, the First Affiliated Hospital, Zhejiang University, between January 2012 and October 2016, were prospectively enrolled. The inclusion criteria were: (1) HBsAg(+) status for more than 6 months; (2) absence of HCV, HDV, or HIV co-infection, autoimmune hepatitis, alcoholic liver disease, and drug-induced liver disease; (3) the absence of decompensated liver cirrhosis and HCC; and, (4) the absence of malignancy.

Laboratory measurements

Serum samples and liver biopsies were obtained on the same day. Complete blood cell counts and biochemical test data, including levels of alanine transaminase (ALT), (aspartate transaminase) AST, albumin, cholinesterase, and total bilirubin, and prothrombin time were obtained using automated techniques. Serum levels of HBsAg and HBeAg were quantified using the Architect assay (Abbott Laboratories, Abbott Park, Chicago, IL, USA). The linear range of the HBsAg assay was 0.05-250 IU/mL. Samples were serially diluted (to 1:500) if the HBsAg levels were >250 IU/mL. The hepatitis B e-antigen titers were directly proportional to the chemiluminescence emitted, measured in relative light units (RLUs). The HBeAg levels were expressed as the ratios of the sample RLUs to the control cut-off RLU (S/CO). An HBeAg titer >1.0 S/CO was considered positive. The Paul Ehrlich Institute (PEI) HBeAg reference standard was used to create the standard curve, and the S/CO values were converted into PEIU/mL [18]. Serum levels of HBV DNA were

Figure 1. Representative photographs of the stages of liver fibrosis. A. No fibrosis (S0). Photograph of a 40-year-old male patient in the S0 stage. B. Portal fibrosis (S1). Photograph of a 50-year-old male patient in the S1 stage. C. Septum formation (S2). Photograph of a 43-year-old female patient in the S2 stage. D. Architectural distortion (S3). Photograph of a 36-year-old male patient in the S3 stage. E. Cirrhosis, either probable or definite (S4). Photograph of a 48-year-old female patient in the S4 stage.
Quantified by real-time fluorescence quantitative polymerase chain reaction on an SLAN-96S platform; the lower limit of detection was 1,000 IU/mL.

Liver biopsy

Percutaneous liver biopsy was performed under ultrasonographic guidance using a 16-gauge needle to obtain a biopsy sample ≥1.5 cm in length with at least six portal tracts. The specimens were fixed, paraffin-embedded, sectioned continuously, and stained with hematoxylin and eosin, Masson’s trichrome, and reticulin. Liver fibrosis was staged using the Scheuer classification [19]: no fibrosis (S0); portal fibrosis (S1); septum formation (S2); architectural distortion (S3); and cirrhosis, either probable or definite (S4). Liver biopsy is the gold standard for diagnosis of liver fibrosis. The degree of hepatic fibrosis is divided into stages S0-S4, as recommended by the Chinese Medical Association Hepatology Society in 2000 [20]. In this study, fibrosis of Scheuer grade < 2 (S0-1) was considered insignificant. Significant fibrosis reflected spread beyond the portal tract (S2-4). Representative photographs of the stages of liver fibrosis in 249 patients are shown in Figure 1.

**Table 1. Association between liver fibrosis and clinical parameters**

<table>
<thead>
<tr>
<th>Variables</th>
<th>S0-1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (Male:Female)</td>
<td>74/24</td>
<td>44/31</td>
<td>27/16</td>
<td>21/7</td>
</tr>
<tr>
<td>Age (years)</td>
<td>36.17±9.40</td>
<td>36.53±9.63</td>
<td>35.79±8.60</td>
<td>41.24±9.86&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HBV DNA (log10 IU/ml)</td>
<td>6.72±2.03</td>
<td>6.48±1.83</td>
<td>5.93±1.70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.72±1.71&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HBsAg (log10 IU/ml)</td>
<td>3.82±0.99</td>
<td>3.70±0.76</td>
<td>3.47±0.59</td>
<td>3.17±0.35&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Anti-HBs (mIU/ml)</td>
<td>0.69±1.40</td>
<td>1.65±4.04</td>
<td>0.79±2.11</td>
<td>0.44±0.68</td>
</tr>
<tr>
<td>HBeAg (log10 PEIU/ml)</td>
<td>2.04±0.85</td>
<td>1.06±1.09</td>
<td>0.77±1.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.68±0.78&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>65.08±69.58</td>
<td>74.91±85.00</td>
<td>67.18±76.91</td>
<td>67.14±66.03</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>41.44±39.36</td>
<td>45.40±31.70</td>
<td>54.02±51.72</td>
<td>45.68±32.29</td>
</tr>
<tr>
<td>ALB (g/L)</td>
<td>45.42±4.78</td>
<td>44.79±3.48</td>
<td>43.40±5.56</td>
<td>43.71±5.24</td>
</tr>
<tr>
<td>CHE (U/L)</td>
<td>8583.01±1895.249</td>
<td>7821.95±2060.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6618.70±1488.98&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>7075.32±2290.60&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>TBIL (μmol/L)</td>
<td>14.16±7.83</td>
<td>13.67±7.07</td>
<td>14.51±8.27</td>
<td>14.89±7.43</td>
</tr>
<tr>
<td>PT (s)</td>
<td>11.41±0.72</td>
<td>11.82±0.75&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.14±1.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.32±1.34&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>PLT (10E9/L)</td>
<td>188.45±44.24</td>
<td>172.73±46.67</td>
<td>156.28±46.37</td>
<td>142.14±71.08</td>
</tr>
<tr>
<td>APRI</td>
<td>0.95±1.15</td>
<td>1.09±1.08</td>
<td>1.10±1.04</td>
<td>1.36±1.25</td>
</tr>
<tr>
<td>FIB-4</td>
<td>1.12±0.99</td>
<td>1.31±0.92</td>
<td>1.53±0.76</td>
<td>2.14±1.56&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; Anti-HBs, Hepatitis B surface antibody; HBeAg, hepatitis E antigen; ALT, alanine transaminase; AST, aspartate transaminase; ALB, albumin; CHE, Cholinesterase; TBIL, Total Bilirubin; PT, Prothrombin time; PLT, Platelets; FIB-4, Fibrosis-4; APRI, AST to Platelet Ratio Index; *compared with S0-1, P < 0.05; *compared with S2, P < 0.05. According to the Scheuer score, liver fibrosis was divided into stages S0-S4. The age, HBV DNA, HBsAg, HBeAg, cholinesterase, prothrombin time and fibrosis-4 were significantly different among the groups. There was no significant difference in the grade or frequency of anti-HBs, ALT, AST, APRI, platelets, total bilirubin, or albumin levels among liver-fibrosis stages. The significance of differences between different stages of liver fibrosis was explored using Pearson’s chi-square test for qualitative data and the t-test and analysis of variance for quantitative data.

**Data analysis**

Continuous variables are expressed as means with SDs. The levels of HBeAg, HBsAg, and HBV DNA were logarithmically transformed. The significance of differences between different stages of liver fibrosis was explored using Pearson’s chi-square test for qualitative data and the t-test and analysis of variance for quantitative data. Correlations between Serum levels of HBV markers and fibrosis stage were analyzed using the Spearman correlation test. Binary logistic regression analyses identified factors independently associated with significant liver fibrosis. An area under the receiver operating characteristic (AUROC) curve was used to determine the cut-off values for serum HBV markers predicting significant liver fibrosis. All tests were two-sided and P values < 0.05 were considered to reflect statistical significance. All statistical analyses were performed with the aid of SPSS software ver. 20.0 (SPSS, Chicago, IL, USA).
Hepatitis B e antigen titers and liver fibrosis

Results

Patient characteristics

We studied 249 treatment-naïve CHB patients, of whom 163 (65%) were HBeAg(+) and 86 (35%) HBeAg(-). There were 171 (69%) males and 78 (31%) females aged 18-63 years old (mean, 36.78±9.47 years old). The mean levels of HBV DNA and HBsAg were 7.20 and 3.99 log10 IU/mL, respectively, in HBeAg(+) patients, significantly higher than in HBeAg(-) patients. In HBeAg(+) patients, the mean HBeAg level was 1.55 PEIU/mL. Compared to patients with insignificant fibrosis, those with significant fibrosis had lower levels of HBsAg, HBeAg, and HBV DNA. We found no significant difference in fibrosis grade or the level of anti-HBs, ALT, AST, APRI, platelets, total bilirubin, or albumin among patients with different grades of liver fibrosis. However, age, prothrombin time, grade 4 fibrosis, and the levels of HBV DNA, HBsAg, HBeAg, and cholinesterase differed significantly between the groups (all \( P < 0.05 \)) (Table 1).

Correlations among serum levels of HBsAg, HBeAg, and HBV DNA

Levels of HBsAg and HBeAg were significantly correlated in HBeAg(+) patients (\( r = 0.572, P < 0.0001 \)) as were levels of HBV DNA and HBeAg (\( r = 0.603, P < 0.0001 \)) and levels of HBsAg and HBV DNA (\( r = 0.623, P < 0.0001 \)). No significant correlation between HBsAg and HBeAg levels or HBVDNA and HBeAg levels was apparent in HBeAg(-) patients. The correlation between HBsAg and HBV DNA levels in HBeAg(+) patients was significantly higher than that in HBeAg(-) patients (\( r = 0.623, P < 0.001 \) and \( r = 0.226, P < 0.05 \), respectively).

Associations between the levels of serum HBV markers and fibrosis stage

As fibrosis became aggravated, levels of HBsAg, HBeAg, and HBV DNA in HBeAg(+) patients trended downward (Figure 2A-C). Levels of HBsAg, HBeAg, and HBV DNA were negatively correlated with the extent of liver fibrosis in HBeAg(+) patients (\( r = -0.506, P < 0.001; r = -0.241, P < 0.0001; r = -0.384, P < 0.0001 \), respectively). The level of HBsAg showed a stronger inverse correlation with liver fibrosis than did the levels of HBeAg and HBV DNA. However, we found no significant correlation between the levels of HBsAg, HBeAg, or HBV DNA among patients with different grades of liver fibrosis.
Hepatitis B e antigen titers and liver fibrosis

HBV marker levels identify significant fibrosis

We stratified patients into two groups on the basis of the extent of fibrosis, and calculated the mean levels of HBV markers (Table 2). HBeAg(+) patients with significant fibrosis (stages S2-4) had significantly lower median levels of HBsAg, HBeAg, and HBV DNA than did those with insignificant fibrosis (stages S0-1). However, we found no significant correlation between levels of HBsAg, HBeAg, and HBV DNA and severity of fibrosis in HBeAg(-) patients.

Logistic regression modeling via ROC analysis

HBeAg(+) patients were divided into two groups in terms of the extent of fibrosis: S0-1 and S2-4. Binary logistic regression analyses showed that the HBeAg level and the prothrombin time were independent predictors of significant liver fibrosis. We drew ROC curves to seek to identify significant fibrosis on the basis of the levels of HBsAg, HBeAg, and HBV DNA. The AUROC curve of the level of HBeAg predicting significant fibrosis was 0.732 (95% CI 0.651-0.812). The cutoff was 2.23 log10 PEIU/mL, with a sensitivity of 72.5% and a specificity of 67.3%. The AUROC curves of HBsAg and HBV DNA levels were 0.755 (95% CI 0.674-0.835) and 0.708 (95% CI 0.626-0.791). The HBsAg cut-off value was 4.15 log10 IU/mL. This predicted significant fibrosis with a sensitivity of 73.6% and a specificity of 70.8%. An HBV DNA cutoff of 7.74 log10 IU/mL predicted significant fibrosis with a sensitivity of 71.4% and a specificity of 61.5% (Figure 4).

Discussion

The annual incidence of cirrhosis in patients with CHB is 2-10% [21]. It is necessary to intervene as early as possible (before cirrhosis develops) to slow the progression of the disease. The 2012 clinical practice guidelines of the European Association for the Study of the Liver and Chinese Medical Association Hepatology Society recommend that patients with moderate to severe fibrosis (S2-4) should receive antiviral treatment [22, 23]. Therefore, it is essential to identify fibrosis early.

HBeAg(+) patients had higher mean HBsAg, HBeAg, and HBV DNA levels than HBeAg(-) patients. Levels of HBsAg and HBV DNA were significantly correlated. Moreover, the HBeAg level was significantly correlated with the
Hepatitis B e antigen titers and liver fibrosis

Table 2. Serum levels of HBV markers according to HBeAg status and fibrosis stage

<table>
<thead>
<tr>
<th>Fibrosis score*</th>
<th>All (n = 249)</th>
<th>HBeAg(+) (n = 163)</th>
<th>HBeAg(-) (n = 86)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean HBsAg level log10 IU/ml</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S0-S1</td>
<td>3.80±0.98 (n = 103)</td>
<td>4.24±0.79 (n = 68)</td>
<td>2.96±0.74 (n = 35)</td>
</tr>
<tr>
<td>S2-S4</td>
<td>3.54±0.67 (n = 146)</td>
<td>3.74±0.64 (n = 95)</td>
<td>3.13±0.56 (n = 51)</td>
</tr>
<tr>
<td>P value</td>
<td>0.017</td>
<td>&lt;0.001</td>
<td>0.211</td>
</tr>
<tr>
<td>Mean Serum HBV DNA log10 IU/ml</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S0-S1</td>
<td>6.71±2.02 (n = 103)</td>
<td>7.76±1.50 (n = 68)</td>
<td>4.71±1.20 (n = 35)</td>
</tr>
<tr>
<td>S2-S4</td>
<td>6.18±1.79 (n = 146)</td>
<td>6.80±1.64 (n = 95)</td>
<td>4.98±1.47 (n = 51)</td>
</tr>
<tr>
<td>P value</td>
<td>0.033</td>
<td>&lt;0.001</td>
<td>0.345</td>
</tr>
<tr>
<td>Mean Serum HBeAg log10 PEIU/ml</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S0-S1</td>
<td>0.91±1.75 (n = 103)</td>
<td>2.04±0.86 (n = 68)</td>
<td>-1.33±0.04 (n = 35)</td>
</tr>
<tr>
<td>S2-S4</td>
<td>0.38±1.52 (n = 146)</td>
<td>1.23±1.12 (n = 95)</td>
<td>-1.31±0.04 (n = 51)</td>
</tr>
<tr>
<td>P value</td>
<td>0.013</td>
<td>&lt;0.001</td>
<td>0.57</td>
</tr>
</tbody>
</table>

*According to the Scheuer score. In HBeAg(+) CHB patients, lower serum HBeAg, HBsAg and HBV DNA levels were more frequent in those with moderate-to-severe fibrosis (stages S2-S4) than patients with no or mild fibrosis (stages S0-S1). There was no significant correlation between HBsAg, HBeAg, and HBV DNA levels with the severity of fibrosis in HBeAg(-) patients. Correlations between Serum levels of HBV markers and fibrosis stage were analyzed using the Spearman correlation test.

Figure 4. The diagnostic performance of HBV serum markers used to identify significant liver fibrosis in HBeAg(+) CHB patients. Binary logistic regression analyses identified factors independently associated with significant liver fibrosis. The AUROC curve of the serum HBeAg level predicting significant fibrosis was 0.732 (95% CI 0.651-0.812). The cut-off was 2.23 log10 PEIU/mL, with a sensitivity of 72.5% and a specificity of 67.3%. The AUROC curves of the serum HBsAg and HBV DNA levels were 0.755 (95% CI 0.674-0.835) and 0.708 (95% CI 0.626-0.791). The HBsAg cut-off value was 4.15 log10 IU/mL. This predicted significant fibrosis with a sensitivity of 73.6% and a specificity of 70.8%. An HBV DNA cut-off of 7.74 log10 IU/mL predicted significant fibrosis with a sensitivity of 71.4% and a specificity of 61.5%.

We found that serum HBV marker levels varied in patients with different stages of fibrosis. Compared to those with insignificant fibrosis, HBeAg(+) patients with significant fibrosis had significantly lower levels of HBsAg, HBeAg, and HBV DNA, as previously described [9, 27]; the reason is unclear. The immune response differs during progression from the immune toler-
Hepatitis B e antigen titers and liver fibrosis

Unconditional logistic regression showed that the HBeAg level and the prothrombin time were independent predictors of significant liver fibrosis in HBeAg(+) patients. The prothrombin time reflects liver coagulation function, and is increases as liver injury progresses. The HBeAg level plays an important role in the identification of treatment-naïve HBeAg(+) CHB patients with significant fibrosis. Although HBeAg is not essential for viral assembly or replication, it is important during natural infection in vivo. Higher serum HBeAg levels may reflect impaired immune status. As the HBeAg level falls, the immune response may increase to induce liver fibrosis. The AUROC curves showed that serum HBeAg, HBsAg, and HBV DNA levels of 0.732, 0.755, and 0.708, respectively, predicted significant fibrosis in HBeAg(+) patients.

Our study had several limitations. We enrolled only Chinese patients and did not assay the HBV genotype or the basal core promoter (BCP)/precore (PC) sequence thought to be associated with liver histological activity and fibrosis [9, 15, 27]. These tests are not routine in our hospital. Repeat inflammation is significant in terms of progression of CHB to cirrhosis and even HCC. Liver biopsy is invasive, but long-term monitoring of serum markers can be used to monitor disease progression.

In conclusion, we found a strong inverse correlation between serum levels of HBeAg and fibrosis severity in HBeAg(+) patients. Patients with moderate to severe fibrosis had lower HBeAg, HBsAg, and HBV DNA levels than did those with no or mild fibrosis.

Acknowledgements

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Disclosure of conflict of interest

None.

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Hepatitis B e antigen titers and liver fibrosis


Hepatitis B e antigen titers and liver fibrosis


