Relationship between Hepatitis B Virus Deoxyribonucleic Acid, HBeAg/anti-HBe Status in Serum and HBcAg in Liver: Its Clinical Significance in Chronic HBsAg Carriers

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Sera and biopsied liver specimens from 45 hepatitis B surface antigen (HBsAg) carriers both with or without overt liver diseases and negative for anti-delta antibody, were examined for markers of hepatitis B virus (HBV) infection: hepatitis B e antigen (HBeAg), hepatitis B virus deoxyribonucleic acid (HBV-DNA) in serum and hepatitis B core antigen (HBcAg) in liver. HBV-DNA in serum was assayed by the spot hybridization technique, and HBcAg in the liver was investigated by the peroxidase anti-peroxidase method. Among the parameters showing active replication of HBV, serum HBeAg, serum HBV-DNA and intrahepatic HBcAg were found in 27 (60%), 27 (60%) and in 22 (48.9%) of 45 HBsAg carriers, respectively. The presence of serum HBV-DNA and of intrahepatic HBcAg in HBeAg positive cases and the absence of serum HBV-DNA and of intrahepatic HBcAg in anti-HBe positive cases was the rule with few disparate cases among those with chronic liver disease. These parameters were not useful in predicting the histology on liver biopsy. The activity of hepatic inflammation in the HBsAg chronic carriers assessed by serum alanine aminotransferase level closely paralleled HBV-DNA in serum but not HBeAg in serum, HBcAg in liver or histologic picture on biopsy. HBV-DNA may be the most sensitive parameter of replication of hepatitis B virus and of the activity of inflammation in the liver.

Key Words: HBV-DNA, HBeAg, HBcAg in liver

The presence of hepatitis B e antigen (HBeAg) in the serum of hepatitis B surface antigen (HBsAg) carriers has generally been considered a reflection of active replication of hepatitis B virus (HBV) and high infectivity, whereas the presence of antibody to hepatitis B e antigen (anti-HBe) in serum has been regarded as an indication of regression of liver disease activity and a low grade of infectivity1,2,3. More recently, however, it has been shown that a substantial number of anti-HBe positive patients have demonstrable hepatitis B core antigen in liver which signifies active HBV replication4,5. Furthermore, recent reports have described patients positive for anti-HBe with active HBV replication shown by hepatitis B virus DNA (HBV-DNA) in serum5,6,7,8,9. Sherlock et al. pointed out that there was a geographical difference in the positive rate of serum HBV-DNA among anti-HBe positive patients10. On the other hand, Matsuyama et al.11 reported that the presence of HBV-DNA in anti-HBe sera and the absence of HBV-DNA in HBeAg-positive sera related to liver disease, i.e., chronic active hepatitis and cirrhosis of the liver, since these phenomena were not seen in asymptomatic HBsAg carriers.

In this study, we investigated HBeAg/anti-HBe status and HBV-DNA in serum and HBcAg in liver of chronic HBsAg carriers. We wished to compare these with histological findings on liver biopsy. We also wished to correlate the respective HBV-markers with liver dysfunction as measured by elevated serum alanine aminotransferase levels.
MATERIALS AND METHODS

Patients:
Serum samples and liver-biopsied specimens from 45 Japanese patients referred to Shinshu University Hospital because they had been HBsAg positive for more than 6 months. There were 38 men and 7 women, ranging in age from 18 to 54. Of 45 HBsAg carriers, 14 were identified at the time blood donation, 10 were found to have HBsAg on family health screening because of liver disease in other family members, 9 were found to have HBsAg on routine health screening, and 12 visited physicians due to nonspecific complaints such as fatigue, anorexia, nausea, and vomiting and were found on laboratory studies due to nonspecific complaints such as fatigue, anorexia, nausea, and vomiting and were found on laboratory studies to be HBsAg positive. All were referred to the University for investigation of the activity of liver disease and infectivity for hepatitis B virus (HBV). None had a history of blood transfusion or illicit self injection. None had received antiviral or immunosuppressive drugs during the 6 months prior to our examination and follow-up. All patients were followed for one year or more after liver biopsy. Each patient liver was biopsied under peritoneoscopy and processed for routine histological staining after fixation in 10% formaline and embedding in paraffine. All serum samples used in this study were taken on the same day that the liver biopsy was performed.

Laboratory tests:
Biochemical determinations including serum alanine aminotransferase (sALT) were done by multichannel autoanalyzer. The normal range for sALT is 4 to 44 Karmen Units/ml. Serum samples were tested for HBsAg by reversed passive hemagglutination (RPHA), and for antibody to HBsAg (anti-HBs) by passive hemagglutination (PHA). Sera were also tested for HBeAg, anti-HBe and antibody to delta-antigen (anti-delta) by radio-immunoassay using commercial kits (Abbott Laboratories, North Chicago, Ill.).

Histologic diagnosis of liver disease:
Chronic hepatitis was classified histologically according to Bianchi et al. as chronic persistent hepatitis (CPH) or chronic active hepatitis (CAH). Cirrhosis of the liver was diagnosed macroscopically at peritoneoscopy and histologically. Biopsy specimens without clearcut histological findings of CPH, CAH or cirrhosis were classified as nonspecific hepatitis (NSH) if some evidence of necroinflammatory disease was present.

Detection for HBeAg in liver:
Intrahepatic HBeAg was examined by the peroxidase anti-peroxidase method using PAP-kits (DAKO Co. LTD, Santa Barbara, CA.) HBeAg in the liver was quantitatively determined by the percentage of hepatocytes positive for HBeAg: +++ >50%, ++ 10-50%, +<10%, – negative.

Detection for HBV-DNA in serum:
HBV-DNA in serum was detected by the method of Scotto et al. with a slight modification. Briefly, the probe was cloned, and plasmid HBV-DNA (3.2 kilobase pairs) was labeled with 32P by nick translation at a specific activity of 2 – 4 x 10^6 dpm/ug. Each sample (150 microliters) was denatured in 150 microliters of 2M NaCl and 300 microliters of 1M NaOH at room temperature for 10 minutes; the mixture was spotted on a nylon membrane (Biodyne A, Pall Corp., Glen Cove, N.Y.) under a negative pressure through a filter apparatus. After the filter was drained, 600 microliters of 0.5M Tris-HCl, 3M NaCl (pH7.4) was passed through it; the membrane was then removed and baked in a vacuum at 80°C for 2 hours. The membrane was incubated at 42°C over night with a prehybridization solution containing 50% (vol/vol) formamide, 6 x SSC (0.9M NaCl, 0.09M sodium citrate), 5 x Denhardt’s solution, 0.5% SDS, 0.01M EDTA, and denatured salmon sperm DNA (100ug/ml). The hybridization was carried out in the presence of a denatured probe at 42°C for 24 hours in the same solutions as was used for prehybridisation. After hybridisation, the nylon membrane was washed at 60°C for 5 hours in Tris-HCl buffer (0.15M, pH7.4) containing 0.45M NaCl, 0.01M EDTA, 0.01% Dextran T 500 (Pharmacia Fine Chemicals, Uppsala, Sweden), 0.01% Ficoll 400 (Pharmacia Fine Chemicals) and 0.2% SDS. Finally, the membrane was dried and autoradiographed at −70°C for 7 days. The amount of HBV-DNA in each spot was estimated by reading on the “standard” spot with a known amount of...
HBV-DNA, HBeAg/anti-HBe in Serum and HBcAg in Liver

Table 1. Correlation of serum HBeAg/anti-HBe status with histological findings of biopsied liver.

<table>
<thead>
<tr>
<th>HBeAg/anti-HBe</th>
<th>No.</th>
<th>male</th>
<th>female</th>
<th>age (years) average±SD</th>
<th>histology*</th>
</tr>
</thead>
<tbody>
<tr>
<td>+/−</td>
<td>27</td>
<td>22</td>
<td>5</td>
<td>34.1±8.8</td>
<td>NRH: 5</td>
</tr>
<tr>
<td>−/−</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>40.7±6.7</td>
<td>CPH: 0</td>
</tr>
<tr>
<td>−/+</td>
<td>15</td>
<td>13</td>
<td>2</td>
<td>42.3±9.2</td>
<td>CAH: 3</td>
</tr>
</tbody>
</table>

NRH: nonspecific reactive hepatitis, CPH: chronic persistent hepatitis, CAH: chronic active hepatitis, LC: cirrhosis of liver

HBV-DNA from a serum proven infectious for chimpanzee\(^{13}\). The minimum amount of HBV-DNA thus detected was 5.0 CIU (chimpanzee infectious unit); thus, samples were considered positive for HBV-DNA if they contained 5 or more CIU.

Statistical analysis:
For statistical evaluation, Student’s t-test was used with a p value less than 0.05 considered significant.

RESULTS

Presence of serum HBeAg/anti-HBe, HBV-DNA and intrahepatic HBcAg: demographic aspects and liver histology.

Of the 45 HBsAg carriers, 27 patients (60%) were HBeAg positive, 15 (33.3%) were anti-HBe positive, and the remaining 3 were negative for both HBeAg and anti-HBe. Serum HBV-DNA was detected in 27 patients (60%) ranging from 5.0 CIU to 8.0 CIU and intrahepatic HBcAg was found in 22 patients (48.9%) ranging from + to ++. Table 1 compares serum HBeAg/anti-HBe status with demographic aspects and histologic diagnoses. Fig. 1 illustrates the correlations between HBeAg/anti-HBe status, HBcAg in liver and HBV-DNA in the serum in relation to histological diagnoses. Of the 27 HBeAg positive patients, 22 had HBV-DNA in serum and 20 HBcAg in liver. Of the 15 patients with anti-HBe, 12 had neither HBV-DNA for HBcAg in liver while 3 had one or both. Of the 3 patients without detectable HBeAg or anti-HBe, one had both HBcAg and HBV-DNA, one HBV-DNA alone, and one neither. The rates of discordance between HBeAg/anti-HBe and HBcAg in liver or HBV-DNA in serum by histologic diagnoses are detailed in Table 2. There were few discordant results; only 3 of the 27 HBeAg positive individuals had neither HBV-DNA nor intrahepatic HBcAg and only 3 of the 15 anti-HBe positive individuals had either serum HBV-DNA or HBcAg in the liver.

Table 2. Discordance of HBeAg/anti-HBe, HBV-DNA in serum and HBcAg in liver.

<table>
<thead>
<tr>
<th>histologic diagnosis</th>
<th>No.</th>
<th>rate of discordance of three HBV markers*</th>
</tr>
</thead>
<tbody>
<tr>
<td>nonspecific reactive hepatitis</td>
<td>8</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>chronic persistent hepatitis</td>
<td>16</td>
<td>7 (48.3%)</td>
</tr>
<tr>
<td>chronic active hepatitis</td>
<td>12</td>
<td>4 (33.3%)</td>
</tr>
<tr>
<td>cirrhosis of liver</td>
<td>6</td>
<td>3 (50%)</td>
</tr>
</tbody>
</table>

* Discordance means the that absence of HBV-DNA in sera and/or HBcAg in liver in HBeAg positive sera or the presence of HBV-DNA in sera and/or HBcAg in liver in anti-HBe positive sera.
Table 3. Relationship between serum alanine aminotransferase levels and HBeAg, HBV-DNA in serum and HBcAg in liver in chronic liver diseases proven by liver biopsy

<table>
<thead>
<tr>
<th>Category</th>
<th>No.</th>
<th>sALT (Karmen Unit)</th>
<th>t-test</th>
<th>range</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mean ± SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HBeAg in serum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>positive</td>
<td>22</td>
<td>109.4 ± 110.5</td>
<td></td>
<td>(26-436) ns</td>
</tr>
<tr>
<td>negative</td>
<td>15</td>
<td>87.3 ± 85.1</td>
<td></td>
<td>(17-267)</td>
</tr>
<tr>
<td>HBV-DNA in serum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>positive</td>
<td>21</td>
<td>138.5 ± 114.6</td>
<td></td>
<td>(21-436) p&lt;0.01</td>
</tr>
<tr>
<td>negative</td>
<td>16</td>
<td>43.4 ± 22.7</td>
<td></td>
<td>(17-96)</td>
</tr>
<tr>
<td>HBcAg in liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>positive</td>
<td>17</td>
<td>138.7 ± 127.8</td>
<td></td>
<td>(21-436) ns</td>
</tr>
<tr>
<td>negative</td>
<td>20</td>
<td>64.2 ± 42.7</td>
<td></td>
<td>(17-165)</td>
</tr>
</tbody>
</table>

ns: not significant

Serum alanine aminotransferase level and its relation to HBeAg/anti-HBe status, HBV-DNA in serum, HBcAg in liver and histology on liver biopsy.

The relationships between sALT level and serum HBeAg/anti-HBe status, serum HBV-DNA, and HBcAg in liver are shown in Table 3. There was a significant difference in sALT level between the 2 groups with and without HBV-DNA in serum (p<0.01). There was no significant difference in mean sALT levels in patients with or without HBeAg in serum and in patients with and without HBcAg in liver. All 8 of the patients with nonspecific hepatitis on biopsy had sALT levels in the normal range. On the other hand, 12 of 15 with CAH, 10 of 16 with CPH and 2 of 6 with cirrhosis had elevated sALT levels.

Anti-delta and anti-HBs.

All patients were negative for anti-delta and anti-HBs in their serum specimens.

DISCUSSION

It has been generally accepted that in chronic HBV infection, the presence of HBeAg in serum reflects a state of active HBV replication\(^{1,2,3}\), whereas the presence of anti-HBe in serum reflects a state of low or absent HBV replication\(^{14,15,16}\). We had confirmed that HBV continues to replicate in a few patients even after seroconversion from HBeAg to anti-HBe\(^{6,7,8,9}\). Further, in this study, we have expanded the observations on the infectivity of HBV in regard to HBeAg/anti-HBe status by measuring both HBcAg in liver and HBV-DNA in serum in chronic HBsAg carriers. We found good correlations between the presence of the so-called markers of infectivity for HBV, i.e., serum HBeAg, serum HBV-DNA, and intrahepatic HBcAg, and their absence. Among 27 HBeAg positive individuals, 22 (81%) also had serum HBV-DNA detectable and 20 (74%) had intrahepatic HBcAg. Among the 15 patients with anti-HBe, who are believed to be less infectious for HBV, 12 had neither detectable serum HBV-DNA for HBcAg in the liver. Thus, anti-HBe positive individuals appear to be less infectious for HBV and to have less replicating HBV.

We analyzed the relationship between the status of three HBV markers; i.e. serum HBeAg/anti-HBe, serum HBV-DNA and HBcAg in liver, and histological change. The discordance of the presence of these HBV markers; i.e., the absence of the serum HBV-DNA and/or HBcAg in liver in HBeAg-positive sera or the presence of the HBV-DNA and/or HBcAg in liver in anti-HBe-positive sera, was observed in patients with chronic liver disease and was not found in patients without obvious liver disease. This means that in asymptomatic HBsAg carriers without liver disease, HBeAg/anti-HBe usually reflect the presence or absence of intrahepatic HBV-replication respectively. On the other hand, in chronic liver disease, HBeAg/anti-HBe do not always reflect the state of intrahepatic HBV replication. Matsuyama et al.\(^{11}\) and Chu et al.\(^{17}\) reported the discordance between serum HBeAg/anti-HBe status and HBV-DNA in patients with chronic hepatitis and cirrhosis. We expanded the observation between HBeAg/anti-HBe, HBV-DNA in serum, and HBcAg in liver and found some discordance here too. Krogsgaard et al.\(^{18}\) reported that patients with type B acute hepatitis showed a discrepancy between the expected presence of HBeAg and HBV-DNA in serum in course of the illness. Since our patients were chronic HBsAg carriers and we did not have serial
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measurements of HBV markers of infectivity, our results cannot be compared with theirs.

When we compared sALT levels between the presence and absence of three HBV replicative markers (HBeAg and HBV-DNA in serum and HBcAg in liver) in chronic liver disease, we found a significant difference in mean sALT level only between those with and without HBV-DNA in serum. This may indicate that in chronic liver diseases the presence of HBV-DNA in serum reflects liver cell damage. However, serum HBV-DNA is also detected in asymptomatic HBsAg carriers without obvious liver damage. This indicates that host-immune response to HBV replication contributes to the development of injury of infected hepatocytes. Thus, the status of immune-response of individuals and the histological situations complicates the relationship among the HBV markers in serum and liver.

Regarding to the discordance of HBeAg and HBV-DNA, the difference of race had been discussed in a couple of papers10,19. Superinfection of delta antigen may influence the relationship between HBV and the degree of hepatic damage; however, none of patients in our study had such a superinfection. All of our patients were from Japan; we have not had the opportunity to study HBV markers of infectivity in HBsAg carriers from other countries in Far East.

ACKNOWLEDGMENT: We gratefully acknowledge Dr. Paul V. Holland, Medical Director of Sacramento Blood Center for valuable criticism and help in preparing the manuscript.

This work was supported by a grant from a Japanese Ministry of Health and Welfare.

REFERENCES


Jpn J Med Vol 27, No 3 (August, 1988) 271