Clinical Features and Hepatitis B Virus (HBV) Genotypes in Pregnant Women Chronically Infected with HBV

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Abstract

Objective The purpose of this study was to clarify the clinical features and hepatitis B virus (HBV) genotypes in pregnant women chronically infected with HBV.

Methods Among 1,489 pregnant women who visited our hospital in 2010, 26 were positive for hepatitis B surface antigens (HBsAg). Of these subjects, 21 from whom informed consent was obtained were included in this study. The clinical features and HBV markers, including genotypes, were investigated.

Results No adverse events were observed in the subjects or the neonates during pregnancy or the perinatal period. The HBV genotypes were C in 14 cases, D in six cases, and undetermined in one case. Hepatitis B e antigens and a high viral load (>7.0 log copies/mL) were found in four and six subjects with genotype C, respectively, and in none of subjects with genotype D. The alanine aminotransferase (ALT) levels and platelet counts were within the normal ranges during pregnancy in all subjects except two and three subjects with genotype C, respectively. Three subjects with genotype C showed transient elevations of ALT after delivery.

Conclusion The majority of subjects were anti-HBe-positive with normal ALT levels; however, some subjects with genotype C showed a high viral load, elevated ALT levels and/or low platelet counts. The pregnancies and deliveries were safe; however, transient elevations of ALT after delivery were observed in some subjects with genotype C.

Key words: hepatitis B virus, genotype, pregnancy

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Introduction

Hepatitis B virus (HBV) is a DNA virus with approximately 3,200 base pairs. HBV induces a variety of liver diseases, ranging from acute or fulminant hepatitis to liver cirrhosis and hepatocellular carcinoma. Approximately 350 million people are chronically infected and two billion people are transiently infected worldwide. HBV can be classified into at least eight genotypes, with a divergence of more than 8% in the nucleotide sequences (1-3). It has been reported that there are differences in the clinical features and routes of transmission between genotypes (4, 5).

In Japan, genotypes B and C are the predominant genotypes; however, the distribution of genotypes is changing (6-8). It has been reported that the main transmission route is vertical in genotypes B and C and horizontal in genotypes A and D (4, 9). Furthermore, the viral states of mothers are closely related to transmission to neonates and children (10-12). There is a paucity of information regarding
HBV markers including HBV genotypes and infectious routes in pregnant women chronically infected with HBV. Moreover, little is known about the differences in clinical features of pregnant women infected with HBV in relation to HBV genotypes. In the present study, we attempted to clarify these issues among patients in the north-western area of Shikoku Island in Japan.

Materials and Methods

Subjects

In 2010, 1,489 pregnant women visited our hospital located in the north-western area of Shikoku Island in Japan. Hepatitis B surface antigens (HBsAg) were assayed in all of the subjects. The aim and protocol of the study were explained to the HBsAg-positive pregnant women, and those from whom written informed consent was obtained were included in the study. This study was conducted prospectively.

Methods

HBsAg were screened using a chemoluminescence immunoassay (CLIA). Hepatitis B e antigens (HBeAg) and anti-HBe were assayed with CLIA, and the HBV-DNA levels were assayed using a real-time polymerase chain reaction (PCR) method. The HBV genotypes were determined using a serial invasive signal amplification reaction assay (Inva-der® assay; BML Inc, Saitama, Japan) (13). When genotype could not be identified with this method, it was also assayed using an enzyme immunoassay (EIA) method (Immunis®, HBV genotype EIA; Institute of Immunology Co., Ltd, Tokyo, Japan) (14). The platelet counts and the levels of alanine aminotransferase (ALT), AFP, HBeAg, anti-HBe and HBV-DNA were assayed during pregnancy, including in the third trimester and one to two months after delivery. Ultrasonography was performed to determine the findings of the liver, especially to screen for findings suspicious of liver cirrhosis or hepatocellular carcinoma.

The clinical diagnoses were determined according to liver function tests, hemograms and ultrasonography findings. In the present study, HBeAg-positive asymptomatic HBV carrier (immune tolerant phase) and inactive HBsAg carrier state were defined as an ALT level within the normal range during pregnancy, a platelet counts over 150,000/μL, no ultrasonographic findings suspicious of liver cirrhosis or hepatocellular carcinoma and HBeAg positive and negative status, respectively. Chronic hepatitis was defined as an elevated ALT level and a platelet count within the normal range (>150,000/μL) with no ultrasonographic findings suspicious of liver cirrhosis. ALT <40 IU/L was defined as the normal range according to the normal range used in our institute. It is known that the level of platelet is related to fibrosis of the liver, and patients with a platelet count <150,000/μL may have fibrosis of the liver. Therefore, regarding the diagnosis of subjects with normal ALT levels and low platelet counts, the diagnoses of these cases were classified as “undetermined”.

Information regarding infectious routes was obtained with medical interviews. When the mothers of the subjects had no history of hepatitis B infection, the infectious route was classified as horizontal; when the mothers had a history of chronic HBV infection, the infectious route was classified as vertical.

Statistical analysis

The statistical analyses were performed using the chi-square test, the Wilcoxon signed-rank test and paired t-test. p<0.05 was considered significant.

Results

Twenty-six of the 1,489 (1.7%) pregnant women were proven to be positive for HBsAg. The details of the study were explained to the 26 women, and written informed consent for participation in the study was obtained from 21 of them. These 21 women were involved in the study. No subjects showed ultrasonographic findings suspicious of liver cirrhosis or hepatocellular carcinoma. The clinical data of the subjects are shown in Table. The route of infection was vertical in six cases (28.6%), horizontal in nine cases (42.9%), and undetermined in six cases (28.6%). HBeAg status was positive in four of the 21 subjects, whereas 16 subjects were positive for anti-HBe and one subject was negative for both HBeAg and anti-HBe. The HBV-DNA level was >7 log copies (LC)/mL in six subjects, 3.5 LC/mL in five subjects, and lower than 3 LC/mL in 10 subjects. The ALT level was within the normal range in 19 subjects and elevated in two subjects during pregnancy. In the present study, the normal range of ALT was defined as <40 IU/L. No subjects showed an ALT level with a range between 30 and 40 IU/L during pregnancy; therefore, the data described above would not have changed if the normal range of ALT was defined as ≤30 IU/L, as has been reported elsewhere (15).

The platelet count was lower than the normal range in three cases (108,000-115,000/μL). These three women had normal ALT levels and were positive for anti-HBe. The clinical diagnosis was HBeAg-positive asymptomatic HBV carrier in four cases, chronic hepatitis in two cases, and inactive HBsAg carrier state in 12 cases. The HBV genotype was determined in 20 subjects: genotype C in 14 subjects and genotype D in six subjects. The genotype was undetermined in one subject due to low levels of HBV-DNA and HBsAg. The infectious route in the genotype C-infected subjects was vertical in six cases and horizontal in four cases, whereas no subjects with genotype D were infected vertically. HBeAg-positive asymptomatic HBV carrier and chronic hepatitis were found in genotype C-infected women only. All of the six genotype D-infected women were diagnosed with inactive HBsAg carrier state. The HBsAg levels ranged from 30.5 to 74,591 IU/mL in genotype C-infected women and 880 to 20,343 IU/mL in the genotype D-
Table. The Clinical Data of the Subjects according to the HBV Genotypes

<table>
<thead>
<tr>
<th>HBV genotype</th>
<th>C</th>
<th>D</th>
<th>Undetermined</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>14</td>
<td>6</td>
<td>1</td>
<td>21</td>
</tr>
<tr>
<td>Age (median)</td>
<td>25 - 40 (34)</td>
<td>32 - 37 (34)</td>
<td>36</td>
<td>25 - 40 (34)</td>
</tr>
<tr>
<td>Infectious route</td>
<td>Vertical</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Horizontal</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>undetermined</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Diagnosis*</td>
<td>HBeAg+ASC</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CH</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>ICS</td>
<td>5</td>
<td>6</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>undetermined**</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>HBeAg/anti-HBe</td>
<td>+/-</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>-/</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>-/+</td>
<td>9</td>
<td>6</td>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td>HBV-DNA (log copies/mL)</td>
<td>0 – 3</td>
<td>6</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>3 - 5</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>&gt;7</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>HBsAg (IU/mL)</td>
<td>Median (min-max)</td>
<td>9,511 (30,5-74,591)</td>
<td>4,090 (880-20,343)</td>
<td>2,281</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>&gt;40</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>&lt;40</td>
<td>12</td>
<td>6</td>
<td>1</td>
<td>19</td>
</tr>
<tr>
<td>Platelet (μL)</td>
<td>&lt;150,000</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>&gt;150,000</td>
<td>11</td>
<td>6</td>
<td>1</td>
<td>18</td>
</tr>
<tr>
<td>Delivery</td>
<td>vaginal delivery</td>
<td>13</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Cesarean section</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

*HBeAg+ASC: HBeAg-positive asymptomatic HBV carrier, CH: chronic hepatitis, ICS: inactive HBsAg carrier state
**Anti-HBe+, a normal level of ALT, a low levels of HBV-DNA (<4.0 LC/mL) with a low platelet count (<150,000/μL)

Figure 1. Changes in the levels of ALT, HBV-DNA and AFP before and after delivery. (a) ALT, (b) HBV-DNA, (c) AFP. Infected genotypes are shown by colors; black: genotype C, red: genotype D, blue: undetermined.

infected women. Although the median level was higher in genotype C-infected women (9,511 vs. 4,090 IU/mL), the difference was not significant. No subjects with genotype D infection showed elevated ALT levels or low platelet counts; however, two and three subjects with genotype C showed elevated ALT levels and low platelet counts, respectively. No adverse events were observed in the subjects or neonates during pregnancy or the perinatal period regardless of HBV genotype. Cesarean sections were performed in one of 15 women infected with genotype C and one of the six women infected with genotype D.

The changes in the levels of ALT, HBV-DNA and AFP before and after delivery are shown in Fig. 1. The ALT levels were significantly higher after delivery (16.7±15.2 IU/L vs. 37.3±35.3 IU/L, p<0.05, paired t-test). There were no significant differences in the HBV-DNA levels before and after delivery. The AFP levels were elevated in all subjects during pregnancy (172.8±109.5 ng/μL), and returned to normal levels after delivery (4.3±3.1 ng/μL) in all subjects regardless of genotype (p<0.001, paired t-test). Three subjects,
with normal ALTs level before delivery, showed transient elevation of ALT after delivery (Fig. 2). Two of these three subjects were positive for HBeAg, and the remaining subject was positive for anti-HBe with a low platelet count. All three subjects were infected with HBV genotype C, with HBsAg levels ranging from 2,893 to 74,591 IU/mL (median: 20,977 IU/mL) and HBV-DNA levels ranging from 3.7 to >9.0 LC/mL (median: 8.3 LC/mL). Although no significant differences in the levels of HBsAg and HBV-DNA were found between these three subjects and those with no elevations of ALT after delivery, two of the three subjects with ALT elevation after delivery showed high levels of HBsAg and HBV-DNA. The HBV-DNA levels were elevated from 3.7 LC/mL to 5.2 LC/mL in one woman and >8.0 LC/mL in other 2 women both before and after delivery. The ALT levels returned to normal several months after delivery in all cases. No changes in the HBV-DNA level were observed in 18 of the 21 women, whereas the HBV-DNA levels were transiently elevated in three women after delivery (two women with genotype C and one woman with genotype D). One subject showed an elevation in the HBV-DNA level from 3.7 LC/mL to 5.2 LC/mL accompanied by an elevation in the ALT level, as shown in Fig. 2(c). The other two women showed elevations in the HBV-DNA level from <2.1 to 3.9 LC/mL and 2.4 to 3.6 LC/mL, respectively; however, no elevations in the ALT levels were observed. No changes in HBeAg or anti-HBe status were observed in any of the subjects.

**Discussion**

The prevalence of HBsAg-positive status in pregnant women was 1.7% in the present study. This is higher than the prevalence in the whole population of Japan, which has been estimated to be approximately 1%. The high rate observed in our study may be associated with the nature of our hospital to which many pregnant women at high risk or with complications are referred; therefore, the present data are probably not indicative of a generally high prevalence of HBV carrier status among pregnant women in this district.

In the majority of the subjects, the clinical diagnosis was inactive HBsAg carrier state, whereas two of the 21 subjects had chronic hepatitis. Three of the 19 women whose ALT levels were within the normal range during pregnancy showed transient elevations in ALT after delivery. Exacerbation of hepatitis B during pregnancy and the postpartum period, accompanied by clearance of HBeAg has been reported elsewhere (16-18). Although no changes in the HBeAg/anti-HBe were observed in the present study, the changes in the ALT levels observed in some of the subjects are consistent with previous reports that disease exacerbation may occur in some patients. Our findings confirm that it is necessary to monitor the ALT levels carefully before as well as after delivery, even if the ALT levels are within the normal range.

![Figure 2. The clinical courses of the women infected with hepatitis B virus genotype C who showed transient elevations of ALT after delivery. (a) A 35-year-old woman, (b) A 25-year-old woman, (c) A 39-year-old woman. The bold line indicates the upper limit of the normal range of ALT.](image-url)
HBV genotypes C and D were identified in the present subjects. In Japan, genotype C is most common followed by genotype B (6). However, in the district where our hospital is situated, the presence of genotype D has also been reported, with a prevalence of genotypes B, C, and D of approximately 5%, 88%, and 6%, respectively (19). The rate of genotype D-infected subjects in the present study was higher than that reported in previous studies, however, another report indicated that the proportion of genotype D in this district is high among HBV carriers born in the 1970s, in which decade the HBV genotype D was supposed to have spread in this area (20). Duong et al. investigated the clinical features of patients infected with genotypes C and D in this district, and reported a high virulence of genotype C compared with genotype D, with the rate of anti-HBe state being higher in patients with genotype D than in those with genotype C (21). In the present study, chronic hepatitis or HBsAg-positive cases were found only in genotype C-infected women, and all women who showed elevations in the ALT levels after delivery were also genotype C-infected subjects. These data are compatible with those of previous reports.

The route of transmission was vertical in six cases and horizontal in nine cases, which may indicate that the most common major route of transmission is horizontal in pregnant women in Japan. Vertical transmission has been reported to be the major route of transmission in East Asia; however, this trend may be changing in Japan. The prevention program against vertical HBV transmission introduced in 1986 in Japan (22) and improvements in hygiene may be associated with this change in transmission. Regarding the infectious routes in relation to the HBV genotype, the majority (or all) of the subjects with genotype D included in this study were presumed to be horizontally infected. Genotype C is known as a genotype for which the main transmission route is vertical. However, in the present study, four of 10 patients with genotype C for whom information regarding transmission route was obtained were presumed to be infected horizontally. Inui et al. reported that the rate of vertical transmission in children is 77% in Japan and that the rate was high in patients infected with genotype C compared with those infected with other genotypes. This report indicates that the major transmission route is vertical; however, there exist a considerable number of HBV carriers infected horizontally, especially those infected with genotypes other than genotype C (23). The infectious routes were determined according to medical interviews in the present study; therefore, the data regarding infectious routes in the present study may not be precise. However, the ratio of vertical transmission to horizontal transmission may be decreasing, especially in areas where genotypes other than genotype C are circulated. These data may indicate that existing prevention programs for vertical infection are not sufficient for preventing HBV transmission, and projects for preventing horizontal infection should be discussed as a national prophylactic strategy in areas or countries where universal vaccination has not been introduced.

In conclusion, HBV genotypes C and D are prevalent among pregnant women in this district. All of the subjects infected with genotype D exhibited the clinical features of the inactive HBsAg carrier state. On the other hand, all of the subjects with elevated ALT levels during pregnancy, transient elevations of ALT after delivery, high viral loads and/or low platelet counts were infected with genotype C.

The authors state that they have no Conflict of Interest (COI).

Acknowledgement

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