Mutations of hepatitis B virus S gene by hepatitis B immunoglobulin administration in late pregnancy

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The safety of hepatitis B immunoglobulin (HBIG) administration in late pregnancy is still controversial, especially the hepatitis B virus (HBV) mutation-generating effects of HBIG. In order to clarify the association between HBV S gene mutations and HBIG administration in late pregnancy, 106 HBV carrier mothers and 60 newborns were recruited for the study. Peripheral blood specimens were collected from mothers before HBIG administration and delivery, as well as from newborns before vaccine immunization. HBV-DNA was detected by fluorescent quantitative-polymerase chain reaction (PCR). HBV S gene was amplified by nested PCR and then directly sequenced. Compared with nucleotide sequences and amino acids in HBV S gene of 73 cases in HBIG group, there were no changes after HBIG injection until delivery. The rate of the mothers infected with genotype C was significantly higher than mothers with genotype B (75.47% vs. 24.53%; P < 0.05). Seven of sixty neonates obtained the results of sequencing. All of the seven newborns were genotype C. But HBV genotypes were not significantly different in HBV intrauterine transmission (P > 0.05). Injection of HBIG does not cause mutations of HBV S gene in Chinese pregnant women. HBV genotypes are not the main factors to intrauterine transmission.

Key words: Hepatitis B virus S gene, mutation, genotype, hepatitis B immunoglobulin.

INTRODUCTION

Hepatitis B virus (HBV) is a worldwide problem that has affected about 350 million people. Approximately 40 to 50% of HBV carriers are the result of mother-to-child transmission (Shi et al., 2011). A recent meta-analysis revealed that injections of hepatitis B immunoglobulin (HBIG) in HBV carrier mothers with a high degree of infectiousness in late pregnancy, effectively prevent HBV intrauterine transmission, which might be due to the reduced maternal HBV DNA load or the development of the newborn's passive immunity (Shi et al., 2010; Xiao et al., 2007). However, the safety of HBIG administration in late pregnancy is still controversial, especially the HBV mutation-generating effects of HBIG. Mutations of HBV S gene have been found in patients who were undergoing HBIG immunoprophylaxis after liver transplantation (Protzer-Knolle et al., 1998).

The S gene of HBV is responsible for the expression of surface antigens and includes the ‘a’-determinant region. Thus, mutations in this region would enable a distinct survival advantage to HBV variants, permitting the mutant virus to escape from the immune system. Whether HBIG administration in late pregnancy would cause the mutations of HBV S gene, the role of HBV genotypes in
intrauterine transmission, guiding treatment, controlling liver diseases, and improving vaccination is still unclear (Kao, 2007).

MATERIALS AND METHODS

Participants

The study was approved by the local ethics committee of the First Affiliated Hospital of Jinan University, and informed consent was obtained from all participants. A total of 106 asymptomatic pregnant women who were HBV carriers were recruited for the study by the Department of Obstetrics and Gynecology of the First Affiliated Hospital of Jinan University. The HBsAg and HBeAg status were checked in the HBV carrier mothers. 73 HBV carrier mothers received HBIG treatment during the third trimester of pregnancy. The maternal HBIG treatment consisted of 200 IU of hepatitis B immunoglobulin (Sichuan Yuan Da Shu Yang Pharmaceutical Co., Ltd. Sichuan, China) by intramuscular injection in the 28th, 32nd and 36th week of pregnancy. The women not treated with HBIG received no other specific treatment. Peripheral blood specimens of HBIG group were collected from the mothers before HBIG administration and delivery. Peripheral blood specimens of control group were collected from the mothers in the 28th and before delivery. Peripheral blood specimens of newborns (only 60 newborns are enrolled with their parents’ agreements) were collected within 24 h after birth before vaccine immunization. Sera were separated and store at -20°C.

DNA isolation and amplification

DNA was isolated from 200 µl serum for each sample according to the method of TIANamp Genomic DNA kit (TIANGEN Biotech Co., Ltd, Beijing). HBV DNA was amplified by nested polymerase chain reaction (PCR). For the first stage PCR, 25 µl reaction mixture contained PCR buffer, dNTP (2.5 mM), primer (sense: 5’-tactgcctcacccatatcgt-3’; antisense: 5’-gctggtaaagtaccccaactt-3’) and Taq DNA polymerase (5 IU/mL). After the first amplification, 1 µl of the PCR products were reamplified with second stage primers (sense: 5’-gctggtaaagtaccccaactt-3’; antisense: 5’-gttaagggagtagccccaac-3’) for 35 cycles. The first amplification was performed with an initial 5 min denaturing step at 95°C, followed by 35 cycles of denaturing at 95°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 30 s, with a final extension at 72°C for 10 min. The second amplification was performed with an initial 5 min denaturing step at 95°C, followed by 35 cycles of denaturing at 95°C for 30 s, annealing at 58°C for 30 s, and elongation at 72°C for 30 s, with a final extension at 72°C for 10 min, and the products of second stage were run on 1% agarose gel, amplified PCR products were 774 bp (Figure 1).

Sequencing

The sequencing analysis of HBV S gene region obtained from the second stage PCR was done by Baolei Co., Ltd in Shanghai. The sequencing primer was 5’-tactgcctcacccatatcgt-3’.

Genotype and mutation analysis

Compared to the standard genetic sequence in GENBANK, we analyzed genotype and S gene region mutation by DNASTAR ALIGN X softwares.

Statistical analyses

Values are expressed as mean ± SEM. All statistical analyses were performed using SPSS software, version 13.0 (SPSS Inc., Chicago, IL, USA). The t and \( \chi^2 \) tests were used. \( P < 0.05 \) was considered statistically significant.

RESULTS

Participants’ characteristics

Specimens from 106 HBV carrier mothers (73 in HBIG group, 33 in control) and 60 newborns were collected. There were no significant differences in age, maternal alanine aminotransferase (ALT), pregnancy duration between the women who had received HBIG treatment during late pregnancy and those who had not (Table 1).
Table 1. Demographic factors in the different study groups.

<table>
<thead>
<tr>
<th>Factor</th>
<th>HBIG</th>
<th>Control</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of women</td>
<td>73</td>
<td>33</td>
<td>-</td>
</tr>
<tr>
<td>HBeAg(+)</td>
<td>57</td>
<td>8</td>
<td>-</td>
</tr>
<tr>
<td>Age (years)</td>
<td>28.21 ± 3.27</td>
<td>29.30 ± 4.16</td>
<td>0.102</td>
</tr>
<tr>
<td>Maternal ALT</td>
<td>22.03 ± 7.23</td>
<td>19.26 ± 7.38</td>
<td>0.242</td>
</tr>
<tr>
<td>Gestational age at birth (weeks)</td>
<td>38.80 ± 1.23</td>
<td>38.89 ± 1.61</td>
<td>0.715</td>
</tr>
</tbody>
</table>

Figure 2. Sequencing of HBV S gene region from sample 20 in HBIG group. a: maternal specimen before administration of HBIG; b: maternal specimen before delivery (110 to 200 are mutation prone areas).

Table 2. HBV genotypes and HBV-DNA titers in the different study groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HBIG</th>
<th>Control</th>
<th>Total</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO. of women</td>
<td>17 (23.29%)</td>
<td>56 (76.71%)</td>
<td>26 (24.53%)</td>
<td>80 (75.47%)</td>
<td>-</td>
</tr>
<tr>
<td>A1: HBV DNA*</td>
<td>5.12 ± 1.12</td>
<td>6.04 ± 0.94</td>
<td>4.31 ± 1.33</td>
<td>4.87 ± 1.52</td>
<td>4.82 ± 0.95</td>
</tr>
</tbody>
</table>

Mutations of HBV S gene

Compared with nucleotide sequences and amino acids in HBV S gene of 73 cases in HBIG group, there were no changes after HBIG injection until delivery (Figure 2). There were no changes of HBV S gene in control group.

Genotypes of HBV

The rate of the mothers infected with genotype C was significantly higher than mothers with genotype B (75.47% vs. 24.53%; P < 0.05). The HBV-DNA titer of genotype C was much higher than genotype B (P < 0.05) (Table 2). There were seven newborns tested positive for HBV-DNA. Seven of them obtained the results of sequencing. All of the seven newborns were genotype C. Although there were seven cases of HBV genotype C transmission, the high prevalence of this genotype in the pregnant women meant that no statistical significance could be found (P > 0.05) (Table 3).

DISCUSSION

HBV is a DNA virus. Since the HBV DNA polymerase lack proofreading function, HBV has a high rate of nucleotide misincorporation during replication or reverse transcription (Carman et al., 1990). From a clinical perspective, the S escape mutant is the most worrisome, because in the absence of surveillance systems, the diagnosis can be difficult to establish. Undiagnosed cases can progress to liver failure and hepatocellular carcinoma.
In addition to genetic variations of natural evolutionary changes, HBV S gene mutation can also emerge as a result of antiviral therapy (Protzer-Knolle et al., 1998). A similar kind of study before had reported that HBV carrier mothers who received injections of HBIG before delivery would not be influenced by HBV S gene mutation, but the samples of that study was limited (only 8 cases in study group)(Chen et al., 2006). In our study, there was no evidence that HBIG administration would cause HBV S gene mutation in late pregnancy. Compared with nucleotide sequences and amino acids in HBV S gene of 73 cases in HBIG group, there were no changes after HBIG injection until delivery. In our study, HBV carrier mothers received HBIG injection of total 600 IU in the third trimester, which was much lower than the dosage (Total 20000 to 100000 IU) after liver transplantation (Protzer-Knolle et al., 1998). Since Sanger sequencing is able to detect mutations when present in >20% of available copies, we can use other techniques, that is, reverse hybridization or pyrosequencing to rule out pre-existing mutations below detection level in next step.

Passive–active immunoprophylaxis with HBIG and hepatitis B vaccine in the infants of HBV carriers is now immunoprophylaxis because they were infected in utero early life even though they received routine neonatal HBIG injection until delivery. In our study, HBV carrier mothers received HBIG injection of total 600 IU in the third trimester, which was much lower than the dosage (Total 20000 to 100000 IU) after liver transplantation (Protzer-Knolle et al., 1998). Since Sanger sequencing is able to detect mutations when present in >20% of available copies, we can use other techniques, that is, reverse hybridization or pyrosequencing to rule out pre-existing mutations below detection level in next step.

In summary, our study indicates that HBIG administration in late pregnancy does not cause HBV S gene mutation, which provides an evidence of the safety of HBIG administration in late pregnancy where HBV genotypes are not the main factors to intrauterine transmission of HBV.

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