Clinical and Virological Aspects of Blood Donors Infected with Hepatitis B Virus Genotypes B and C

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Pathogenic and therapeutic differences among hepatitis B virus (HBV) genotypes have been documented. However, the association of virological characteristics with clinical differences among HBV genotypes remains unclear. We therefore studied the clinical and virological characteristics of Taiwanese volunteer blood donors infected with HBV genotypes B and C. HBV genotypes were determined in 300 candidate blood donors positive for HBV surface antigen (HBsAg), and sequences of the precore gene of the HBV genome were determined in 50 HBV e antigen (HBeAg)-positive and 50 HBeAg-negative blood donors. Of 300 HBeAg-positive blood donors, 10% had elevated serum aminotransferase levels and 27% were positive for HBeAg. HBV genotype distribution in 264 viremic carriers was as follows: B, 221 (83.7%); C, 39 (14.8%); F, 1 (0.4%); and mixed infection, 3 (1.1%). Blood donors with genotype C infection tended to have a higher frequency of HBeAg positivity and a higher serum HBV DNA level than those with genotype B infection. The frequency of precore stop codon mutation was significantly higher in HBeAg-negative blood donors than HBeAg-positive ones, irrespective of HBV genotypes. Meanwhile, only 5% of blood donors with genotype C infection had C-1858 strains. In conclusion, mixed infection of HBV genotypes indeed occurs, and genotype C has a higher serum HBV DNA level than genotype B. Precore stop codon mutation is common in HBeAg-negative HBV carriers, irrespective of HBV genotypes. In contrast, precore C-1858 strains are rarely identified in Taiwanese HBV genotype C.

Hepatitis B virus (HBV) infection is a global health problem, and more than 350 million people of the world population are chronic carriers of the virus (7). The infection is associated with a wide clinical spectrum, ranging from acute or fulminant hepatitis to various forms of chronic infection, including asymptomatic carrier status, chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC) (3, 7). Although serological and genotypic classifications of HBV have been well documented (20, 24), the clinical significance of HBV genotypes in terms of clinical outcomes and therapeutic response to antiviral therapy in patients with chronic HBV infection remained largely unknown until recently. Our previous studies indicated that HBV genotypes B and C are the most prevalent viral strains in Taiwan, and genotype C is associated with the development of cirrhosis and HCC while genotype B may be associated with the development of HCC in young patients (8). In addition, HBV genotype C is associated with a higher frequency of core promoter mutation and a lower response rate to alpha interferon therapy compared to genotype B (10). Taken together, these data suggest the possible pathogenic and therapeutic differences among HBV genotypes. However, the association of virological characteristics, including efficiency of viral replication as well as viral genome variability, with these clinical differences among HBV genotypes remains unclear. We therefore studied the clinical features and virological characteristics, with special reference to the precore mutations, of Taiwanese volunteer blood donors infected with HBV genotypes B and C.

MATERIALS AND METHODS

Subjects. Plasma samples from 300 volunteers (197 men and 103 women; mean age, 31 ± 10 years) eliminated as blood donors because of positivity for HBV surface antigen (HBsAg) were collected and stored at −70°C until use. These samples were negative for antibodies to HCV and HDV (anti-HCV and anti-HDV), and were selected from blood donations at Taipei (northern), Taichung (central), and Kaohsiung (southern) Blood Donation Centers through the Blood Services Foundation of the Republic of China, which collects virtually all of the blood donations in Taiwan. The foundation is a nongovernment and nonprofit organization and has an annual voluntary donation of nearly 2 million units. Around 1.8% of them were HBsAg positive in 2000.

Serological tests. Serum alanine aminotransferase (ALT) was tested with routine automated techniques (upper limit of normal, 40 U/liter). Serum HBsAg and HBV e antigen (HBeAg) were assayed by Ausria-II and IMx HBe 2.0 (Abbott Laboratories, North Chicago, Ill., respectively). Anti-HCV and anti-HDV were tested by commercially available assays (HCV EIA II, Anti-Delta; Abbott Laboratories).

Genotyping of HBV. HBV genotypes were determined by using the nested PCR-restriction fragment length polymorphism of the surface gene of HBV as previously described (18). Six genotypes (A to F) of HBV could be identified by the restriction patterns of DNA fragments. To avoid false-positive results, instructions to prevent cross-contaminations were strictly followed, and results were considered valid only when they were obtained in duplicate. The sensitivities of our first-round and second-round PCR assays were 103 and 10 copies of HBV DNA per specimen, respectively, by testing serial 10-fold dilutions of HBV DNA transcripts with known amounts (103 copies/ml).

Amplification and sequencing of the precore gene. The entire precore gene was amplified in samples from 50 HBeAg-positive (34 with genotype B infection and 16 with genotype C infection) and 50 HBeAg-negative (27 with genotype B infection and 23 with genotype C infection) blood donors as previously described (10), and precore mutations at codons 1, 2, 28, and 29 as well as variability at nucleotide 1858 were subsequently analyzed. Nucleotide sequences of amplified products were directly determined by using fluorescence-labeled primers with a 373A Sequencer (Applied Biosystems, Foster City, Calif.). Sequencing conditions were specified in the protocol for the Taq DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems). The inner primer pair was used as sequencing primers for both directions.
TABLE 1. Clinical and virological features of volunteer blood donors infected with HBV genotype B or C

<table>
<thead>
<tr>
<th>Feature</th>
<th>Genotype B</th>
<th>Genotype C</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of donors</td>
<td>221</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>Gender (no. male/no. female)</td>
<td>150/71</td>
<td>22/17</td>
<td>NS8</td>
</tr>
<tr>
<td>Mean age ± SD (yr)</td>
<td>31 ± 11</td>
<td>30 ± 9</td>
<td>NS</td>
</tr>
<tr>
<td>Positivity for HBeAg</td>
<td>66 (30)</td>
<td>16 (41)</td>
<td>NS</td>
</tr>
<tr>
<td>Mean ALT (U/liter)</td>
<td>26 ± 34</td>
<td>18 ± 12</td>
<td>NS</td>
</tr>
<tr>
<td>ALT &gt; 40 U/liter</td>
<td>27 (12)</td>
<td>2 (5)</td>
<td>NS</td>
</tr>
<tr>
<td>Positivity of PCR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First round</td>
<td>122 (55)</td>
<td>30 (77)</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Second round</td>
<td>99 (45)</td>
<td>9 (23)</td>
<td></td>
</tr>
</tbody>
</table>

* Unless otherwise noted, results are presented as number (percentage).
8 NS, not significant.

Statistical analysis. Fisher’s exact test, chi-square test with Yates’ correction, and Student’s t test were used where appropriate. A P value of <0.05 was considered statistically significant.

RESULTS

Of 300 HBsAg-positive volunteer blood donors, 30 (10%) had elevated serum ALT levels (>40 U/liter) and 82 (27%) were positive for HBeAg. Among them, 264 (88%) were positive for serum HBV DNA by the sensitive PCR assay and HBV genotype distribution was as follows: B, 221 (83.7%); C, 39 (14.8%); F, 1 (0.4%); and mixed infection of B and C, 3 (1.1%). Accordingly, genotypes B and C were the predominant strains among these candidate volunteer blood donors persistently infected with HBV. The clinical and virological features of HBsAg-positive volunteer blood donors infected with HBV genotype B or C are shown in Table 1. Those with genotype B or C infection were comparable in terms of gender, mean age, seropositivity for HBeAg, mean serum ALT level, and the frequency of elevated serum ALT level. However, those with genotype C infection had a higher frequency of first-round PCR positivity than those with genotype B infection (77 versus 55%; P < 0.02).

The prevalence of precore mutations (codons 1, 2, 28, and 29) and variability at nucleotide 1858 (codon 15) and precore mutations (codons 28 and 29) according to the positivity for HBeAg and HBV genotypes

<table>
<thead>
<tr>
<th>Variability [no. (%)]</th>
<th>Genotype No.</th>
<th>Codon 15</th>
<th>Codon 28</th>
<th>Codon 29</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C-1858</td>
<td>T-1858</td>
<td>(A-1896)</td>
</tr>
<tr>
<td>HBeAg+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>34</td>
<td>0</td>
<td>34 (100)</td>
<td>4 (12)b</td>
</tr>
<tr>
<td>C</td>
<td>16</td>
<td>1 (6)</td>
<td>15 (95)</td>
<td>1 (6)f</td>
</tr>
<tr>
<td>HBeAg−</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>27</td>
<td>0</td>
<td>27 (100)</td>
<td>20 (74)b</td>
</tr>
<tr>
<td>C</td>
<td>23</td>
<td>1 (4)‡</td>
<td>22 (96)</td>
<td>15 (65)f</td>
</tr>
</tbody>
</table>

* This patient had a wild-type codon 28 (G-1896).
‡ P < 0.001.
8 P < 0.001.
‡ All had codon 28 mutations (A-1896).

DISCUSSION

In addition to the serological classification of HBV isolates into nine subtypes according to the antigenic determinants of their HBsAg (4, 19), a genetic classification based on the comparison of complete genomes has recently defined seven genotypes of HBV (A to G) (20, 24). HBV genotypes have distinct geographical distributions (11, 21). In general, genotypes B and C are prevalent in Asia (8, 26), whereas genotypes A and D prevail in Western countries (17). Genotype E is restricted to Africa, and genotype F prevails in Central America. Genotype G has been identified in France and North America very recently (24). Our previous clinic-based study indicated that genotypes B and C are the most common HBV genotypes in Taiwan (8); however, the study population may not be well controlled, and thus a selection bias may be present. To avoid the possibility of biased selection, studies based on a general population such as first time blood donors should be more appropriate to address this important issue. By using a simple HBV genotyping method (18), the distribution of HBV genotypes was studied in the general population of Taiwan, and our data consistently showed that genotype B was the most predominant HBV, followed by genotype C. Other genotypes accounted for only a minimal proportion. In addition, mixed infection of genotypes B and C was found in 1% of the HBsAg-positive candidate volunteer blood donors, suggesting that coinfection of different HBV genotypes or superinfection of heterologous HBV strains on top of HBV carriers may occur (6, 29), as is the case in chronic HCV or HDV (5, 9, 30). In addition, the frequency of HBeAg positivity (27%) and abnormal serum ALT level (10%) in our HBsAg-positive volunteer blood donors was comparable to that reported in asymptomatic HBV carriers (28).

The clinical, virological, and therapeutic implications of HBV genotypes in patients with chronic HBV infection have been partially clarified. Our previous data suggested that HBV genotype C is associated with the development of cirrhosis and HCC as well as a lower response rate to interferon therapy compared to genotype B (8, 10). Lindh et al. also indicated that genotype C, compared to genotype B, is associated with a higher frequency of HBeAg positivity and HBV DNA level, more pronounced liver inflammation, and a lower frequency of...
precore mutants as well as a higher frequency of core promoter mutants (13, 15). Similarly, Orito et al. reported that HBeAg and core promoter mutants were less frequent in genotype B than C but that the frequencies of precore mutants were comparable between genotypes B and C (22). Nevertheless, the association of molecular virological characteristics, including efficiency of viral replication as well as viral genome variability, with these differences among HBV genotypes remains to be established further.

In the present study, the results showed that the clinical and laboratory features were comparable between volunteer blood donors with genotype B or C infection (Table 1). Although HBeAg was less common in samples from genotype B- than C-infected volunteer blood donors (30 versus 41%), the difference was not statistically significant. However, samples from volunteer blood donors with genotype C infection had a higher frequency of first-round PCR positivity than those with genotype B infection (77 versus 55%; P < 0.02), suggesting that genotype C may yield a higher HBV DNA level than genotype B as previously reported (13, 15).

Mutations in the precore and basal core promoter regions of the HBV genome have been observed in patients with chronic HBV infection (1, 25). The major missense or nonsense mutations in the precore region are found in codons 1, 2, 28, and 29 (14, 16). Among them is a G-to-A change at nucleotide 1896 (codon 28), which creates a premature stop codon (precore stop codon mutant). This mutation prevents the translation of the precore protein and completely abolishes the production of HBeAg (27). In addition, nucleotide variability (T or C) at position 1858 (codon 15) is commonly observed (11). The relationship between HBV genotypes and types of precore mutation as well as nucleotide variability at position 1858 has been reported (6, 14). For example, genotypes other than A have a T at nucleotide 1858 (T-1858) which makes a wobble pairing with G-1896 in the stem of the e encapsidation signal. The mutation for A-1896 (precore stop codon mutant) tightens the stem structure by making a T-A pair. In contrast, genotype A possesses C-1858, making C-G pair with G-1896 in the wild type. Since mutation for A-1896 breaks this stable pair, it does not occur except in combination with another mutation from C-1858 to T-1858. Accordingly, precore stop codon mutation is restricted to HBV strains with T-1858 and rarely occurs in those with C-1858, and this may explain why genotype A rarely circulates as an HBe mutant and why genotype D is the most frequent HBV genotype among the precore mutants in Ag-negative Western countries (6, 12, 16, 23). Recently, C-1858 is also frequently observed in Chinese patients with genotype C infection (2); however, the sample size of the study was limited, and further large studies are needed to confirm the findings. In the present study, we sequenced the precore region of the HBV genome in samples from 50 HBeAg-positive and 50 HBeAg-negative volunteer blood donors with genotype B or C infection (Table 2). Our data showed that none of them had a codon 1 or 2 mutation. In contrast, the frequency of precore stop codon mutation was significantly higher in samples from HBeAg-negative volunteer blood donors than HBeAg-positive ones, irrespective of HBV genotypes, confirming that this mutation can universally terminate the production of HBeAg in different genotypes. In addition, codon 29 mutation was less frequently found, and all codon 29 mutations were in genotype B strains and in combination with codon 28 mutation as previously described (16). Sequence analysis of the nucleotide variability at nucleotide 1858 showed that all of the genotype B and most (95%) of the genotype C strains had T-1858, and the only two patients with genotype C infection who had C-1858 all possessed G-1896. These data were consistent with previous reports (6, 12, 16, 23) and suggested that precore C-1858 strains rarely circulate in Taiwanese HBV carriers, even in those infected with genotype C strains.

In summary, our data indicate that HBV genotype B is the most predominant genotype in Taiwan and mixed infection of genotypes B and C may occur. Patients with genotype C infection, compared to those with genotype B infection, have a higher frequency of HBeAg positivity and a higher HBV DNA level that may contribute to multiple episodes of acute flares and progression of liver disease. In addition, precore stop codon mutations are rather prevalent in HBeAg-negative HBV carriers; however, precore C-1858 strains are rare in HBV genotype C in Taiwan.

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REFERENCES


