High Levels of Hepatitis B Virus After the Onset of Disease Lead to Chronic Infection in Patients With Acute Hepatitis B

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Background. Some patients with acute hepatitis B virus (HBV) infection develop chronic infection. However, the method for identifying these patients has not been established.

Methods. We followed 215 Japanese patients with acute HBV infection until the clearance of hepatitis B surface antigen (HBsAg) or the development of chronic infection. Levels of HBsAg and HBV DNA were serially monitored from the onset.

Results. Of the 215 patients, 113 (52.5%) possessed HBV genotype A, 26 (12.0%) genotype B, and 73 (34.0%) genotype C. Twenty-one of the 215 (9.8%) developed chronic infection, with the persistence of HBsAg for >6 months. The rate of chronicity of genotype A, B, and C was 12.4%, 3.8%, and 8.2%. Of the 21 patients, only 6 (2.8%) patients, including 5 with genotype A, failed to clear HBsAg within 12 months. Levels of HBsAg at 12 weeks and HBV DNA at 4 weeks were useful for distinguishing the patients who became chronic from those who did not (P < .001 and P < .001, respectively). Likewise, the levels of HBsAg at 12 weeks and HBV DNA at 8 weeks were useful for discriminating between the patients who lost HBsAg within 12 months and those who did not (P < .01 and P < .05, respectively).

Conclusions. In acute HBV infection, clearance of HBV may happen between 6 and 12 months from the onset. Only those who fail to clear HBV within 12 months from the onset may develop chronic infection.

Keywords. hepatitis B virus antigen; hepatitis B virus; genotype.

The clinical outcome of acute hepatitis B is self-limited in the majority of immunocompetent adults. However, some patients run a prolonged or even chronic course, or are complicated by acute liver failure. Several factors are implicated in different clinical courses.

Hepatitis B virus (HBV) genotypes and subtypes are known to influence the clinical outcome of acute hepatitis B. For instance, HBV subgenotype B1 is associated with fulminant hepatic failure in acute hepatitis B [1]. On the other hand, genotype A is associated with chronic sequelae [2–5]. Furthermore, patients with subgenotype C2 are more likely to develop chronic infection than those with subgenotype B2 [6]. These characteristics may reflect viral kinetics in acute HBV infection that would differ among HBV infections with distinct genotypes/subgenotypes, but little is known about them.

Quantitation of hepatitis B surface antigen (HBsAg), in addition to HBV DNA, has been introduced to analysis of viral kinetics in patients with chronic hepatitis B in recent years. HBsAg levels are also useful for estimating...
viral loads and predicting the response to antiviral treatments [7–9], and for determining the natural history of chronic hepatitis B [10, 11]. Therefore, HBsAg and HBV DNA would be instrumental in foretelling the outcome of acute hepatitis B. However, the clinical utility of these markers in patients with acute hepatitis B is largely unknown.

Therefore, the aim of the present study was to examine differences in viral kinetics among patients with acute hepatitis B, who were infected with HBV of different genotypes, and evaluate the usefulness of quantifying HBsAg and HBV DNA for predicting the clinical outcome.

**PATIENTS AND METHODS**

**Patients**

This was a retrospective study of patients who were diagnosed with acute hepatitis B in our institutions during 1994 through 2010. Criteria for the diagnosis of acute hepatitis B were (1) acute onset of liver injury without a previous history of liver dysfunction; (2) detection of HBsAg in the serum; (3) immunoglobulin M (IgM) antibody to HBV core (anti-HBc) in high titers (detectable in serum samples diluted 10-fold) [3]; (4) absence of a past or family history of chronic HBV infection; and (5) exclusion of coinfection with hepatitis A virus, hepatitis C virus, or other hepatotropic viruses by serologic testing.

Among the 232 patients who met these criteria, 215 patients (159 men and 56 women with a mean age of 31.8 ± 10.0 years) whose serum samples were available for virologic analyses were included in the study. No patient developed liver failure.

No patient received antiviral treatment. Of the 215 patients, 159 (74.0%) patients could be regularly followed up until the confirmation of clinical outcomes. Based on the duration of HBsAg (defined as the interval between the onset [defined by the first visit] and the last visit with detectable HBsAg), we classified the 159 patients into the following 4 groups (the duration of HBsAg is indicated in parentheses): group 1 (<3 months); group 2 (3–6 months); group 3 (>6–12 months); and group 4 (>12 months). Changes in virologic parameters were analyzed in relation with clinical characteristics. The study was approved by the ethics committees of our institutions, and written informed consent was obtained from each patient.

**Quantification of Serologic Markers for HBV Infection and HBV DNA**

HBsAg had been measured quantitatively by chemiluminescent enzyme-linked immunosorbent assay (ELISA; Sysmex JAPAN Co, Ltd, Kobe, Japan) every 2–4 weeks, until the clinical outcome was known. It has a dynamic range of 0.03–2, 500 IU/mL. Serum samples scaling out from this range were diluted so as to contain them within it. Antibody to hepatitis B s antigen (anti-HBs), hepatitis B e antigen (HBeAg), and IgM anti- HBc were determined by ELISA (Abbott JAPAN Co, Ltd, Tokyo, Japan). Levels of HBV DNA were determined using the COBAS TaqMan HBV v.2.0 kit (Roche Diagnostics, Basel, Switzerland), which has a dynamic range over 2.1–9.0 log copies/mL.

**HBV Genotyping**

The HBV genotype was determined by a genotype-specific probe assay (Smittest HBV genotyping Kit, Genome Science, Fukushima, Japan) as previously reported [12].

**Molecular Evolutionary Analyses**

HBV genotype A started to prevail in Japan merely several years ago, suggesting that it was imported to Japan only recently [3, 13]. Therefore, genomic sequences of HBV genotype A (HBV/A), recovered from sera of patients with acute HBV infection, would be closely related to one another and with those reported from abroad. To evaluate this possibility, 20 HBV/A samples were selected randomly and sequenced by the method reported previously [14].

The number of nucleotide substitutions per site was estimated by the 6-parameter method [15], and a phylogenetic tree was constructed by the neighbor-joining method [16] based on the numbers of substitutions. To confirm the credibility of phylogenetic analyses, bootstrap resampling tests were carried out 1000 times [17].

**Statistical Analyses**

Categorical variables were compared by χ² test or Fisher exact test, and continuous variables by the Mann-Whitney U test. P < .05 was considered statistically significant. Receiver operating characteristic (ROC) analysis was performed to compute the area under the ROC curves for viral markers to determine cutoff points for predicting the outcome.

**RESULTS**

**Distribution of HBV Genotypes in Patients With Acute Hepatitis B**

HBV genotypes were determined in 215 of the 232 (93%) patients with acute hepatitis B. Of the 215 patients, genotype A was detected in 113 (52%), B in 26 (12%), C in 73 (33%), D in 1 (1%), E in 1 (1%), and F in 1 (1%). The distribution of genotypes was compared among 4 periods during 1994 through 2010 (Table 1). The proportion of patients with genotype A gradually increased to 65.9% in 2007–2010; it was higher than those in the earlier periods (34.4% in 1994–1998 [P = .002], 36.8% in 1999–2002 [P = .002], and 51.9% in 2003–2006 [P = .093]).

**Phylogenetic Relationship Among HBV Strains of Genotype A**

We randomly selected 11 HBV/A strains sampled in 2007–2010 and 9 of those in 2001–2006, and constructed a molecular evolutionary tree (Figure 1). All 20 samples had similar nucleotide sequences with a concordance of 99%. They were close to previously
Table 1. Prevalence of Hepatitis B Virus Genotypes in Patients With Acute Hepatitis B During 4 Chronologic Periods

<table>
<thead>
<tr>
<th>Period</th>
<th>Genotype A</th>
<th>Genotype B</th>
<th>Genotype C</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>1994–1998</td>
<td>11a (34.4%)</td>
<td>3 (9.3%)</td>
<td>18 (56.3%)</td>
<td>0</td>
</tr>
<tr>
<td>(n = 32)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1994–1998</td>
<td>14b (36.8%)</td>
<td>4 (10.5%)</td>
<td>20 (52.7%)</td>
<td>0</td>
</tr>
<tr>
<td>(n = 38)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1994–1998</td>
<td>28c (51.9%)</td>
<td>6 (11.1%)</td>
<td>19 (35.1%)</td>
<td>1 (1.9%)</td>
</tr>
<tr>
<td>(n = 54)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1994–1998</td>
<td>60a,b,c (65.9%)</td>
<td>13 (14.3%)</td>
<td>16 (17.6%)</td>
<td>2 (2.2%)</td>
</tr>
<tr>
<td>(n = 91)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>113 (52.5%)</td>
<td>26 (12.0%)</td>
<td>73 (34.0%)</td>
<td>3 (1.5%)</td>
</tr>
<tr>
<td>(N = 215)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- a P = .0032.
- b P = .0014.
- c P = .02.

reported genotype A2 sequences from Western countries. The results support the possibility that HBV/A was imported to Japan only recently and has been spreading throughout the country.

Clinical Features Among Patients Infected With HBV of Different Genotypes

Clinical features of patients with acute hepatitis B of different genotypes are compared in Table 2. The mean age was no different among patients infected with HBV of different genotypes. The proportion of men was higher in genotype A or B than C infection (93.8% or 80.7% vs 39.7% [A vs C, P < .001; B vs C, P < .001]).

The maximum alanine aminotransferase (ALT) level was lower in patients with genotype A than in those with genotype C (2126 ± 938 vs 2857 ± 1668 IU/L, P = .002). The maximum bilirubin level was higher in patients with genotype A (7.1 ± 6.4 mg/dL) or C (9.0 ± 7.5 mg/dL) than in those with genotype B (4.8 ± 3.3 mg/dL) (A vs B, P = .003; B vs C, P < .001).

Regarding viral markers, the peak HBV DNA level was higher in patients with genotype A than in those with genotype C (6.3 ± 1.7 vs 4.9 ± 1.5 log copies/mL, P < .001). HBeAg was detected in 95 of the 121 (77.3%) patients with genotype A, 24 of the 28 (88.5%) with genotype B, and 37 of the 58 (65.5%) with genotype C (A vs C, P = .036). Men who have sex with men were more frequently represented among patients with genotype A than B or C (31.4% vs 4.8% or 11.3% [A vs B, P = .017; A vs C, P = .002]). Antibody to human immunodeficiency virus (anti-HIV) was examined in 72 of the 113 (63.7%) patients with genotype A, 7 of the 26 (26.9%) with genotype B, 58 of the 73 (79.5%) with genotype C, and 1 with genotype E. Anti-HIV was detected in 7 of the 72 (9.7%) patients with genotype A, and the other 96 patients tested for anti-HIV showed negative results. All of the 7 patients with anti-HIV cleared HBeAg from the serum within 6 months without antiviral treatment.

Among the 215 patients whose HBV genotypes were determined, 159 could be followed until the confirmation of clinical outcomes. The distribution of HBeAg-positive period is compared among patients with different genotypes. Group 1 (HBeAg persisting for <3 months) comprised 84 patients; group 2 (3–6 months) comprised 54 patients; group 3 (>6–12 months) comprised 15 patients; and group 4 (>12 months) comprised 6 patients. HBeAg remained >6 months in 21 of the 215 (9.8%) patients, including 14 of the 113 (12.4%) with genotype A, 1 of the 26 (3.8%) with genotype B, and 6 of the 73 (8.2%) with genotype C. Among the 21 patients, 15 (71.4%) cleared HBeAg within 12 months from the onset, and were classified into group 3. The remaining 6 (5 with genotype A and 1 with genotype B) who failed to clear HBeAg within 12 months were classified into group 4. All of the 6 were negative for anti-HIV. The proportion of group 4 was 6.0% in the patients with genotype A, 4.0% in those with genotype B, and 0% in those with genotype C.

The mean duration of HBeAg was 13.9 ± 8.7 weeks in patients with genotype A, 7.1 ± 5.3 weeks in those with genotype B, and 9.6 ± 7.6 weeks in those with genotype C, presuming the duration of HBeAg in group 4 at 12 months. The duration was longer in patients with genotype A than in those with B or C (A vs B, P < .001; A vs C, P = .04).

Prediction of the Outcome by the Duration of HBeAg

Table 2 shows that the duration of HBeAg among patients with genotype A varied to a higher extent than that among those with other genotypes. Therefore, we determined HBeAg and HBV DNA levels serially, and evaluated them for the ability to predict the outcome of acute hepatitis B in patients with genotype A.

Serial changes in HBeAg levels are shown in Supplementary Figure 1A. HBeAg levels declined more slowly in group 2 than group 1, as well as in group 3 than group 2. In group 4, HBeAg reelevated at 12 weeks after the onset. Figure 2 compares HBeAg levels among groups 1–4 at different intervals from the onset. HBeAg at 8 weeks from the onset was useful for distinguishing group 3 or 4 from group 1 or 2. Likewise, HBeAg at 12 weeks from the onset was helpful for discriminating among groups 2, 3, and 4.

Prediction of the Outcome by HBV DNA

We also studied serial changes of HBV DNA in patients with genotype A, and examined if they also were useful for predicting the clinical outcome of acute hepatitis B. Supplementary Figure 1B shows serial changes in HBV DNA levels in patients in 4 groups. Although the reelevation of HBV DNA was not observed, the decline of HBV DNA was quite slow in group 4. Figure 3 compares HBV DNA levels among groups 1–4 at different intervals from the onset. HBV DNA at 4 weeks from
the onset was useful for distinguishing group 3 or 4 from group 1 or 2. Likewise, HBV DNA levels at 8 weeks from the onset were useful for discriminating between group 4 and group 3, as well as for distinguishing group 3 or 4 from group 1 or 2.

Levels of HBsAg and HBV DNA for Predicting Persistent Infection
As the levels of HBsAg at 12 weeks and HBV DNA at 8 weeks from the onset were useful for distinguishing group 4 from the other groups, we evaluated the appropriate levels for predicting persistent infection in patients with genotype A. When we set the cutoff value of HBsAg at 1000 IU/mL based on the ROC analysis, both the positive predictive value and the negative predictive value were 100% with high sensitivity (100%) and specificity (98.1%). Likewise, when we set the cutoff value of HBV DNA at $10^6$ log IU/mL based on the ROC analysis, both the positive predictive value and the negative predictive value were 100% with high sensitivity (100%) and specificity (96.4%). Therefore, HBsAg at 12 weeks $>$1000 IU/mL or HBV DNA at 8 weeks $>$10^6 log copies/mL is useful for predicting persistent infection.

DISCUSSION
In Japan, as shown in Table 1, the dominant HBV in acute hepatitis has been shifting from genotype C to A [3, 5, 14, 18]. The fact that nucleotide sequences of HBV/A isolates from patients

Figure 1. Evolutionary relationships of 86 hepatitis B virus genotype A taxa, including 20 from the present cases. The evolutionary history, inferred using the neighbor-joining method, shows that all 20 samples had similar nucleotide sequences close to previously reported genotype A2 sequences from Western countries.
with acute hepatitis B in this study were very close to one another suggests that most HBV/A strains were imported recently and have spread rapidly, which may be attributed to the features of HBV/A in transmission routes and viral kinetics. We have reported that patients with genotype A tend to have multiple sexual partners [5]. Consequently, chances of secondary transmission of HBV/A would be higher than those of other genotypes, which may increase the number of patients who contract HBV/A infections. On the other hand, HBsAg persisted longer in patients with genotype A than B or C, which is consistent with the in vivo experiment using chimera mice carrying human hepatocytes showing that proliferation of HBV starts later and lasts longer in genotype A than in B or C infection [19].

Our results have shown that 6% of the patients with genotype A develop persistent infection. Because liver cirrhosis or hepatocellular carcinoma can develop in a substantial population of HBV carriers [20, 21], it is important to distinguish the patients in whom HBV infection becomes chronic, and follow them carefully. Although polymorphisms in host genes may be useful for identifying patients who are prone to develop chronic HBV infection [22], simple surrogate markers for the outcome have not been reported. Our data indicate that it would be difficult to predict the clinical outcome based on serum levels of viral markers at the first visit alone. This is understandable, because the dose of infecting virus, as well as the interval between infection and the first visit, can vary widely. Hence, we set out to analyze changes in serum levels of viral markers.

As seen in Figure 2, HBsAg levels at 12 weeks from the onset were most useful for discriminating among groups 2, 3, and 4 in the genotype A infection. Therefore, the outcome of acute hepatitis B may be predictable at this time point. Of note is the reelevation of HBsAg observed in group IV (Supplementary Figure 1A). Reelevation of viral markers suggests prolonged viral proliferation in the liver, and may be useful to identify the patients who may develop chronic infection.

<table>
<thead>
<tr>
<th>HBV Genotypes</th>
<th>A (n = 113)</th>
<th>B (n = 26)</th>
<th>C (n = 73)</th>
<th>D (n = 1)</th>
<th>E (n = 1)</th>
<th>F (n = 1)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, y</strong></td>
<td>30.8 ± 9.5</td>
<td>32.3 ± 9.5</td>
<td>33.3 ± 10.9</td>
<td>27</td>
<td>26</td>
<td>58</td>
</tr>
<tr>
<td><strong>Male</strong></td>
<td>106 (93.8%)</td>
<td>21 (80.7%)</td>
<td>29 (39.7%)</td>
<td>0</td>
<td>0</td>
<td>1 (100%)</td>
</tr>
<tr>
<td><strong>Transmission routes Identified</strong></td>
<td>102 (90.2%)</td>
<td>21 (80.8%)</td>
<td>53 (72.6%)</td>
<td>1 (100%)</td>
<td>1 (100%)</td>
<td>1 (100%)</td>
</tr>
<tr>
<td><strong>Heterosexual</strong></td>
<td>70 (68.6%)</td>
<td>19 (90.4%)</td>
<td>47 (88.7%)</td>
<td>1 (100%)</td>
<td>1 (100%)</td>
<td>1 (100%)</td>
</tr>
<tr>
<td><strong>MSM</strong></td>
<td>32 (31.4%)</td>
<td>1 (4.8%)</td>
<td>6 (11.3%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>ALT, IU/L</strong></td>
<td>2126 ± 938*</td>
<td>2394 ± 820</td>
<td>2857 ± 1668*</td>
<td>4180</td>
<td>1175</td>
<td>1533</td>
</tr>
<tr>
<td><strong>Bilirubin, mg/dL</strong></td>
<td>7.1 ± 6.4*</td>
<td>4.8 ± 3.3*</td>
<td>9.0 ± 7.5*</td>
<td>6.8</td>
<td>3.9</td>
<td>3.5</td>
</tr>
<tr>
<td><strong>HBV DNA, log copies/mL</strong></td>
<td>6.3 ± 1.7*</td>
<td>5.5 ± 2.3</td>
<td>4.9 ± 1.5*</td>
<td>5.2</td>
<td>7.4</td>
<td>4.8</td>
</tr>
<tr>
<td><strong>HBeAg</strong></td>
<td>95/121 (77.3%)*</td>
<td>24/28 (85.5%)</td>
<td>37/58 (65.5%)</td>
<td>1/1 (100%)</td>
<td>1/1 (100%)</td>
<td>1/1 (100%)</td>
</tr>
<tr>
<td><strong>Anti-HIV</strong></td>
<td>7/72 (9.7%)</td>
<td>0/7 (0%)</td>
<td>0/23 (0%)</td>
<td>Not tested</td>
<td>0/1 (0%)</td>
<td>Not tested</td>
</tr>
<tr>
<td><strong>Duration of HBsAg</strong></td>
<td>7/72 (9.7%)</td>
<td>0/7 (0%)</td>
<td>0/23 (0%)</td>
<td>Not tested</td>
<td>0/1 (0%)</td>
<td>Not tested</td>
</tr>
</tbody>
</table>

Abbreviations: ALT, alanine aminotransferase; anti-HIV, antibody to human immunodeficiency virus; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; MSM, men who have sex with men.

* P < .001.
* P < .001.
* P = .017.
* P = .002.
* P = .002.
* P = .003.
* P < .001.
* P < .001.
* P < .001.
* P = .036.

* Data from anti-HIV-positive patients are excluded.
Figure 2. Levels of hepatitis B surface antigen in patients with different durations of infection compared at various weeks after the onset of acute hepatitis B genotype A. *P < .05; **P < .01; ***P < .001. Abbreviation: HBsAg, hepatitis B surface antigen.

Figure 3. Levels of hepatitis B virus DNA in patients with different durations of infection compared at various weeks after the onset of acute hepatitis B genotype A. *P < .05; **P < .01; ***P < .001. Abbreviations: HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus.
As shown in Figure 3, HBV DNA levels at 4 weeks from the onset can discriminate groups 1/2 from groups 3/4. Furthermore, HBV DNA levels at 8 weeks from the onset can distinguish group 4 from group 1, 2, or 3. Therefore, the combination of HBV DNA levels at weeks 4 and 8 would be useful for predicting the outcome. For the prediction of a chronic outcome, HBV DNA level at 8 weeks from the onset is a useful surrogate marker of the outcome as well as HBsAg level at 12 weeks. There were differences in viral kinetics among groups 1, 2, 3, and 4.

Our present study showed that 15 of the 215 patients (7.0%) cleared HBsAg from >6 to 12 months after the onset. Sixty percent of the 15 patients had HBV/A. Although these patients met the criteria of chronic infection, they finally cleared HBsAg from the sera. Therefore, we would like to propose that transition to chronic infection in acute hepatitis B be judged at 12 months from onset in patients with genotype A; further studies in larger cohorts are necessary. One reason for our proposal is the indication of antiviral treatment. Antiviral treatment in patients with acute hepatitis B is not indicated because previous studies failed to show the efficacy of antiviral treatments in the patients with acute hepatitis B [23, 24]. However, if patients who actually develop chronic infection can be identified and treated by antiviral treatment, the number of those who develop secondary infection may be markedly reduced. Evaluation of the efficacy of antiviral treatments by prospective studies, based on surrogate markers for the outcome, should be conducted as the next step. HBsAg, which was reported to be useful as a surrogate marker for chronicity, should also be assessed as a surrogate marker [25, 26].

Our study has some limitations. First, the lack of data in early stages made it difficult to study viral kinetics precisely. Second, viral kinetics in the infection with each HBV genotype were obtained from a restricted number of patients, not large enough to establish the usefulness of changes in viral markers in earlier stages of HBV infection. Third, anti-HIV was not checked in all patients due to the lack of informed consent. Fourth, HBsAg and HBV DNA were not determined 24 weeks after onset when discrimination between groups 3 and 4 may be possible more easily. Fifth, the maximum levels of ALT and bilirubin may be affected as the next step. HBeAg, which was reported to be useful as a surrogate marker for chronicity, should also be assessed as a surrogate marker [25, 26].

In conclusion, we have shown that viral kinetics and the clinical outcome are different among patients with acute hepatitis B who are infected with HBV of distinct genotypes. HBsAg levels at 12 weeks and HBV DNA at 8 weeks after the onset would be useful to predict the clinical outcome of patients with acute hepatitis B.

Supplementary Data

Supplementary materials are available at Clinical Infectious Diseases online (http://cid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References


