Prevalence of and Risk Factors for Hepatitis B Viremia After Spontaneous Hepatitis B Surface Antigen Seroclearance in Hepatitis B Carriers

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In 118 previous hepatitis B surface antigen (HBsAg) carriers, low-level hepatitis B (HBV) viremia persisted at a rate of 15%–20% for >10 years after HBsAg seroclearance. The frequency of HBV viremia was significantly (P < 0.002) lower in patients with anti-HBsAg seroconversion (6 of 69 [8.7%]) than in those without seroconversion (15 of 49 [30.6%]).

Spontaneous hepatitis B surface antigen (HBsAg) seroclearance in the natural history of chronic hepatitis B virus (HBV) infection has been increasingly recognized recently [1]. The annual incidence of HBsAg seroclearance ranged from 0.45% to 2.38% in cohorts from Asian countries and from 0.54% to 1.98% in cohorts from Western countries [2]. Most patients with HBsAg seroclearance have an excellent outcome and are at a state closest to a “cure” [2].

Virtually all HBsAg carriers test negative for HBV DNA by hybridization assays (<2 × 10^4 IU/mL or 10^5 copies/mL) after HBsAg seroclearance, but in some of them HBV DNA still can be detected by polymerase chain reaction (PCR)–based assays that can detect HBV DNA levels as low as 10–100 copies/mL [3–11]. The persistence of low-level viremia after HBsAg seroclearance may be a potential source of HBV transmission through blood transfusion or transplantation and can account for reactivation of hepatitis B with chemotherapy or immunosuppression. However, the rate of HBV viremia varied considerably in previously published reports: the overall frequency ranged from 0% to 60% [4, 5, 9, 11]; HBV viremia was detected in 0.9%–71.4% of participants within 1 year after HBsAg seroclearance [3, 6–8, 10]. The reasons for such a remarkable discrepancy remain unclear. In most studies, HBV DNA was assayed using in-house PCR [3–5, 8–11]. The sensitivity and specificity of each in-house PCR assay were largely unknown and might vary considerably among different laboratories. The sensitivity of 2 in-house PCR assays was reported to be <100 copies/mL [8, 10], and varied from 6.38 copies/mL [7] to 400 copies/mL [6] in 2 commercial kits. In addition, most studies used stored serum specimens that might have interfered with the accuracy of the assays, especially when the level of HBV DNA tended to be low. The clarification of the actual rate of HBV viremia after HBsAg seroclearance by overcoming these drawbacks may have pathophysiological significance for research of the natural history of chronic HBV infection. Furthermore, factors that predict the presence of HBV viremia after HBsAg seroclearance have rarely been addressed.

Materials and Methods

Patients

HBsAg carriers with HBsAg seroclearance were advised to be regularly followed up once every 6–12 months. A total of 118 consecutive HBsAg carriers with documented HBsAg seroclearance who received follow-up evaluations at our outpatient clinic during a 1-year period from January 2010 to December 2010 were enrolled in this study as they fulfilled the following criteria: (1) HBsAg positive for at least 12 months before HBsAg seroclearance; (2) loss of serum HBsAg on 2 occasions at least 6 months apart during follow-up and remaining HBsAg negative up to the previous visit; (3) no concomitant hepatitis C virus (HCV) or hepatitis D virus (HDV) infection; (4) no antiviral or immunomodulatory therapy before or during follow-up; (5) no other causes of liver disease, including alcoholism, drug use, or autoimmune liver diseases. No patient in this cohort admitted intravenous drug abuse or same-sex behavior. Serum specimens at the most recent visit were collected and stored at −80°C. Assays of HBV DNA were performed within 6 weeks after collection.

Methods

HBsAg, anti-HBsAg, and antibody against HDV were assayed by radioimmunoassay or enzyme immunoassay (Abbott Diagnostics). Antibodies against HCV were tested by a second- or third-generation enzyme immunoassay (Abbott Diagnostics). Serum specimens were assayed for HBV DNA by using a fully automated, real-time PCR assay (Cobas AmpliPrep/Cobas
TaqMan HBV test; Roche Diagnostics). According to the manufacturer’s instructions, serum specimens may be frozen at \(-20^\circ\text{C}\) to \(-80^\circ\text{C}\) for at least 6 weeks. This assay has a specificity of 100% and sensitivity (95% hit rate) of 4–12 IU/mL (23–69 copies/mL) [12]; 1 IU is equivalent to 5.82 HBV DNA copies.

**Statistical Analysis**

To compare frequency of HBV viremia between groups, we used either \(\chi^2\) test or Fisher exact test. We analyzed actors that correlated with the presence of HBV viremia with logistic regression. We performed statistical procedures with SAS statistical software (version 8.1; SAS Institute). We considered \(P\) values <.05 significant.

**Results**

Eighty-five men and 33 women were included in the study. The mean age (standard deviation) at HBsAg seroclearance was 51.1 (9.4) years. In all, 10 patients had already experienced liver cirrhosis, as documented by ultrasonography, before HBsAg seroclearance. Ultrasonographic features of fatty livers were identified in 65 patients.

The intervals between HBsAg seroclearance and enrollment for HBV DNA assay were <1 year in 21 patients, 1–3 years in 21 patients, 4–6 years in 26 patients, 7–10 years in 22 patients and >10 years in 28 patients. At these intervals, 19.0% (4 of 21), 52.4% (11 of 21), 69.2% (18 of 26), 68.2% (15 of 22), and 75% (21 of 28), respectively, tested positive for anti-HBsAg. In addition, 15 patients (12.7%) had abnormal aspartate aminotransferase (AST) levels and 20 (16.9%) had abnormal alanine aminotransferase (ALT) levels.

The HBV viral target was detected in 21 of 118 carriers (17.8%; 95% confidence interval [CI], 10.9%–24.7%) after HBsAg seroclearance. Of these carriers, 16 (76.2%) had levels of HBV DNA below the sensitivity of hybridization assays, as documented by ultrasonography, before HBsAg seroclearance. Ultrasonographic features of fatty livers were identified in 65 patients.

The prevalence of HBV viremia varied considerably in the previously published reports [3–11]. In this investigation, we applied a widely used, commercially available, real-time PCR assay with well-recognized sensitivity and specificity for detection of HBV DNA [12]. Moreover, serum was assayed within 6 weeks of collection to avoid the potential interference of stored serum on assay accuracy. The present results thus should more accurately represent the actual rate of HBV viremia in HBsAg carriers after HBsAg seroclearance. Our data revealed that the overall prevalence of HBV viremia after HBsAg seroclearance was 17.8% (95% CI, 10.9%–24.7%). Whereas all patients had levels of HBV DNA below the sensitivity of hybridization assays (2 \(\times\) 10^4 IU/mL or 10^5 copies/mL), 2.5% (3/118) had levels of HBV DNA above the lower limit of detection of the Cobas

**Table 1. Detection of Hepatitis B Viremia After Hepatitis B Surface Antigen (HBsAg) Seroclearance by Interval After HBsAG Seroclearance and Anti-HBsAg Seroconversion**

<table>
<thead>
<tr>
<th>Interval after HBsAg seroclearance</th>
<th>With anti-HBsAg seroconversion</th>
<th>Without anti-HBsAg seroconversion</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of cases</td>
<td>No. (%) with HBV viremia</td>
<td>No. of cases</td>
</tr>
<tr>
<td>&lt;1 y</td>
<td>4</td>
<td>0 (0)</td>
<td>17</td>
</tr>
<tr>
<td>1–3 y</td>
<td>11</td>
<td>1 (9.1)</td>
<td>10</td>
</tr>
<tr>
<td>4–6 y</td>
<td>18</td>
<td>2 (11.1)</td>
<td>8</td>
</tr>
<tr>
<td>7–10 y</td>
<td>15</td>
<td>1 (6.7)</td>
<td>7</td>
</tr>
<tr>
<td>&gt;10 y</td>
<td>21</td>
<td>2 (9.5)</td>
<td>7</td>
</tr>
<tr>
<td>Subtotal</td>
<td>69</td>
<td>6 (8.7%)</td>
<td>49</td>
</tr>
</tbody>
</table>

Abbreviations: HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; anti-HBsAg, antibody against HBsAg.

* \(P = .0022\).
Amplicor HBV monitor assay (400 copies/mL). These data suggest that HBsAg seroclearance in HBsAg carriers is most likely a result of decreasing levels of HBV replication, but in rare instances, mutation in the “a” determinant of HBsAg may account for failure to detect HBsAg in serum.

This investigation showed that the presence of HBV viremia after HBsAg seroclearance was not significantly related to sex, age at HBsAg seroclearance (which is almost equivalent to duration of HBV infection in southeastern Asia), abnormal ALT levels, or the presence of fatty liver or cirrhosis. The frequency of HBV viremia did not significantly correlate with the interval after HBsAg seroclearance. The frequency of HBV viremia was 23.8% during the first 3 years after HBsAg seroclearance, and it tended to decrease to about 15% afterward (Table 1). However, this difference did not reach statistical significance. These data contradict previous observation by other investigators that the frequency of HBV viremia decreased remarkably, from 71.4% during the first year after HBsAg seroclearance to 14.3% at 10 years after HBsAg seroclearance [10]. In this investigation, the only factor significantly related to the presence of HBV viremia after HBsAg seroclearance that we identified was anti-HBsAg seroconversion. Anti-HBsAg seroconversion was associated with a significantly lower odds ratio of 0.22 for HBV viremia. HBV viremia persisted at rates of 5%–10% in patients with anti-HBsAg seroconversion and 25%–30% in those without anti-HBsAg seroconversion, without much difference at intervals from within 1 year to >10 years after HBsAg seroclearance (Table 1).

In conclusion, we found the overall prevalence of HBV viremia in HBsAg carriers with HBsAg seroclearance to be 17.8%. Low-level viremia persisted at a rate of 15%–20% at different intervals from within 1 year to >10 years after HBsAg seroclearance. Although the presence of HBV viremia correlated negatively with anti-HBsAg seroconversion, HBV viremia still can be detected in approximately 5%–10% of patients with anti-HBsAg seroconversion.

Notes

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Potential conflicts of interest. Y. F. L. has been involved in clinical trials or served as a global advisory board member for Roche, Bristol-Myers Squibb, GlaxoSmithKine, Novartis, and Gilead Sciences. 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.

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