Hepatitis B Virus DNA in Persons with Isolated Antibody to Hepatitis B Core Antigen Who Subsequently Received Hepatitis B Vaccine

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Serum samples from 133 persons who were positive only for antibody to hepatitis B core antigen (anti-HBc) by enzyme immunoassay (EIA) were retested for seromarkers of hepatitis B virus (HBV) by radioimmunoassay and for HBV DNA by polymerase chain reaction analysis. All persons were subsequently vaccinated with hepatitis B vaccine. HBV DNA was found in only five persons, four of whom remained positive during retesting. Most persons had a primary antibody response with three doses of hepatitis B vaccine. Evidence of HBV DNA was not detected in 96% of persons with isolated anti-HBc by EIA.

Antibody to hepatitis B core antigen (anti-HBc) is considered a sensitive and specific serum marker of hepatitis B virus (HBV) infection [1]. Anti-HBc can be found in serum samples from persons who have been exposed to HBV. Most persons who have been infected with HBV and have recovered have antibody to hepatitis B surface antigen (anti-HBs), and those who are actively infected also have hepatitis B surface antigen (HBsAg).

The significance of finding anti-HBc as the sole marker of HBV infection remains uncertain. This finding could be due to false-positive results of reactivity tests, particularly when testing is performed by EIA (the method most used by commercial laboratories and blood banks [2, 3]); the loss of anti-HBs with time or failure of persons to develop anti-HBs after HBV infection; and the "window" phase of acute HBV infection, which occurs after the loss of HBsAg and before anti-HBs appears. In these cases anti-HBc IgM is almost always present, an HBV carrier state where there is undetectable HBsAg and low levels of HBV replication [4].

In this study, we utilized two methods to measure seromarkers of HBV infection in serum samples from 133 persons who had anti-HBc as the sole marker of HBV infection. We also used PCR analysis to determine if HBV DNA was present. We correlated our findings with responses to hepatitis B vaccine that we have previously reported [2].

Patients and Methods

All 133 subjects had participated in a study examining the response to hepatitis B vaccine in persons with only anti-HBc [2]. Participants were persons who were positive for anti-HBc but negative for HBsAg and anti-HBs when tested by EIA (Auszyme, Auszab, and Corzyme, Abbott Laboratories, North Chicago, IL) before vaccination. Precrnavacination serum samples were also tested for HBsAg, anti-HBs, and anti-HBc by RIAs (AUSR A II, Ausab, and Corab, Abbott Laboratories). Subjects were divided into four groups according to their anti-HBc and anti-HBs status (table 1). Anti-HBs levels in participants were again measured by RIA 1 month after the first and third doses of plasma-derived hepatitis B vaccine (20 μg per dose; Heptavax, Merck Pharmaceuticals, West Point, PA). A booster response was defined as development of ≥50 sample ratio units (SRU) of anti-HBs after one dose of vaccine, and a primary response was defined as ≥10 SRU of anti-HBs 1 month after the third dose of vaccine [2].

Precrnavacination serum samples were tested for HBV DNA by PCR analysis with use of nested primers from the precore/core region of the HBV genome [5]. This assay is able to detect as little as 10 genome equivalents of HBV DNA per sample. General measures to avoid contamination were utilized [6].

Results

Only five (3.8%) of 133 persons were positive for HBV DNA by PCR analysis (table 1). Repeated PCR testing of the
Table 1. Groups of subjects positive only for anti-HBc by EIA according to anti-HBc and anti-HBs status measured by EIA and RIA.

<table>
<thead>
<tr>
<th>Finding</th>
<th>Group A (n = 39)</th>
<th>Group B (n = 49)</th>
<th>Group C (n = 36)</th>
<th>Group D (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presence of anti-HBc by EIA</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Presence of anti-HBc by RIA</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Presence of anti-HBs (&lt;10 SRU) by RIA</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Presence of anti-HBs (≥10 SRU) by RIA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>No. with HBV DNA by PCR analysis</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>No. with booster response* / total no. tested</td>
<td>3/35</td>
<td>3/48</td>
<td>15/40</td>
<td>4/7</td>
</tr>
<tr>
<td>No. with primary response* / total no. tested</td>
<td>24/26</td>
<td>27/33</td>
<td>14/21</td>
<td>1/2</td>
</tr>
</tbody>
</table>

NOTE. anti-HBc = antibody to hepatitis B core antigen; anti-HBs = antibody to hepatitis B surface antigen; HBV = hepatitis B virus; SRU = sample ratio units; + = positive; − = negative.

* Development of ≥50 SRU of anti-HBs after one dose of hepatitis B vaccine.

In four other studies investigating serum samples positive only for anti-HBc by either RIA or EIA, PCR analysis revealed HBV DNA in <10% of samples in three studies [7, 9, 10] and in 35% of samples in one study from China [8]. It is likely that the proportion of persons with sera positive only for anti-HBc who are positive for HBV DNA will be higher among populations with a high prevalence of HBV infection.

Although a few reports have implicated persons with only anti-HBc as the source of posttransfusion hepatitis B, it would be both expensive and time-consuming for blood banks to screen samples that are HBsAg-negative but anti-HBc-positive. In this study, 26% of persons who were positive for anti-HBc by EIA were negative by RIA; only three (9%) of 35 persons had a booster response to one dose of hepatitis B vaccine, while 24 (92%) of 26 of those who did not have a booster response had a primary response. In addition, we have previously shown by comparing these two methods that most serum samples positive for anti-HBc by EIA, but negative by RIA, have low levels of anti-HBc [3]. In contrast, serum samples positive only for anti-HBc by both methods have high levels of anti-HBc when quantitated by RIA. Thus, raising the positive cutoff level for EIA or employing RIA for determination of anti-HBc would eliminate some of the false-positive results.

In conclusion, in this study, HBV DNA was not found in 96% of serum samples from individuals with only anti-HBc when tested by EIA. Since most of these persons had a primary response to hepatitis B vaccine, the finding of isolated anti-HBc is most likely to be a false-positive result. When a person who is screened for hepatitis B vaccination by EIA is found to have isolated anti-HBc in their serum, the most practical approach would be to offer the person the hepatitis B vaccine and evaluate their response to vaccination.

References


