Concurrence of Hepatitis B Surface Antigen and Antibody in Acute and Chronic Hepatitis B Cases

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Summary
A total of 250 hepatitis B surface antigen positive sera were screened for antibody to hepatitis B surface antigen. It was found that seven (3%) sera showed concurrently circulating surface antigen and surface antibody to hepatitis B virus. The level of antibody to surface antigen was not affected by HBeAg and most of the cases were found in chronic hepatitis B carriers.

Key Words: Hepatitis B surface antigen, Antibody to hepatitis B surface antigen, Chronic hepatitis B carriers

Introduction
In viral hepatitis B infection, the presence of hepatitis B surface antigen (HBsAg) in the serum implies infectivity, and antibody to hepatitis B surface antigen (anti-HBs) becomes detectable as HBsAg begins to clear from the serum. Its presence suggests recovery from the hepatitis B infection, life-long immunity from subsequent infection and non-infectivity of the serum. The persistence of HBsAg in the blood for more than six months indicates chronic infection.

On occasion, HBsAg and anti-HBs are present simultaneously in the serum of a patient. This phenomenon was first reported in 1976\(^1\). Its incidence ranged from 2.1\(^3\) to 32 per cent\(^4\) with greater frequency among chronic hepatitis B infected patients\(^5\).

The objective of this study is to determine the occurrence of concurrent presence of circulating HBsAg and anti-HBs in the HBsAg-positive sera collected in University Hospital, Kuala Lumpur.

Materials and Methods

Blood specimens
A total of 250 HBsAg-positive sera detected in the Viral Hepatitis & AIDS Laboratory, University Hospital, Kuala Lumpur between April 1991 and May 1992 was used in this study. The sera were stored at -20°C before being assessed. One hundred and eighty-four (74%) of the sera were male, 66 (27%) were female. The study group was composed of 170 (68%) Chinese, 63 (25%) Malays, 15 (6%) Indians and two (1%) from other races. The youngest patient was two-years-old.
and the oldest 85. The majority of the patients, however, were between 20 to 40 years old. The clinical diagnosis of the patients is summarised in Table I.

Detection of HBsAg
All 250 sera were retested for HBsAg using Ausria II-125 RIA (Abbott Diagnostic, USA). The sera which were stored at -20°C, were thawed and tested on the same day. The sera with counts per minute (cpm) equal to or greater than the cut-off value were considered as positive for HBsAg. All 250 sera were again found to be HBsAg-positive.

Detection of Anti-HBs
Antibody to hepatitis B surface antigen was detected by Ausab RIA (Abbott Diagnostic, USA). The counts per minute (CPM) was determined by a gamma counter. The antibody level in mIU/ml was determined using a standard graph plotted with count per minute against the standard of known values of antibody to hepatitis B surface antigen (i.e. 0, 15, 40, 75 and 150 mIU/ml).

Detection of HBeAg
Hepatitis B e antigen (HBeAg) was detected by Abbott HBe (rDNA) RIA. The specimen with cpm equal to or greater than the cut off values are considered reactive for HBeAg.

<table>
<thead>
<tr>
<th>Clinical diagnosis</th>
<th>No. of patients</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute viral hepatitis</td>
<td>168</td>
<td>67</td>
</tr>
<tr>
<td>Chronic hepatitis B carriers</td>
<td>42</td>
<td>17</td>
</tr>
<tr>
<td>Hepatoma</td>
<td>28</td>
<td>11</td>
</tr>
<tr>
<td>Liver cirrhosis</td>
<td>12</td>
<td>5</td>
</tr>
</tbody>
</table>

Results
All 250 HBsAg positive sera tested, 97 (39%) sera were HBeAg positive, 132 (53%) were HBeAg negative. Nineteen (8%) anti-HBs negative sera were not tested for HBeAg due to insufficient serum.

Concurrently circulating HBsAg and anti-HBs was detected in seven (3%) out of 250 sera. Three of the seven sera were positive for HBeAg of whom two were males (aged 9 and 43) and one a female (aged 18). In general, all three showed low anti-HBs levels ranged from 10 to 15 mIU/ml except for one HBeAg-negative patient who showed a antibody level of 113 mIU/ml (Table II). The concurrence of HBsAg and anti-HBs was not influenced by the age. The detection of HBeAg did not seem to affect the antibody level.

It was found that six of the seven patients showed concurrently circulating HBsAg and anti-HBs in this study were chronic hepatitis B carriers (Table III). Anti-HBs was not detected in patients with acute hepatitis B infection nor liver cirrhosis. Anti-HBs was detected in only one (4%) of the 28 patients with hepatoma.
CONCURRENCE OF HEPATITIS B SURFACE ANTIGEN AND ANTIBODY

Table II
Age, sex distribution of patients with concurrent HBsAg and anti-HBs

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age (Years)</th>
<th>HBeAg</th>
<th>Anti-HBs titre (mIU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>9</td>
<td>+</td>
<td>14</td>
</tr>
<tr>
<td>Male</td>
<td>23</td>
<td>-</td>
<td>12</td>
</tr>
<tr>
<td>Male</td>
<td>42</td>
<td>+</td>
<td>10</td>
</tr>
<tr>
<td>Male</td>
<td>51</td>
<td>-</td>
<td>113</td>
</tr>
<tr>
<td>Female</td>
<td>9</td>
<td>-</td>
<td>12</td>
</tr>
<tr>
<td>Female</td>
<td>10</td>
<td>-</td>
<td>15</td>
</tr>
<tr>
<td>Female</td>
<td>18</td>
<td>+</td>
<td>13</td>
</tr>
</tbody>
</table>

Table III
Clinical diagnosis of patients with concurrent circulating HBsAg and Anti-HBs

<table>
<thead>
<tr>
<th>Clinical diagnosis</th>
<th>No. of HBsAg-positive sera tested</th>
<th>No. of Anti-HBs-positive</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute viral hepatitis</td>
<td>168</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Chronic hepatitis B carriers</td>
<td>42</td>
<td>6</td>
<td>14</td>
</tr>
<tr>
<td>Hepatoma</td>
<td>28</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Liver cirrhosis</td>
<td>12</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Discussion
When compared with other studies, this study indicates that concurrence of HBsAg and anti-HBs appear to be a fairly rare serological finding with the prevalence rate of 3 per cent. Shields et al.4 had reported that 32 per cent of patients with hepatitis B infection showed concurrently circulating HBsAg and anti-HBs whereas Tsang et al.5 found that 25 per cent of HBsAg-positive individuals carried anti-HBs. Heijtink et al.6 reported that 49 per cent (20/41) chronic active hepatitis, and 100 per cent (5/5) patients with chronic active hepatitis and cirrhosis exhibited concurrence of HBsAg and anti-HBs. These variations in the frequency of detection may be due to the differences in the sensitivity of the diagnostic kits used by these investigators.

Shields et al.4 also found that HBeAg positivity occurred more frequently in patients with concurrent markers than those who are HBsAg positive alone (68% vs 42%). Foutch et al.7 reported that nine of 13 patients with concurrent HBsAg and anti-HBs were HBeAg positive. As HBeAg is known to be a marker of a high degree of viral infectivity8, and concurrence of HBsAg and anti-HBs may independently reflect the stage and activity of the chronic liver disease, it can
be suggested that these patients are a reservoir for transmission of hepatitis B virus. In our study, 97 of the 231 HBsAg-positive sera tested for HBeAg were found to be positive. Among the seven sera with simultaneously circulating HBsAg and anti-HBs, HBeAg was detected in only three patients. This indicates that HBeAg positivity did not influence the appearance of anti-HBs as suggested by Shields et. al.\textsuperscript{4}. Recently, HBeAg was found to be derived from the cleavage of the translation product of the precore region and core gene. Omata et. al.\textsuperscript{9} in analysing the entire precore region of hepatitis B virus, found that a point mutation can occur, resulting in the introduction of a stop codon. Therefore, HBeAg is not a suitable marker for infectivity as it may be absent in a patient with fulminant hepatitis.

The coexistence of HBsAg and anti-HBs may occur as a result of different antigenic determinants for HBsAg. It is well known that HBsAg has a common group-reactive antigenic determinant \(a\) and mutually exclusive antigenic specificities \(d\) or \(y\) and \(w\) or \(r\)\textsuperscript{10}. Based on these antigenic determinants, the major subtypes of HBsAg are \(adw\), \(ayw\), \(adr\) and \(ayr\)\textsuperscript{11} but the number of HBsAg subtypes have recently been expanded to eight: \(aywl\), \(ayw2\), \(ayw3\), \(ayw4\), \(ayr\), \(adw2\), \(adw3\) and \(adr\) depending on which of the \(w\) subterminants could be demonstrated\textsuperscript{12}. Therefore a patient can be chronically infected with one subtype of HBsAg and on exposure to a second HBV subtype, produce a monotypic anti-HBs to the second HBsAg subtype determinant. The antibody level detected is usually low, and it is generally believed that the production of any anti-HBs after HBV infection can confer protection against reinfection with either homologous or heterologous subtypes due to the presence of common \(a\) determinant\textsuperscript{13}. However, the failure of the presence of anti-HBs in preventing reinfection of HBV has been reported\textsuperscript{14}.

It has been found that concomitant HBsAg and anti-HBs tend to be more prevalent in the chronic HBsAg carriers, intravenous drug abusers and patients with chronic renal disease on haemodialysis\textsuperscript{15}. Six of the seven patients found to have concurrent HBsAg and anti-HBs were chronic hepatitis B carriers, suggesting that this is a phenomenon commonly found in hepatitis B carriers.

While it has been reported that simultaneous circulation of HBsAg and anti-HBs in patients with chronic hepatitis has no practical, clinical or prognostic significance\textsuperscript{16}; from the histological aspect, Heijtink et. al.\textsuperscript{6} postulated that concurrent HBsAg and anti-HBs positivity was a marker of severe chronic liver disease. They noted that as the degree of liver disease progressed, the frequency of concurrence increased, and the patients with histologically advanced hepatitis B infection frequently developed heterotypic antibody.

The gene products of the pre-s/s-gene region of the HBV genome are associated with stages of HBV infection\textsuperscript{17}. In future work, the study of the pre-s and anti-pre-s antibodies is warranted to assess the significance of concurrent HBsAg and anti-HBs to HBV replication, HBsAg subtype and histological changes of the disease.

**References**


