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Hepatitis B Surface Antigen Carriers and in Primary Hepatocellular Carcinoma Patients

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Abstract
A total of 246 HBsAg-positive subjects were tested for the presence of Hepatitis B, antigen, and total anti-delta IgG. Of the 246, 174 were HBsAg-positive asymptomatic chronic carriers and 72 were primary hepatocellular carcinoma patients attending the two teaching hospitals, i.e. Parirenyatwa and Harare Central Hospital.

All sera were examined by the enzyme-linked amunosorben assay (ELISA), and results were spectrophotometrically at a wavelength of 82 nm. 82 (33.3%) were HBsAg-positive. Of 246, 56 (39.4%) males and 26 (25%) females had HBeAg present in the serum. However, the prevalence of HBsAg from only the chronic symptomatic HBsAg-positive carriers in males and females was 17 out of 103 (16.5%) and 12 out of 71 (16.9%), respectively—thus with an overall prevalence of 31 (17.8%) from the symptomatic group. Consequently, the prevalence of HBsAg from the primary hepatocellular carcinoma (PHC) group was 32 of 72 (44.4%).

INTRODUCTION

Hepatitis B surface antigen (HBsAg) is intimately associated with viral hepatitis type B. It exists in the form of 20 nm spheres, 20 nm diameter lamellae and on the surface of 42 nm Dane particles. The e-antigen is potentially usable as a prognostic marker of the chronicity of hepatitis B virus (HBV) infection, and would give information concerning the infectivity of HBsAg-positive individuals.

Although HBsAg can be present in non-infectious or mildly infectious blood, the additional finding of HBeAg in serum apparently indicates the presence of circulating infectious HBV particles. Blood positive for HBeAg has accordingly been found to transmit hepatitis B in a mother-infant series and in exposure studies to a HBsAg, but positive for the corresponding antibody, anti-HBc.

Previous studies on HBsAg in Zimbabwe showed a high prevalence (13.7%) of positive results obtained from the healthy population. This study, however, was undertaken to determine the proportion of HBeAg positivity, in sera from both asymptomatic HBsAg chronic carriers and primary hepatocellular carcinoma patients. In this study, "chronic asymptomatic carriers" is used to refer to those healthy individuals who are carriers of HBsAg and do not show any clinical symptoms.

Methods and Materials
The study population consisted of 104 females and 142 males. Thus the total number examined was 246. Of the 104 females, 71 were HBsAg-positive asymptomatic carriers whereas 33 were primary hepatocellular carcinoma patients who were positive for HBsAg. Among the 142 males, 103 were HBsAg-positive asymptomatic carriers and 39 were patients suffering from primary hepatocellular carcinoma and at the same time positive for HBsAg.

The age range of the HBsAg-positive asymptomatic carriers was from 11 to 60 years with a mean of 24 years. The 0–10 age group was not included in the study because of problems that the investigator faced in obtaining specimens (5–8 ml of blood). But the ages of the primary hepatocellular carcinoma subjects were not available, although all of the subjects were adults.

Samples from the HBsAg-positive asymptomatic chronic carriers were collected from Gweru, Masvingo, Harare, Kariba and Gokwe areas. However, sera from confirmed cases of hepatocellular carcinoma were collected only from patients attending Parirenyatwa and Harare Central Hospitals.

Procedures
Samples of 5 ml venous blood were collected into 10 ml clotted test tubes and were immediately
transported to the laboratory for serum separation. Separated sera were transferred into 3 ml vials and stored at -20°C if not examined on the same day.

For serological detection of HBsAg, sera were tested by the Ortho Enzyme-linked immunosorbent assay following the manufacturers' procedures with few modifications to suit our laboratory's conditions. Briefly, 100 ul of each test specimen was added into separate wells of the anti-HB, coated microplate. Similarly 100 ul of both the negative and positive controls were added into four and two wells respectively, and plates were incubated at 37°C in a water bath for two hours. After incubation the contents of each well were removed and plates were washed four times with distilled water following which 100 ul of the enzyme conjugate was added to each well and plates incubated for two hours in similar conditions as above. After two hours incubation plates were washed as above and 200 ul substrate solution which was prepared by adding 4 0 - phenylenediamine 2 HCl (OPD) to 12 ml deionized water in a clean plastic container. Following complete dissolution of OPD tables 5 ul of 30% hydrogen peroxide was added and the substrate used within 30 minutes. Finally, plates were incubated at room temperature in the dark for 20 minutes. The reaction was then stopped by adding one drop (50 ul) of 2,5 N HCL to each well. Results were read visually by taking all the wells with yellow colour change as positive, and these were recorded.

Only sera positive for HBsAg were stored at -20°C and later shipped to the Centre for Diseases Control in the USA, where they were examined for the presence of both HBsAg and anti-Delta IgG. This portion of the work was undertaken by the investigator with some assistance from Dr Howard Fields, a WHO consultant in the Hepatitis Branch at CDC.

Commercial kits for the detection of HBsAg and anti-delta IgG from Abbot Diagnostic Laboratories were used. Results for both HBsAg and anti-delta IgG were read spectrophotometrically using the Abbot Quantum 11th at a wavelength of 492 nm.

Patients queried for primary hepatocellular carcinomas were screened for the presence of PHC by detecting the rise of alpha-fetoprotein. Only patients with 100 ng/ml were considered positive for primary hepatocellular carcinomas.

RESULTS

Of the 246 examined sera, 82 (33.3%) were positive for HBsAg, 56 (39.4%) of the males and 26 (25%) of the females had HBsAg in their sera. However, the prevalence of the HBsAg in chronic asymptomatic hepatitis B surface antigen carriers in males and females were 17 (16.5%) and 12 (16.9%) respectively, but the overall prevalence in this group was 31 (17.8%).

Consequently, the relationship between age-group and percentage distribution of hepatitis B, antigen marker in the healthy chronic asymptomatic HBsAg – positive carriers is as shown in Table I. The frequency of HBsAg – reactivity decreases with increasing age.

Because of the unavailability of information on age from the primary hepatocellular carcinoma patients, this group, therefore, was not included in Table I, but its prevalence was found to be 32 (44.4%) out of the 72 tested subjects. The positivity rate in males and females from this group was 18 of 39 (46.2%) and 14 of 33 (42.4%) respectively.

Interestingly, total anti-delta IgG was not detected in all the subjects examined, i.e. in all the 246 HBsAg – positive carriers.

DISCUSSION

Hepatitis B, antigen, associated with HBV was originally described by Magnus and Espmark,1 and has not been fully characterised, although it exists primarily in a soluble, non-particulate form in contrast to HBsAg and HBcAg. The e-antigen is usually found in sera that are reactive for HBsAg. HBsAg and HBcAg has a variable distribution throughout the world. In Taiwanese,7 in blood donors from Paris,8 in Indochinese refugee blood donors,9 the prevalence of HBsAg was 32%, 33.7% and 54.8% respectively. However, from this study the prevalence of 17.8% was drastically lower than the aforementioned studies from similar subjects, although higher than studies from Senegalese,10 American Red Cross blood donors in Bethesda Maryland11 or in Japanese2 which indicated a prevalence of 6%, 7.4% and 6.2% respectively.

It was clearly indicated in the study that the presence of the HB markers was correlated with age and the findings were compatible with other similar studies.12,13,14 Thus the prevalence of the HBsAg decreased with increasing age. Therefore, findings in this study suggest that HBsAg positivity in HBsAg carriers is of limited duration.

Although there is no established difference in the acquisition of HBV in relationship to set under conditions of equal exposure, it has been shown that males are probably more prone to become asymptomatic chronic carriers of HBsAg,3,15 thus increasing the chance of detecting the antigen.

This study showed a statistical significa
TABLE I—Relationship between age group and percentage distribution of HB eAg marker in chronic HB s Ag carriers

<table>
<thead>
<tr>
<th>Age Group</th>
<th>No. exam.</th>
<th>Total</th>
<th>No. (%) Positive</th>
<th>F</th>
<th>M</th>
<th>Total (%) Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>M</td>
<td>Total</td>
<td>F</td>
<td>M</td>
<td>Total (%) Positive</td>
</tr>
<tr>
<td>11-15</td>
<td>5</td>
<td>13</td>
<td>18</td>
<td>1 (20)</td>
<td>4 (30.8)</td>
<td>5 (27.8)</td>
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<tr>
<td>16-20</td>
<td>25</td>
<td>20</td>
<td>46</td>
<td>5 (19.2)</td>
<td>6 (30)</td>
<td>11 (23.9)</td>
</tr>
<tr>
<td>21-25</td>
<td>12</td>
<td>9</td>
<td>21</td>
<td>2 (16.7)</td>
<td>4 (44.4)</td>
<td>8 (38.1)</td>
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<tr>
<td>26-30</td>
<td>7</td>
<td>25</td>
<td>32</td>
<td>1 (14.3)</td>
<td>1 (4)</td>
<td>2 (6.3)</td>
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<tr>
<td>31-35</td>
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<td>8</td>
<td>17</td>
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<td>0 (0)</td>
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<tr>
<td>36-40</td>
<td>3</td>
<td>8</td>
<td>11</td>
<td>1 (33.3)</td>
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<tr>
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<tr>
<td>56-60</td>
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<td>3</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Total</td>
<td>71</td>
<td>103</td>
<td>174</td>
<td>12 (16.9)</td>
<td>17 (16.5)</td>
<td>31 (17.8)</td>
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</table>

difference p ≤ 0.001 in prevalence of HB s Ag with regards to sex. It was, however, comparable to similar studies in Japan, but studies somewhere showed a significant difference (29% and 46.6% positivity rate) as per male versus female groups.

In the primary hepatocellular carcinoma patients the HB s – positivity rate was significantly higher than that of the asymptomatic carriers. It was also shown that HB s Ag – positive carrier males showing a higher prevalence of that antigen showed a statistical significant difference p ≤ 0.001 of the e – antigen as compared to females. But results of males from the two groups, i.e. the symptomatic carriers and the PHC subjects when analysed together, indicated a higher prevalence, 39.4% in males to 25% in females.

The fact that the HB s Ag was more common in the PHC subjects could probably be as a result of these subjects having been currently infected with HBV. As a result, investigations are underway to determine the correlation between HBV markers and PHC in patients attending Parirenaira and Harare Central Hospitals.

Anti-delta is normally detectable in on-going or recent infections with HBV. Consequently, the delta-antigen can exist as a co-infection or super-infection. Although no anti-delta antibodies were detected in this study, this may not suggest a total absence of the delta viral antigen because several factors could have contributed to negative results. Detectability of anti-delta antibody highly depends on the high concentration or circulating antibodies in sera, which could be during current infection. Studies consisting of patients with active viral hepatitis are underway in order to establish an acceptable status of anti-delta in Zimbabwe.

In conclusion the study revealed that the prevalence of HB s Ag in HB s Ag – positive asymptomatic carriers was lower compared to that found in the Far East, but reasonably similar to several studies conducted in the West. Secondly, the positive rate of HB s Ag from PHC was higher than that of the asymptomatic carriers. However, there was no significant difference in the positive rate between males and females, but a frequency of HB s reactivity was shown to be decreasing with increasing age.

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