Detection of HBs-Ags in sera of hepatitis B virus infected patients

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Abstract

Objective and background: this study is aiming to evaluate the prevalence of HBV among patients attending AL-Ramadi Hospital for cases of hepatitis HBV is worldwide distributed and it is the etiological agent of acute and chronic liver disease in human. Two hundred and ninety four serum samples were the subject of this study in which each serum sample was subjected to two well-known serological methods for detection of HBs-Ag i.e. Health mate and HBs-Ag ELISA kits. Out of the total number (294) of serum samples it has been found that 96 serum were positive (32.7%) for HBs-Ag while 198 serum were negative (67.4%). It has been found also that HBs-Ag were higher in male (40.8%) than female (26.6%) in age group of 21-30 years of age. It is concluded that HBV is prevalent in age group less than 20 years (32.7%) while it is least in age group ≥ 60 years (4.4%) and that age and gender can be considered as two important risk factors in acquiring HBV.

Key words: HBV, HBs-Ag, HBs-Ab.

Introduction

Hepatitis is defined the inflammation of the liver and characterized by the presence of inflammatory cells in the tissue of the organ. It can be self-limiting or can progress to fibrosis and cirrhosis (1). Hepatitis may occur with limited or no symptoms but often leads to jaundice, anorexia and malaise, it is acute when it lasts less than six months and chronic when, it persist longer (2).

Hepatitis is caused by a group of viruses known as the hepatitis viruses which causes most cases of hepatitis worldwide, but it can be due to toxic alcohol, certain medications, some industrial organic solvents and plants (3).

Hepatitis B virus can infect the liver cells resulting in an acute infection or persist with chronic inflammation of the liver and implicated in most cases of hepatocellular carcinoma (4)(5). Hepatitis B virus spreads through contact with the body fluids of an infected person and sexual contact and carrier of HBV can spread the virus among different contacts (5). Hepatitis B virus consist of a core containing (HBV-DNA) with an enzyme known as DNA polymerase that assist with viral replication and is surround by a surface proteins (HBs Ag) (3, 4). Two other proteins, HBC Ag which stays within the infected liver cells for assemble of new viruses, while another protein in the core known as HBC Ag is released into blood stream during active viral replication (3).
Hepatitis B Ag (HBs Ag) if detected by blood test, it is an indicator of an active infection and it is detectable within 3 to 4 weeks of the infection and possibly up to 5 months in an acute infection, while if this antigen is still detectable after 6 months then it is indication of chronic infection (6).

Antibodies to HBs-Ag (anti-HBs) can be detected after 3 to 6 months or it may be detectable for several years or even throughout life and these Anti-HBs can be detected in case of previous infection or vaccination (7).

Hepatitis B core antigen is not detectable in the blood of infected person at any time, while the antibody formed against it (anti-HBC) can be detected soon after infection and rapidly, rises, then is followed by a gradual decline but persist over the long time (8). These anti-HBC are evident in an infection but not with vaccination and can therefore help to differential whether the presence of anti-HBs is due to an infection or vaccination (9, 10, 11).

This work is aimed to evaluate the prevalence of HBV among patients attending AL-Ramadi Teaching Hospital.

**Patients and methods**

**Patients:** Two hundred and ninety four patients suspected of having hepatitis B attending AL-Ramadi Teaching Hospital were the source of blood samples (consisted of 5ml blood from each patient).

**Methodology:** specimen collection. Blood samples of 5ml were collected aseptically from each patient suspected of having hepatitis B, sera were separated as usual.

**Serological tests:** Processing of blood samples. Each blood sample was left at room temperature for the purpose of coagulation, and then centrifuged at 3000 RPM for 20 minutes. The supernatants which represented the sera were separated and stored at -20°C when it is not immediately tested.

The two tests used in this work were applied according to manufacture-instructions in which:

A- Health mate HBs strip kits which is a chromatographic immunoassay kit used for rapid and qualitative detection of HBs-Ag from human serum or plasma were performed for each serum sample.

B- HBs-Ag ELISA kits were also performed which employs monoclonal and polyclonal Abs specific for HBs-Ag in which microtiter wells are coated with monoclonal Ab specific for HBs-Ag, then mixed with serum specimen and with enzyme conjugated polyclonal antibodies. Presence of HBs-Ag will result in the formation of an antibody-HBs Ag-antibody enzyme complex seen as blue color when examined by using the OD at 450nm with an EIA plate reader.

**Results**

The result of this study in which 294 patients were the source of blood samples revealed (table 1 & 2) that male were 144 patients (49%) while female 150 patients (51%) and that the no. of HBV (HBs-Ag) positive serum sample were 96 (32.7%) and
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The negative HBV serum samples were 198 (67.4%).

In table 2 which shows the relation between age and gender which showed that the highest attendance of the hospital within the male groups were in those of less than 20 years of age and represent 44 patient (30.5%) out of 144 patient (41.0%) while those in age higher than 60 years of age which represented only 4 patient (2.8%).

In female group as in the table 3 it is seen that female within the age group less than 20 years of age were the highest among the group which 52 patient (32.7%) out of 150 patients. The lowest attendances were 6 patients (4%) at the age higher than 60 years of age out of 150 patients.

In the same table (3) it is seen also the total no. of HBV infected patient were 96 patients (32.7%) male and female within the age group less than 20 years of age while least no. of infected of both sexes were 14 patients (4.8%) at the age higher than 60 years of age.

Interpretation of our result were based on the basis of that a negative when there is only one band in the control line case of using health HBs strip kit while of HBs-Ag ELISA kit, the result is considered positive when the ratio of P/N value is equal or greater than 2.1 while negative result when the P/N value is less than 2.1.

From table (4) it is seen that the highest percentage of HBs-Ag is within the age group of less than 20 years of age (43.8%) while the lowest percentage is within the age groups of 51-60 years of age followed by zero percent of HBsAg in the age group of patients at age higher than 60 years.

### Table 1 percentage of HBV in serum samples according to HBs-Ag detection

<table>
<thead>
<tr>
<th>No. of samples</th>
<th>Positive samples</th>
<th>Percent</th>
<th>Negative samples</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBs-Ag</td>
<td>294</td>
<td>96</td>
<td>198</td>
<td>67.4%</td>
</tr>
</tbody>
</table>

### Table 2 Prevalence of HBs-Ag in patients according to gender

<table>
<thead>
<tr>
<th>Gender</th>
<th>No. of patients</th>
<th>No. of HBV infected patients</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>144</td>
<td>58</td>
<td>40.8%</td>
</tr>
<tr>
<td>Female</td>
<td>150</td>
<td>38</td>
<td>26.6%</td>
</tr>
<tr>
<td>Total</td>
<td>294</td>
<td>96</td>
<td>32.7%</td>
</tr>
</tbody>
</table>

### Table 3 Distribution of patients according to age and gender

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Male</th>
<th>Female</th>
<th>total</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 20</td>
<td>44</td>
<td>52</td>
<td>96</td>
<td>32.7%</td>
</tr>
<tr>
<td>21-30</td>
<td>38</td>
<td>24</td>
<td>62</td>
<td>21.1%</td>
</tr>
<tr>
<td>31-40</td>
<td>26</td>
<td>22</td>
<td>48</td>
<td>16.3%</td>
</tr>
<tr>
<td>41-50</td>
<td>18</td>
<td>40</td>
<td>58</td>
<td>19.4%</td>
</tr>
<tr>
<td>51-60</td>
<td>10</td>
<td>6</td>
<td>16</td>
<td>5.2%</td>
</tr>
<tr>
<td>&gt; 60</td>
<td>8</td>
<td>6</td>
<td>14</td>
<td>4.4%</td>
</tr>
<tr>
<td>Total</td>
<td>144</td>
<td>150</td>
<td>294</td>
<td>100%</td>
</tr>
</tbody>
</table>
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| Table 4 age related prevalence of HBs-Ag infected patients |
|-----------------------------------------------|----------------|----------------|-------------|
| Age (year) | No. subject | No. of HBs-Ag | Percent |
| <20   | 96          | 42            | 43.8%    |
| 21-30 | 62          | 16            | 26        |
| 31-40 | 48          | 20            | 41.6%    |
| 41-50 | 58          | 16            | 27.6%    |
| 51-60 | 16          | 2             | 12%      |
| > 60  | 14          | 0             | -         |
| Total | 294         | 96            | 32.7%    |

Discussion

Hepatitis B virus (HBS) is one of the most human pathogens in which more than 350 million people worldwide are persistently infected with HBV and are at risk of developing chronic liver diseases, cirrhosis and hepatocellular carcinoma (16, 17, 18).

Vertical transmission of HBV from mother to neonates always results in chronic hepatitis (19) and infection during adulthood results in lifelong protective immunity (20, 21). Hepatitis B surface antigen (HBs-Ag) is an important viral envelope protein which appear shortly after infection and is a key serological marker for detection and diagnosis of HBV (22).

The present study documents high prevalence of HBV infection in the study area and thus confirms finding in previous epidemiologic studies as that done by others (23, 24) who found that the prevalence rates of HBV infection in the range of 8 to 25 percent a result which is confirmed by our study in which the prevalence of HBV infection is 32.7%.

Risk factor for HBV infection may vary considerably depending on epidemiological data, the geographic region and the social, cultural characteristics of different population (16).

In our study two risk factors were included i.e. gender and age in which it has been found that HBV infection in women is higher (51%) than that in men (48.9%) within the age group of 20-60 years.

In this work in which 294 HBV infection were included at showed that 96 (32.5%) were positive for HBs-Ag and 198 (67.4%) were negative as in table 1 & 2 shows also that HBs-Ag was higher in men 58 (40.0%) than female 28 (25%) a result which agrees with others (26, 27, 28) who found that men had a higher range of HBV infection, it is generally agreed that risk of chronic HBV infection is inversely related to age of onset of infection and that children become chronic most often and represent the most important reservoir of infection in the community (29, 30, 31).

The proportion of patients with clinically apparent HBV infected increase from less than 10% in children below 10 years to 33-45% in adults an observation which is confirmed in our study in which the age related prevalence of HBs-Ag showed that age group < 20 year had the highest prevalence 42 (43.7%) as in tables 3 & 4. This is because infection occurs in young
adults because of life style or occupational exposure\(^{(38, 39)}\).

It is uncertain whether most of the positive hepatitis B cases are the active or they are as carrier states.

In conclusion, male has the highest prevalence of HBV infection than female.

**Conclusion:**

It is concluded from this work that HBV is highly prevalent in female (17.7%) in age less than 20 years than male (15%) at the same age and that male is higher in HBV prevalence (2.8%) at age higher than 60 years than that of female (2.1%) at the same age.

**References**

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