Schistosoma mansoni infection and its association with hepatitis B virus in Keryab Village

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Abstract
The study was conducted in Keryab village to investigate the association, if any, between S. mansoni infection and hepatitis B surface antigen. The study revealed that the overall infection rate was 15% (45 out of 300 stool samples examined). The prevalence in females was higher than that of males (17.8% and 13.4% respectively).

The highest infection rate (17.5%) was reported among the 11-15 years age group while the infection was less (14%) among the 16-20 years age group, and lowest infection (11.1%) was reported among the 5-10 years age group. On the other hand, 20 blood samples out of 300 were positive for hepatitis B surface antigen which constitutes 6.7% prevalence rate.

The highest rate of hepatitis B surface antigen (10%) was reported among the 16-20 years age group while the infection was less (6.25%) among the 11-15 years age group.

Out of 45 positive S. mansoni cases, 11 were found positive with hepatitis B surface antigen which constitutes 24.44% of the positive cases. On the other hand, 9 positive hepatitis B surface antigens were found among the negative S. mansoni cases which constitute 3.52% of the negative cases.

Introduction:
Schistosomiasis is considered to be the most important helminthic disease of man since it infects about 200 million individuals in about 74 countries in the world. Subjects are affected in different manners and most infected individuals are asymptomatic carriers of the parasite. The most serious form is the hepatosplenic one, whose major complication is upper digestive hemorrhage secondary to portal hypertension (¹).

In pure and uncomplicated cases, hepatocellular function is usually preserved up to the more advanced phases of the disease. Two aspects motivated the study of the role of viral hepatitis in shistosomiasis. The first was the observation that in case of schistosomiasis that progressed to the decompensated form, unusual cirrhotic changes could be observed in liver
paraenchyma. The second was the fact that patients with schistosomiasis show altered behavior when in contact with different pathogens, with possible modifications occurring in the course of the disease in the associated presence of enterobacteria, other helminthes and different protozoa \(^{(1, 2, 3 \& 4)}\). Pioneering studies by Daneshmend et al \(^{(5)}\) demonstrated a significantly higher incidence of B virus surface antigen (HBsAg) in individuals with hepatosplenic schistosomiasis (HSS) and suggested that the antigenemia of patients tended to persist. Anatomopathological studies demonstrated the role of active chronic hepatitis as a decomposing factor in schistosomiasis \(^{(2, 6)}\).

**Materials and methods:**

The study was conducted in Keryab village, sharg Al Nil which is located 30 kilometers far from Khartoum centre.

**Study population:** The study was conducted on 300 individuals selected randomly. Search for Hbs antigens was done for both positive and negative S. mansoni cases. The population was divided according to gender and age groups (5-10, 11-15 and 16-20 years).

**Sample collection:** Stool samples were collected in sterile containers. Blood samples were collected in filter papers.

**Stool examination:** Faecal samples collected were examined by the locally modified Kato method described by Teesdate and Amin \(^{(7)}\).

**Blood examination:**

**Specimen processing:** About 300 \(\mu l\) of whole blood was obtained from each suspected patient in filter papers (whatman) type and allowed to dry in room temperature and stored in closed container to avoid contamination and touch of each paper.

**Preparation of eluate:** 24 hrs before testing, the spot of filter paper was soaked by 300 \(\mu l\) of PBS. The tube was tightly sealed and kept at room temperature over night to be tested the next day by ELISA test (DIA. PRO, Diagnostic Bioprobes Srl. Italy).

**Test procedures:**

1. The required numbers of strips was placed in the plastic holder and washed once to hydrate the well. The wells for controls, calibrator and sample were carefully identified.
2. Well Al was left empty for blanking purposes.
3. 150 \(\mu l\) of the negative control were pipetted in triplicate, 150 \(\mu l\) of the calibrator in duplicate and 150 \(\mu l\) of the positive control was all pipetted into the assigned wells. This was followed by addition of 150 \(\mu l\) of the eluate of each sample.
4. Presence of samples in wells was checked by naked eye (there is a marked color difference between empty and full wells) or by reading at 450/620nm (samples show OD values higher than 0.100).
5. 100 \(\mu l\) of diluted enzymatic conjugate were dispensed in all wells, except for Al, used for blanking operations.
6. Addition of the conjugate was followed, checking that the color of the samples has changed from yellowish to red and then the micro plate was incubated for 120 min at 37\(^{\circ}\)C.
7. When the first incubation is over; the micro wells were washed very well.
8. 200 µl chromogen/substrate were pipetted into all the wells, including Al.
9. The micro plate was incubated and protected from light at 18-24 ºC for 30 min. wells were dispensed with the positive control, the calibrator and the positive samples will be turned from clear to blue.
10. 100 µl Sulphuric acid were added into all the wells to stop the enzymatic reaction, using the same pipetting sequence as in step 8. Addition of the acid solution turned the positive control, the calibrator and positive samples from blue to yellow.
11. The color intensity of the solution in each well was measured using a 540 nm filter (reading) and if possible a 620-630 nm filters, blanking the instrument on Al.

Statistical analysis: Statistical analyses were performed using statistical package for social sciences (SPSS). Proportions were compared using Chi Square test. Significance was determined at the 0.05 probability level in all analysis.

Results:
Out of the 300 faecal samples examined, 45 were found positive for *S.mansoni*. This constitutes a 15% prevalence rate (table 1). Out of the 193 male examined, 26 were found positive for *S.mansoni* which constitutes 13.4% prevalence rate, while out of the 107 females examined, 19 were found positive for *S.mansoni* which constitutes 17.8% prevalence rate (table 2). This difference was found to be statically insignificant (P value=0.268).

The results showed that, the highest prevalence rate of *S. mansoni* (17.5%) was observed among the (11-15 years) age group while the lowest prevalence rate (11.1%) was detected among the (5-10 years) age group (table 3). This difference was found to be statistically insignificant (P value=0.268).

The detection of antibodies against Hepatitis-B surface Ag (HBs Ag) among the school children in Kerayab village revealed prevalence of 6.7% (table 4). Among those who were infected with *S.manson*, the detection rate was 24.44% while among those who were negative for *S.manson*, the detection rate was 3.52% (table 5). This difference was found to be statistically significant (P value=0.01).

The result showed that the highest prevalence rate of Hepatitis-B surface Ag (10%) was observed among the 16-20 years age group while the lowest prevalence rate (5.5%) was detected among the 5-10 years age group (table 6). This difference was found to be statistically insignificant (P value=0.663).

Out of the 193 males examined, 16 were found positive for Hepatitis-B surface Ag (HBs Ag) which constitutes 8.3% prevalence rate, while out of the 107 females examined, 4 were found positive for Hepatitis-B surface Ag (HBs Ag) which constitutes 3.7% prevalence rate (table 7). This difference was found to be statistically insignificant (P value=0.168).
Table 1: Overall prevalence rate of *S. mansoni* among school children in Keryab village:

<table>
<thead>
<tr>
<th>Number examined</th>
<th>Number Positive</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>300</td>
<td>45</td>
<td>15%</td>
</tr>
</tbody>
</table>

Table 2: The prevalence rate of *S. mansoni* among school children in Keryab village according to gender:

<table>
<thead>
<tr>
<th>Gender</th>
<th>Number examined</th>
<th>Number positive</th>
<th>Prevalence</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>193</td>
<td>26</td>
<td>13.4%</td>
<td>0.268</td>
</tr>
<tr>
<td>Female</td>
<td>107</td>
<td>19</td>
<td>17.8%</td>
<td></td>
</tr>
</tbody>
</table>

Table 3: The prevalence rate of *S. mansoni* among school children in Keryab village according to age group:

<table>
<thead>
<tr>
<th>Age group</th>
<th>Number examined</th>
<th>Number positive</th>
<th>Prevalence</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 – 10</td>
<td>90</td>
<td>10</td>
<td>11.1%</td>
<td>0.268</td>
</tr>
<tr>
<td>11 – 15</td>
<td>160</td>
<td>28</td>
<td>17.5%</td>
<td></td>
</tr>
<tr>
<td>16 - 20</td>
<td>50</td>
<td>7</td>
<td>14%</td>
<td></td>
</tr>
</tbody>
</table>

Table 4: Seropositivity of hepatitis B surface antigen among school children in Keryab village:

<table>
<thead>
<tr>
<th>Number examined</th>
<th>Number Positive</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>300</td>
<td>20</td>
<td>6.7%</td>
</tr>
</tbody>
</table>

Table 5: Seropositivity of hepatitis B surface antigen among Schistosoma mansoni positive and negative cases in Keryab village:

<table>
<thead>
<tr>
<th>Number tested</th>
<th>Number positive</th>
<th>Prevalence</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ve</td>
<td>45</td>
<td>11</td>
<td>24.44%</td>
</tr>
<tr>
<td>-ve</td>
<td>255</td>
<td>9</td>
<td>3.52%</td>
</tr>
</tbody>
</table>

Table 6: The prevalence rate of hepatitis B surface antigen among school children in Keryab village according to age group:

<table>
<thead>
<tr>
<th>Age group</th>
<th>Number examined</th>
<th>Number positive</th>
<th>Prevalence</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 – 10</td>
<td>90</td>
<td>5</td>
<td>5.5%</td>
<td>0.663</td>
</tr>
<tr>
<td>11 – 15</td>
<td>160</td>
<td>10</td>
<td>6.25%</td>
<td></td>
</tr>
<tr>
<td>16 - 20</td>
<td>50</td>
<td>5</td>
<td>10%</td>
<td></td>
</tr>
</tbody>
</table>
Table 7: The prevalence rate of hepatitis B surface antigen among school children in Keryab village according to gender:

<table>
<thead>
<tr>
<th>Sex</th>
<th>Number examined</th>
<th>Number positive</th>
<th>Prevalence</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>193</td>
<td>16</td>
<td>8.3%</td>
<td>0.168</td>
</tr>
<tr>
<td>Female</td>
<td>107</td>
<td>4</td>
<td>3.7%</td>
<td></td>
</tr>
</tbody>
</table>

Discussion:
The result obtained revealed that the overall prevalence rate of *S. mansoni* in Kerayab village was found to be 15%. This rate was lesser than the rate reported by Tagwa (8) who reported 40% prevalence rate. This marked reduction in the prevalence rate of *S. mansoni* in the area might be attributed to control measures launched in the area before the start of our study.

In this investigation, there was no correlation between age and the occurrence of *S. mansoni* infection among the studied population. This finding is in contrast with the findings of Nasr (9) and Butterworth *et al* (10) who attributed the correlation to age dependent acquisition of immunity to super-infection. The prevalence and intensity of infection was higher in females (17.8%) than in males (14.3%). In spite of exposure rate and water contact which is usually higher in males by virtue of their activities. Although this is an agriculture scheme, farming is normally practiced by male and female particularly the young and middle aged ones. The land is irrigated by canals which harbor infected snails. These same canals act as swimming pools for children and youngest boys and girls. Since this is one of the main items for recreation, it is customary to find them daily swimming from early to late afternoon or washing clothes. The result obtained showed that HBs Ag was high among bilharzial patient than in non-bilharzial individuals. Similar results were reported from Egypt (11, 12, 13, & 14), Brazil (15), Kuwait (16) and Sudan (5). Such results can be regarded as evidence for the existence of an association between HBV and *S. mansoni* infection.

In contrast, some investigators (17, 18, 19, 20, & 21), showed that there is no link between the two diseases. Some of them referred the association found in the former studies to selection bias *i.e* those studies showed positive correlation in selected hospitalized patients with *S. mansoni* infection. As is generally accepted that *S. mansoni* infection alone does not lead to cirrhosis or chronic active hepatitis (18 & 22), interaction between HBV and *S. mansoni* infection was suggested to cause a serious form (14).

The result obtained showed that the bilharzial patients with or without oraganomegally had comparable seropositivity for HBsAg. This indicates that they are similary susceptible to HBV infection. Similar finding were reported by Khalil *et al* (13) and Ghaffar *et al* (14).
contrast, Bassily et al (11) and Al Nakib et al (16) reported that HBV infection was higher in hepatosplenic than in intestinal schistosomiasis. However, the organomegally found in these subjects might not be due to only Schistosoma infection, because the study area is endemic for other diseases such as malaria. Tosswil and Rideiy (23) suggested the presence of shared antigens between S.mansoni and HBV infection, however, the introduction of highly specific methods for detection of HBsAg made this possibility rather remote. The initial defense mechanisms against viral infection are the innate immunity defense such as interferon-γ (INF-γ), Natural Killer (NK) and macrophages. INF-γ inhibits viral replication and enhances adaptive immune response by stimulating and increasing the expression of major histocompatibility complex (MHC) class 1 and it is also potential activator of macrophages and NK cells. Since HBV is an intracellular organism, MHC1 is the most important mechanism for eliminating the virus infected cells through CD-8 T cells (14).

The overall prevalence rate of the present study was 24.4%. This rate was found to be higher than the rate reported by Eltoum et al (20), Hyams et al (18), and Taha (24) who reported 18.5%, 10%, and 9% rate respectively. However, our rate was less than the rate reported by Nasr (9) who reported 30% rate in Sudan.
References:


