Seroprevalence of Hepatitis B and C Viral Infections among Blood Donors in Shendi, River Nile State, Sudan

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Abstract: The main objective of this study was to determine the seroprevalence and the possible risk factors for hepatitis B and C virus infections among the blood donors attending Shendi Teaching Hospitals, River Nile State, Sudan. Seventy eight (n=78) subjects were investigated during the period from May to July, 2005. All the subjects examined were males, age ranging from 20-50 years. The hepatitis B surface antigen (HBsAg), the main serologic marker for hepatitis B virus (HBV) infection was detected among 5.1% blood donors using both immunochromatographic test (ICT) strips and the enzyme-linked immunosorbent assay (ELISA). Hepatitis C virus (HCV) specific antibodies (anti-HCV Abs) were detected among 1.3% blood donors using ICT only. In all subjects examined, dual infection with both viruses was never detected. ELISA was found to be fairly sensitive in detecting HBsAg compared to ICT; however, there was no significant difference (P>0.05) between the two techniques used. Previous blood transfusion was found to be a significant (P<0.05) predisposing risk factor to both viral infections. However, no other possible risk factors (e.g. drug abuse, previous surgical operations, needle injuries) were found to be significant (P>0.05) to contract both diseases. There was no significant difference (P>0.05) between the prevalence of HBsAg among married blood donors compared to the single donors.

Keywords: Hepatitis, HBV, HCV, serological markers, HBsAg, Blood donors, ELISA, ICT Shendi, Sudan

INTRODUCTION

Hepatitis B virus (HBV) and hepatitis C virus (HCV) can cause a spectrum of clinical conditions in humans ranging from the symptoms of free-carrier state through chronic hepatitis, liver cirrhosis to hepatocellular carcinoma[3,6,13]. Worldwide there are almost 350 million chronic carriers of HBV[3] and more than 170 million of HCV chronic carriers who are at risk of developing liver cirrhosis, liver cancer or both[3, 6, 13,17].

Human infection with both viruses can cause acute or chronic hepatitis which seem to be determined by the individual's immune response. Detection of hepatitis B surface antigen (HBsAg) in the blood indicates ongoing active infection. During viral pathogenesis in the liver, large amounts of HBsAg are released into the blood, in addition to the complete virion[6,13,17].

Both HBsAg and its specific antibodies are good serologic markers to diagnose the disease during the acute or chronic carrier state[3,17]. Therefore, sensitive serologic techniques, like enzyme-linked immunosorbent assay (ELISA) and immunochromatographic test (ICT) are, usually, used to detect these markers in the blood and sera of suspected individuals[12,13,17].

Infection with HCV was first indentified by Choo et al.[7], and was found to cause, like HBV, acute and chronic human hepatitis. However, it causes mild infection compared to HBV. Both human viral infections can be transmitted by various routes, i.e. blood and blood products (e.g. blood transfusions) sexual, oral, vertical and horizontal transmission[3, 6, 17]. Because both viral infections are of clinical importance in Sudan, various investigators have studied them in different regions of the country[2,18,11,12,16]. Therefore, this preliminary sero-survey investigation was performed in Shendi area, River Nile State, Sudan, in order to demonstrate the possible seroprevalence status of both viral infections. The well known and routinely used screening serologic techniques (ELISA and ICT) were both employed to detect HBsAg, and anti-HCV antibodies among different male blood donors attending Shendi Teaching Hospitals. Demographic studies using interviewing questionnaires were also used to illustrate the possible risk factors for both viral infections.

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MATERIALS AND METHODS

Time and Location of Study: This study was conducted during the period from May to July, 2005, as a hospital-based study in the city of Shendi, River Nile State, Sudan. The main reason for selecting this area was that no previous studies were done to investigate the prevalence of both human viral infections. The population of Shendi area is approximately, 250000. The health services are mainly through the primary health care units, which consist of 16 clinics, 22 health centers and 4 rural hospitals. In addition, the recently established Al Mak Nimir University Hospital added more to all health services in the area.

Study Population: All apparently healthy male blood donors (n=78) attending Shendi Teaching Hospital and Al Mak Nimir University Hospital from May to July, 2005, for blood donation were randomly, selected as study subjects.

Data Collection: After explaining the purpose of the study, data were collected from each subject by interviewing questionnaire. The data included the demographic information (age, sex and residence), history of previous blood transfusions and other possible risk factors (e.g. needle exchanges).

Specimen collection: With extreme precaution and under strict sterile conditions, 5 ml aliquots of whole venous blood samples were withdrawn from each blood donor under investigation. The blood samples were collected in sterile containers without any additives. Serum was, aseptically, separated after clot retraction by centrifugation at 2000 rpm for 5 minutes. Serum samples were then, stored at -4°C until tested (not more than a month).

Laboratory Techniques: Using both immunochromatographic test (ICT) and enzyme-linked immuno-sorbent assay (ELISA) to detect HBsAg, and using ICT alone to detect the specific antibodies for HCV.

ICT for detection of HBsAg: For test strips from Advanced Quality, the following technique was conducted according to Wisdom ([15]). Before proceeding with the assay, all reagents and specimens were brought to room temperature. Briefly, the test strip was removed from the foil pouch and placed on a clean dry surface. Aliquots of 80 μl of the sample or control, were dispensed on the sample pad. The results were interpreted after 15 minutes according to presence of color band.

ELISA for Detection of HBsAg: This assay was performed according to Magnus et al. ([15]) with some modifications. As above, all reagents and specimens were brought to room temperature before proceeding with the assay. When commencing the assay all specimens and controls were carefully recorded on the sheet supplied with the kit.

The required number of microtiter strips were selected and placed, firmly, on the holder. Extreme care was taken for proper heat transfer during the 80 minutes incubation at 37°C. The microtiter wells were placed on a prewarmed humid towel or metal block in the incubator. Careful and repeated washing was highly critical, because insufficient washing might result in poor precision and false elevated absorbance.

Exposure to strong light was avoided during color development. Each pipetting step was followed by gentle rocking of the plates to ensure thorough mixing without spilling the solutions. Air bubbles were removed prior to incubations as well as reading absorbance.

Briefly, aliquots of 50 μl of negative control, positive control, sample and enzyme conjugate were pipetted into the assigned wells. The sample and enzyme conjugate were mixed well by gentle rocking for 20 seconds. The microtiter strips were covered with adhesive film or foil. The samples were incubated for 80 minutes at 37°C, and the contents of the wells were aspirated into 5% sodium hypochlorite solution. The wells were washed 5 times using 400 μl washing buffer. The remaining solutions were removed by tapping the plate upside down on tissue paper.

Aliquots of 50 μl of substrate reagent A and substrate reagent B were dispensed into the wells, and all were mixed and incubated for 30 minutes at room temperature. Then aliquots of 100 μl stop solution (1.0 mol/l sulfuric acid) were added into each well and mixed well. The absorbance was read at 450 nm against blank. The result obtained was read within 30 minutes after adding the stop solution as followed:

- Mean negative control value: MNC< 0.100
- Mean positive control value:
- MPC>0.600MPC-MNC>:0.500
- Cut-off value (COV)=MNC+0.025

Interpretation of Results:

Positive: Specimen with absorbance values equal to or greater than the cut-off value was considered HBsAg positive (reactive).

Negative: Specimen with absorbance values less than the cut-off value was considered HBsAg negative (non-reactive).
ICT for detection of specific anti-hepatitis C antibodies: The following technique was performed according to Choo et al.,[8] with some modifications. Before proceeding with the assay, all reagents and specimens were brought to room temperature. Briefly the test strips (Advanced Quality) were removed from the foil pouch and placed on a clean dry surface. Aliquots of 10 μl of specimen or control were dispensed on the sample pad. The test results were obtained after 15 minutes as followed:

Positive: Both purplish red test band and purplish red control appeared on the membrane.

Negative: Only the purplish red control band appeared on the membrane.

Statistical Analysis: Processing and analysis of data were performed by means of the statistical package for social sciences (SPSS-PC version 10.0, computer software). Chi-square test was used to assess the difference between the various groups. Statistical significance was taken as P<0.05.

RESULTS AND DISCUSSIONS

A total of 78 subjects were included in the study to show the prevalence of HBV and HCV among the blood donors in Shendi and Al Mak Nimir Teaching hospitals and to determine the possible risk factors in the study group.

Distribution of Blood Donors According to Age:
Most of the blood donors examined were within the age group 20-29 (43.6%) and 30-39 (43.6%) for each. However, only 1.3% of blood donors were above 50 years of age (data not shown).

Distribution of Blood Donors According to Residence:
Most of the blood donors were from Shendi city (62.8%), while only few of them (32.2%) were from the rural area (villages) around Shendi city (data not shown).

Distribution of Blood Donors According to Marital Status:
The married blood donors were shown to be fairly higher in number (56.0%) compared to single blood donors (44.0%) showing no significant difference (P>0.05) (data not shown).

Detection of HBsAg and Anti-HCV Antibodies: Only 4 blood donors (5.1%) were found positive for HBsAg (P<0.05) using ICT and ELISA techniques, while anti-HCV antibodies were detected in only one person (1.3%; P<0.05) among the subjects investigated using table 1: Positive cases of HBsAg and specific anti-HCV antibodies among the tested individuals.

<table>
<thead>
<tr>
<th>Serological markers</th>
<th>Number of positive cases</th>
<th>Percent(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBsAg</td>
<td>4</td>
<td>5.1</td>
</tr>
<tr>
<td>Anti-HCV</td>
<td>1</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Chi-square for HBsAg =62.821 (p<0.05)  
Chi-square for Anti-HCV Abs =74.05 (p<0.05)

Table 2: Effect of risk factors in detection of both HBsAg and the specific anti-HCV Abs.

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>No. tested</th>
<th>No. positive</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood transfusion</td>
<td>13*</td>
<td>2*</td>
<td>15.4</td>
</tr>
<tr>
<td>Accidental needle stick injury</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Minor surgical operation</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>16</td>
<td>2</td>
<td>15.4</td>
</tr>
</tbody>
</table>

Chi-square=0.400 (p>0.05)

Fig. 1: The relationship between marital status and infection with HBV and HCV.

ICT only (table 1). During performing this study, ELISA was not available to detect anti-HCV antibodies.

The Relationship of Marital Status to HBV and HCV Infection: Figure 1 demonstrates that there was no significant difference (P>0.05) between the prevalence of HBsAg and anti-HCV antibodies among the married blood donors in comparison to the single ones.

Possible Risk Factors for Both Viral Infections: The results displayed in table 2 revealed that the only possible risk factor that had significant effect (P<0.05) on both viral infections was the previous blood transfusions. All other possible risk factors either had minimal or no effect at all in contracting both diseases.

Effect of Age on Both Viral Infections: Figure 2 exhibits no effect of age of all blood donors examined (P>0.05) in detection of both serological markers of the two diseases.
The prevalence of HBsAg obtained in this study was 5.1% (table 1). These findings were low compared to those obtained by Hyam et al. in Al Gazira rural area (18.7%), and those obtained by Michael et al. in Juba city (26%), southern Sudan. This discrepancy could, possibly, be due to the fact that both investigator groups had used large sample size (e.g. 666 subjects in Juba). Furthermore, these variations could be attributed to some possible racial and socioeconomic differences between the different areas of the studies.

For HCV infection, only one case was shown to be positive (1.3%) according to the detection of the specific anti-HCV antibodies (table 1). These results were in agreement with those reported by Sirchia et al. in Italy (1%) and Choo et al. in France (0.57%). Almost similar results were also obtained by Dominque et al. in Ethiopia (1.4%) and Aceti et al. in Somalia (0.97%).

Similar studies carried out in Saudi Arabia revealed that HCV infection was more prevalent among apparently healthy individuals who were over 50 years of age (5%). However, studies conducted in Egypt among university student blood donors showed HCV prevalence of 9.2%, while a prevalence of 26.6% was obtained among blood donors in Cairo.

The studies done in Juba city, southern Sudan, by Michoel et al. disclosed that HCV prevalence was 3% among the study subjects compared to 1.3% obtained in this study. Once again, this discordance could be due to either the small sample size used in this study or the different techniques used to detect the specific anti-HCV antibodies. However, possible socioeconomic and racial differences between the two areas of investigation should be considered when interpreting the results of such kinds of studies.

The results obtained in this study, clearly demonstrated that previous blood transfusion was a main risk factor for HBsAg detection (15.4%) among the blood donors investigated. This could, possibly, be due to the fact that either blood screening programs were not endorsed in the past, or urgent blood transfusions were performed without screening tests.

Although the results obtained for HBsAg showed that ELISA was fairly sensitive in comparison to ICT no significant difference (P>0.05) was observed between the two techniques. The possibility exists that the sample size used in this study and the financial difficulties faced the investigators to perform further sensitive laboratory techniques (e.g. Western Immunoblot, PCR) were the main reasons for these preliminary findings. Further in-depth studies, using large sample size at different hospitals and health centers and employing confirmatory techniques (e.g. recombinant immunoblot assay, PCR) are needed to explore more information about these two clinically important human diseases.

ACKNOWLEDGMENTS

Our thanks and appreciations must go to the doctors, laboratory technologists and the staff of Al Mak Nimir University Hospital and Shendi Teaching Hospital for their kind help and cooperation. We are also thankful to Maha M. El Mukhtar for typing this manuscript.

REFERENCES