Seroprevalence of HBV infection and viral loads in outpatients attending a district hospital located in Mardan, Pakistan

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Abstract

Hepatitis B is a major public health problem in Pakistan like other developing countries. HBV infection is a more contagious disease than HCV and HIV infection. The aim of the current study is to compare seroprevalence of HBV infection in outpatients who attended a district headquarter hospital of district Mardan, Pakistan. A total number of 270 patients were selected for this research work. Serum of patients were tested by HBsAg and ICT strip method and those found positive were sent to GCMBDR to confirmed for HBV DNA by Real Time PCR. Out of the total 270 screened patients, HBV DNA was confirmed in 94(34.81%) patients using Real Time PCR of which 56(59.6%) were male while 38(40.4%) were female outpatients. All the positive patients were divided into three categories on the bases of viral load categories such as low (<100000 IU/ml), intermediate (100,000-10,000,000 IU/ml) and high viral load (>10,000,000 IU/ml). On the bases of these categories 50 %( n=47) patients were found with low viral load, 33 %( n=31) with intermediate and 17 %( n=16) were found having high viral load. Female patients were slightly more susceptible to HBV infection compared to male patients. The highest HBV infection seroprevalence was found in patients of 21-40 age group. Majority of the sick subjects were however seen with low viral titers.

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Introduction

Hepatitis is a major health problem in both developed and under developed countries as it affects about 3.5 billion individuals globally (Wittet, 2010., Komars et al., 2010, Awan et al., 2010, Awan et al., 2010). HBV infection is a more contagious disease than HCV and HIV infection. It is 10 times more contagious disease than HCV and about 50-100 times more contagious disease than HIV (Samuel et al., 2004). Hepatitis B virus belongs to hepadnaviridae family and to the genus Orthohepadna virus (Hunt, 2007). Genome of HBV is partially circular and double-stranded circular DNA virus (Barker et al., 1996). In 1988 six major serotypes (A-F) of HBV were identified worldwide (Norder et al., 1994) including genotypes G and H (Shibayama et al., 2005) and nowadays each genotype is further divided into multiple subtypes with distinct properties (Schaefer et al., 2007).

Hepatitis B prevalence is high among developing countries of Africa, the Pacific Islands and Asia while HBV prevalence is low among the developed countries of USA, Australia and Western Europe. In Pakistan, HBV is also a major health problem (Khan et al., 2010). In Japan, Eastern Europe and Russia, about 2-8% of their populations are infected by HBV. According to 2010 report, it is estimated that 120 million individuals are infected with HBV in China followed by India with 40 million and Indonesia with 12 million infected individual. World Health Organization reported that 600,000 deaths may occur annually due to Hepatitis B virus infection (Jakarta Post, 2011)

HBV transmission occurs as a result of exposure to infected human blood or body fluids. It may also be transmitted to healthy individual by sexual contact with HBV infected persons (Fairley and Read, 2012). It may be transmitted through contaminated blood transfusion (Buddeberg et al., 2008) re-use of contaminated syringes (Hughes, 2000) and from infected mother to child during child birth (Hughes, 2000). Within family members, HBV can be transmitted through contact with non intact skin or mucous membrane with secretions or saliva containing HBV (Petersen et al., 1976). Different methods were used for detection of HBV infection. Diagnostic assays are used to detect HBV infections using serum or whole blood which may contain viral antigens or antibodies produced by the host (Bonino et al., 1987). The most frequently used method for the detection of HBV infection is HBsAg screening. HBsAg is the first viral antigen that may appear and easily detectable during infection (Karayiannis et al., 2009).

ALT is also easy method for detection of viral hepatitis in infected individual (Dufour et al., 2000). Nowadays, PCR test is the most specific method used for the detection of HBV infection and also used to measure its viral load (Zoulim et al., 2006). HBV infection may be prevented by using safe methods i.e. proper screening of blood before transfusion, avoiding re-use of used syringes and needles, and by vaccination at birth (O'Connor, 2008).

Very few number of studies are available from district Mardan about seroprevalence of HBV infection. Therefore, the present study was designed to evaluate the seroprevalence of Hepatitis B virus infection in different age groups of outpatients that attended a district hospital in Mardan, Pakistan.

Materials and methods

Study Area

For the current study general population of District Mardan was selected. Mardan the second largest city of the Khyber Paktunkhwa Province of Pakistan with a population of 1.46 million according to 1998 census and de facto headquarters of the Yousafzai tribe (Government of Pakistan). The study was conducted from December 2011 to December 2012.

Sampling

All the patients included in this study were found HBV positive by HBsAg and strip methods previously. Whole blood samples were obtained from these 270 outpatients who attended a district hospital in Mardan, Pakistan. Blood samples were centrifuge and sera was separated and for further investigation serum samples of the patients were sent to Genome
Center for Molecular Diagnostics & Research Lahore (GCMBDR). All the samples were received with proper consent of the patients at GCMBDR.

**Viral DNA Isolation/Confirmation by Real Time Polymerase Chain Reaction**

Viral DNA was isolated from sera samples by using NucleoSpin DNA Virus/Virus F kits by Machery and Nagel. Nucleic acid isolation was done using 100 ul serum samples according to the kit protocol. Isolated HBV DNA was confirmed by Smart Cycler II Real-time PCR (Cepheid, USA). Amplification was done using real time amplification kit by Sacace Biotechnologies Italy. Total reaction volume was 25 ul, containing 15.25 ul HBV amplification mix, 0.5 ul Taq polymerase and 10 ul extracted DNA. For precise check of the reaction negative and positive controls were also included with the run. Sensitivity of the assay is 20 IU per ml blood sample. Specificity of the assay is about 99%.

**Statistical Analysis**

Statistical 9.0 software was used for analyses and summarization of data obtained. Chi-square test was used-value less than 0.05 was considered as significant.

**Results**

**Gender and Age wise Prevalence rate of HBV infection**

Out of the total 270 blood samples made of 162(60.0%) males and 108(40.0%) females, HBV DNA was observed in 94 (34.8%) patients. Of the total 162 male patients 56(34.5%) were found positive for HBV DNA while of the 108 female patients, 38(35.1%) were found positive for HBV DNA by Real-Time PCR.

**Table 1.** Sex wise distribution of HBV infection among outpatients studied.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Total samples</th>
<th>Total HBV positive samples</th>
<th>Percentage</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>162</td>
<td>56</td>
<td>34.5%</td>
<td>&lt;0.05 Significant through Statistix 9 (Chi-Square)</td>
</tr>
<tr>
<td>Female</td>
<td>108</td>
<td>38</td>
<td>35.1%</td>
<td>&lt;0.05 Significant through Statistix 9 (Chi-Square)</td>
</tr>
<tr>
<td>Total</td>
<td>270</td>
<td>94</td>
<td>34.8%</td>
<td></td>
</tr>
</tbody>
</table>

Table 2 shows the seroprevalence of HBV infection among the different age groups. All the outpatients were divided into four groups i.e. 0-20, 21-40, 41-60 and above 60yrs age groups. HBV infection rate was highest (38.9%) in 20-40 age group followed by 60 age group which recorded infection rate of 36.1%, as well as 23.1% infection rate in age group 40-60 and low prevalence of HBV infection observed in outpatients above 60 years of age.

**Table 2.** Age wise distribution of HBV infection among outpatients studied.

<table>
<thead>
<tr>
<th>Age</th>
<th>Total samples</th>
<th>Total HBV positive samples</th>
<th>Percentage</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-20</td>
<td>39</td>
<td>9</td>
<td>23.1%</td>
<td>&lt;0.0001 Significant through Statistix 9 (Chi-Square)</td>
</tr>
<tr>
<td>21-40</td>
<td>175</td>
<td>68</td>
<td>38.9%</td>
<td></td>
</tr>
<tr>
<td>41-60</td>
<td>36</td>
<td>13</td>
<td>36.1%</td>
<td></td>
</tr>
<tr>
<td>&gt;60</td>
<td>20</td>
<td>4</td>
<td>20%</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>270</td>
<td>94</td>
<td>34.8%</td>
<td></td>
</tr>
</tbody>
</table>

**HBV DNA viral loads among both gender and various age groups**

On the basis of viral loads all the positive patients were analyzed in three categories such as low (<100000 IU/ml), intermediate (100,000-10,000,000 IU/ml) and high viral load (>10,000,000 IU/ml). HBV viral load in both sexes are shown in Table 3. Out of the total of 94 seropositive patients 50%( n=47) were found to have low viral load, 33 %( n=31) with intermediate and 17 %( n=16) were found with high viral load. HBV viral load in various age groups was analyzed in Table 3. All the outpatients were divided into four groups based on their ages. Of the 9 patients of age group 0-20, 4.2 %( n=4) were
found with low viral load, 1% (n=1) with intermediate and 4.2% (n=4) with high viral load. In age group 21-40, 35.1% patients were found with low, 26.5% with intermediate and 11.7% with high viral load. 8.5% outpatients of 41-60 age group recorded low viral load, 3.1% with intermediate and 1% with high viral load while 2.1% greater than 60yr old recorded patients showed both low and high viral loads.

### Table 3. Sex wise distribution of HBV viral loads among outpatients studied.

<table>
<thead>
<tr>
<th>Gender</th>
<th>HBV Viral load (IU/ml)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;100000</td>
<td>100,000&gt;10,000,000</td>
</tr>
<tr>
<td>Male</td>
<td>27(28.7%)</td>
<td>23(24.4%)</td>
</tr>
<tr>
<td>Female</td>
<td>20(21.2%)</td>
<td>08(8.5%)</td>
</tr>
<tr>
<td>Total</td>
<td>47(50%)</td>
<td>31(33%)</td>
</tr>
</tbody>
</table>

**Discussion**

Mardan, a large district in Khyber Pukhtunkhwa Pakistan, with an area of about 1632 sq.km, like other northern areas of Pakistan lag behind in literacy rate and health facilities, although measure towards improvement are under way. According to a survey in 2005/06, poverty in Khyber Pukhtunkhwa is estimated to be 29% while the literacy is 50% in the gap in female literacy is 31% (unic.org.pk). Therefore it goes without saying that extensive researches on pertinent health issues like prevalence of hepatitis B are scarce despite the fact that due to the same lack of advancement infectious disease like hepatitis is continuously on the rise, this justifies the purpose of our research. Mardan is important also because owing to the scarcity of jobs and natural disaster like flood and political instability a large numbers of its natives migrates to large cities of other provinces every year, resulting in interchanging of infectious diseases like hepatitis B (as of 6th may 2010, 341,768 people of KPK have become internally displace people and have been living in host communities) (WHO).

### Table 4. Age distribution of HBV viral loads among outpatients studied.

<table>
<thead>
<tr>
<th>Age</th>
<th>HBV Viral load (IU/ml)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;100000</td>
<td>100,000&gt;10,000,000</td>
</tr>
<tr>
<td>0—20</td>
<td>04(4.2%)</td>
<td>01(1%)</td>
</tr>
<tr>
<td>21—40</td>
<td>33(35.1%)</td>
<td>25(26.5%)</td>
</tr>
<tr>
<td>41—60</td>
<td>08(8.5%)</td>
<td>03(3.1%)</td>
</tr>
<tr>
<td>&gt;60</td>
<td>02(2.1%)</td>
<td>02(2.1%)</td>
</tr>
<tr>
<td>Total</td>
<td>47(50%)</td>
<td>31(32.9%)</td>
</tr>
</tbody>
</table>

Few Number of study is available on molecular epidemiology of HBV infection in district Mardan. Our research shows that gender wise, the percentage of female affected (35.1%) is slightly more as compare to that of males (34.5%), this contrasts with the results from other regional studies in which more male patients were affected than female (Khan et al., 2011). This may be due to the fact that males migrate to other cities and provinces in search of better jobs and as a result acquire different infections in the process. During their stay in these developed cities they may get diagnosed and treated early for their infections and may not be counted in the infected population, but during their visits to their native land, they may transfer these infections to their wives and other female occupants. These females are usually confined to their homes and city may not get diagnosed and treated due to the poor health facilities of the city and may get counted in the infected population. Our study also depicts a predominance of male patients in the low and intermediate categories. Only in the high viral load category females showed a significant preponderance.

As far as prevalence of HBV infection is concerned, results obtained in this study are consistent or not
consistent with previous similar studies both showing highest rate of infection in persons aged 21-40 years (Alam et al., 2007). High rate of HBV infection in males compare to females may be due to the fact that they are employed outside their homes visiting barber shops and also their involvement in blood donation and transfusion practices, a Pakistani study shows that in countries like Pakistan, Iran, India, Bangladesh, Israel and Italy HBV can be transmitted through blade sharing and barber related instruments (Waheed et al., 2010). Blood transfusion has been reported as potential risk factor for getting hepatitis C and B virus infections. Poor health facilities can leads to improper screening as well as unsterilized dental instruments can lead to the spread of hepatitis B. ( in a survey conducted in 2010 in Mohmand agency KPK it was discovered that all health sectors lacked facilities for modern screening, dental services and detection and management of sexually transmitted diseases (Qureshi et al., 2009).

Used syringes/needles appear to be the most significant factor in the spread of hepatitis B and C virus in general population of Pakistan (Ali et al., 2009). Two major factors contributing to high re-use of used needles/syringes leading to prostration is the major cause of earlier while a bur dance of un professional practitioners and lack of awareness among the patients seems to be root cause of the latter. As has been impisize in Mardan both these causes are present. Un safe sexual practices also contribute a major group of risk factors (Norman, 1997) staying frequently away from home is common in immigrant in Mardan can lead to acquisition of high risk behavior such as injectable drug use and unsex paid sex.

This Study shows that out of 270 HbsAg positive patients 94 were positive for HBV PCR. Previous studies show that HbsAg is cleared by young carriers earlier as compared to adults. The patients in their early infected stage have positive HbsAg and high viral load and as the infection grows older DNA levels in serum decreases which is associated with the detectable antibody that results in the clearance of HbsAg (Norman, 1997).

According to the present study of viral loads all the positive patients were analyzed and grouped in three categories such as low (<100000 IU/ml), intermediate (100,000-10,000,000 IU/ml) and high viral load (>10,000,000 IU/ml). Total 94 positive patients 50 % (n=47) were found with low viral load, 33 % (n=31) with intermediate and 17 % (n=16) were found with high viral load. Data shows that more females had high viral load (10.6%) as compared to males (6.3%), which may be due to the fact that males have an excess to treatment during the early stage of disease than female.

Our results indicate that the viral load declined with age which is supported by the previous studies conducted in Gambia (Mendy et al., 2008). Patients that occurred in age group 0-20, had similar percentages for both low and high viral loads. In age group 21-40 years 35.1% patients were found with low, 26.5% with intermediate and 11.7% with high viral load. Age group 41-60 year show 8.5 % patients found with low viral load, 3.1% with intermediate and 1% with high viral load while patients with age more than 60 year show that only 2.1% of the patients were found with both low and high viral load and none of the patients were found with high viral load, supporting earlier studies and indicating the complex pattern of HBV infection that is highly dependent upon the age of the infection and the clearance of the virus is greatly influenced by the host response to the virus. According to a documented report, it has been noticed that 95% of the adult patients develop acute hepatitis B recover soon and remaining 5% remain persistently infected (Norman et al., 1997).

Progression of the viral load is dependent upon the particular genotype that the patient carries (Kumar et al., 2011). In this particular study we did not work of the genotype that the patient carried. Further work has to be done.

Conclusion
From the present study it is concluded that female outpatients were slightly susceptible to HBV infection compared to the male outpatients. The highest HBV infection rate was found in patients of 21-40 age group and majority of the infected subjects recorded low viral loads.

Acknowledgement
We are thankful to GCMD for supporting of this study. Also Thanks to Mr. Murad khan and Yahya Khan for his co-operation in this study.

Competing interest
Declare none.

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