ABSTRACT: Hepatitis B is a life-threatening disease of liver caused by Hepatitis B Virus (HBV). It is considered as a serious global risk infecting 25.7 crores (257 million) people around the globe. This virus (HBV) is responsible for causing deaths of 887000 people in the world due to Hepatocellular carcinoma and cirrhosis. Keeping this in view, the study was designed to investigate the seroprevalence of HBV along its various risk factors in sex workers of Bahawalpur City. Present study was conducted in district hospital of the Bahawalpur city from year 2016 to 2017. Samples (n = 9370) from general population were randomly collected from different geographical areas of city. Convenience samples of 33 female sex workers were collected to check the incidence in sex workers. Initially, samples were screened by ICT and confirmed by direct ELISA and PCR. Out of 9370 samples 224 (2.39%) were found positive with HBV. PCR identification using PR2a primers revealed that out of 224, 148 were seriously infected and were recommended for immediate medication. Out of these 33 females sex workers 22 were found infected, with high prevalence of about 66.67%. Foremost reasons for transmission of virus were probably blood transfusions, piercing, unsafe sexual intercourse and poor hygiene conditions due to unawareness and low literacy rate. Further planning to control the dissemination of HBV infection includes mass level screening followed by awareness campaigns & workshops for the enhancement and betterment of livelihood principles and hygiene of locals.

Keywords: Rectal douching, sexual transmission, low literacy rate, hygiene conditions.

INTRODUCTION

Pakistan is developing country with high prevalence of hepatitis B & C viruses. The country is highly endemic for different diseases including hepatitis with 7-9 million people living with hepatitis B virus (Ali et al., 2011). Bahawalpur is under developed city of south Punjab with low resources for...
health sector. The literacy rate of the city is also very low. The people of the city are not well aware of the disease and how it transmits (Khan, 2010).

The disease is caused by HBV which is a double stranded DNA virus belonging to family Hepadnaviridae with Orthohepadnavirus genus having small circular genome of 3.2 kb size (Tiollais and Buendia, 1991). Hepatitis is the inflammation of liver and is caused by different types Hepatitis viruses. Liver being an imperative organ performs multiple tasks like nutrient processing, blood filtration and fights against infections. These functions are affected by intense alcohol consumption, toxins, medication and some clinical disorders (Lucey et al., 2008). Hepatitis A, B, C & E are more prevalent in Pakistan. HAV and HEV infection are acute in nature and reported in outbreaks as both have same route of transmission (fecal oral route). They are also known as self-limiting viruses and body swiftly cures itself (Yayli et al., 2002). HBV & HCV are chronic in nature and leads to carcinoma formation if untreated or if infection persists for long time. Acute Hepatitis B, being solemn viral infection of liver is short term disorder of about 6 months while persistent (chronic) Hepatitis B is lifelong infection (WHO, 2018).

About 2 billion people are exposed to this virus and 357 million people are chronically infected every year and it is reported as global health problem (Jefferies et al., 2018). In humans HBV infection occurs due to mutant genotypes, immune suppression and interaction of HBV with host (Oakes, 2014). HBV is transmitted from person to person through blood transfusion, semen and other body fluids. It can transfer from infected mother to offspring during pregnancy or at the time of birth (Greenfield et al., 1986). Intravenous drug users are at higher risk due needle/syringe sharing with other individuals (Samuel et al., 2001). Rarely It often spread in the form of outbreaks due to congested community, poor sanitation and poor infection control by health centers (Control and Prevention, 2001). Body piercing and tattooing are also the causes of virus spreading. Intercourse with positive spouses can spread HBV, especially in adolescents and immature adults (Akhtar et al., 2018).

Female sex workers at high risk of getting disease due to multiple partners. Oral-anal intercourse, anal-genital intercourse (Rosenblum, 1992) and rectal douching were considerably associated with transmission of HBV(Control et al, 2018). While no confirmation of HBV transmission through oral-oral and oral-genital contact (Judson, 1981). Intravenous drug users (IVDU) are at higher risk of getting HBV infection due to needle sharing. Human Immuno Deficiency virus (HIV) infection in IVDUs facilitates HBV infection and show adverse immunological effect of HB vaccine (Piot and Goilav, 1990).

Mother-to-infant transmission of HBV contributes notably to the higher number of HBV cases in newborns due to highly weak immune status (Han et
al., 2011). The risk of developing chronic infection is about 90% in infants while maximum of 5% in adults (Wiseman et al., 2009). In United States in 2008, HBV infection due to sexual intercourse in female were 7025 and 11900 in males making approximately 50% of total cases with 622 females and 825 males, were aged between 15-24 years old (Satterwhite et al., 2013). Barber shavings are also considerable cause of disease transmission throughout community due to sharing of blades, scissors etc (Khaliq and Smego, 2008).

This research was conducted to check the frequency of HBV infections in Bahawalpur, Pakistan. For this purpose, hospital records were used as a basis of secondary data. Records of year 2017 from January to December were studied for comparative prevalence of HBV & HCV.

MATERIALS AND METHODS

Sampling

During the year 2016-17, samples were collected randomly from patients who visited District Head Quarter Hospital. Sample size (n) was of 9370 people. Convenience sampling technique was used to collect samples (n=33) from female sex workers.

Sample Processing and Techniques

9370 blood samples from general population and 33 blood samples from sex workers were collected in sterile blood collecting tubes and stored at room temperature for 15-30 minutes for extraction of serum. This serum was further used along with buffer for screening through ICTs kits that were manufactured by Bio Check. Manufacturer's instructions and prescribed protocols were strictly followed.

The positive samples were further processed by ELISA. HBV competition Ab test based on competition between serum to be tested with monoclonal antibody fixed in ELISA plate. For this purpose, Human HbsAg (hepatitis B virus Surface Antigen) ELISA Kit manufactured by Wuhan Fine Biotech Co., Ltd under cat# EH4002 were used as per instructions mentioned by manufacturer.

ELISA positive samples were subjected to PCR because it is considered as the most sensitive and specific gold standard tool for diagnostic purposes. HBV PR 2a primers were used in Polygenetic analysis using serum sample. With an annealing temperature of 50°C, the sequence of both forward and reverse primers is mentioned as,

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequencing</th>
<th>Position(nt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2300 F</td>
<td>CCACMWAATGCCCTATC</td>
<td>2300-2317</td>
</tr>
<tr>
<td>215 R</td>
<td>AGRAAMACMCCGCTGT</td>
<td>215-200</td>
</tr>
</tbody>
</table>
RESULTS

Prevalence of HBV was studied using hospital records of year 2016-17 of District Headquarter Hospital, Bahawalpur. It was found that disease is quite prevalent in different areas of city. About 9370 persons were screened for HBV using ICT kits. Table 1 illustrates the results of ICT screening. The positive samples from ICT were subjected to ELISA, out of 224 samples 181 were found having high titer. For further confirmation, PCR was performed for positive samples. Out of 181 samples, 148 were found positive with high number of viral copies. Table 2 presents the results of ELISA along with PCR. Samples from 33 female sex workers were taken and screened for HBV. Out of 33 samples, 22 were found positive for HBV. Figure 1 illustrates the data about Female sex workers.

Table 1: Screening Results of HBV in Year 2016-17

<table>
<thead>
<tr>
<th>Months (2017)</th>
<th>Total screened persons</th>
<th>HBV +ve</th>
<th>Prevalence of HBV</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>735</td>
<td>9</td>
<td>1.22%</td>
</tr>
<tr>
<td>February</td>
<td>695</td>
<td>14</td>
<td>2.014%</td>
</tr>
<tr>
<td>March</td>
<td>469</td>
<td>13</td>
<td>2.77%</td>
</tr>
<tr>
<td>April</td>
<td>881</td>
<td>13</td>
<td>1.47%</td>
</tr>
<tr>
<td>May</td>
<td>882</td>
<td>4</td>
<td>0.453%</td>
</tr>
<tr>
<td>June</td>
<td>583</td>
<td>9</td>
<td>1.54%</td>
</tr>
<tr>
<td>July</td>
<td>1013</td>
<td>26</td>
<td>2.56%</td>
</tr>
<tr>
<td>August</td>
<td>1134</td>
<td>30</td>
<td>2.64%</td>
</tr>
<tr>
<td>September</td>
<td>959</td>
<td>26</td>
<td>2.71%</td>
</tr>
<tr>
<td>October</td>
<td>842</td>
<td>32</td>
<td>3.80%</td>
</tr>
<tr>
<td>November</td>
<td>325</td>
<td>19</td>
<td>5.84%</td>
</tr>
<tr>
<td>December</td>
<td>852</td>
<td>29</td>
<td>3.40%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>9370</strong></td>
<td><strong>224</strong></td>
<td><strong>2.39%</strong></td>
</tr>
</tbody>
</table>

Prevalence = no. of cases/ diagnosed population×100
Prevalence = 224/9370×100
Prevalence = 2.39%

Table 2: Screening Results of ELISA and PCR

<table>
<thead>
<tr>
<th>Categories</th>
<th>Total samples</th>
<th>Positive results</th>
<th>Negative results</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA</td>
<td>224</td>
<td>181</td>
<td>43</td>
</tr>
<tr>
<td>PCR</td>
<td>181</td>
<td>148</td>
<td>32</td>
</tr>
</tbody>
</table>
Seroprevalence of Hepatitis B virus

Fig. 1: Data of Female Sex Workers, Their Work Duration & HBV status

Total screened individuals = 33
Positive individuals = 22
Expected prevalence = \( \frac{22}{33} \times 100 = 66.67\% \) (only in female sex workers)

DISCUSSION

Pakistan is under developing country and is in endemic zone for HBV. This study showed that HBV infections are very prevailing with prevalence of about 2.39% in the city. The virus is blood-borne and spreads through different routes (Greenfield et al., 1986). Intravenous drug users are at higher risk due to needle/syringe sharing with other individuals (Bialek et al., 2005). Body piercing and tattooing are also the cause of virus spreading (Braithwaite and Stephens, 1999). Intercourse with positive spouses can spread HBV, especially in adolescents and immature adults (Fattovich and Bortolotti, 2008). Mother-to-infant transmission of HBV contributes notably to the higher number of HBV cases in newborns due to highly weak immune status (Wiseman et al., 2009). It rarely spread in the form of outbreaks due to congested community, poor sanitation and poor infection control by heath care centers. Investigation about the transmission of virus through barbers showed positive results. There were no sterilizers or antiseptics (Candan and Alagözlı, 2002)

The next thing to be investigated was the spread of HBV infections by means of sexual contact with HBV +ve sex workers (Rai et al., 2007). For this, basic screening was performed to check the health status of sex workers regarding HBV infection.
Only 33 females agreed to go through screening process. 22 out of 33 were found HBV +ve. Investigation revealed that no safety measures are adopted while having sex. Due to this they were found responsible for spreading of disease especially in teenagers in city.

Our study correlates with a research study conduct in Nigeria that showed similar kind of results. Relative HBV prevalence among sex workers in Nigeria is a sign that active sexual dissemination has significant impact in the spread of virus. Sex workers act as pool of a reservoir group for the maintenance and transmission of the virus as they are sexually active and has multiple partners (Forbi, 2008). Dissemination by means of anal sex was also reported (Rosenblum, 1992). This also relates with a similar study with similar results that was conducted in Mexico on pregnant women whose spouse were HBV positive (Vázquez-Martínez, 2003).

In developed western countries, a study revealed that HBV infection rate is about 20-27% more in spouses of hepatitis B patients, female sex workers and sexually active multi-partner young adults as compared to "controls" (Judson, 1981). In United States, a study showed that out of 3816 examined homosexual men, 6.1% had HBsAg, 52.4% had antibodies to HBsAg and 3.0% had anti-HBc (no indication of HBV infection at start of study) (Schreeder et al., 1982).

Our study is useful in designing public health programs for the control of HBV in Bahawalpur. We call for innovative and immediate implementation of best possible ways to control such dissemination patterns.

REFERENCES


