ABSTRACT: Background and Aim: Hepatitis B continues to pose a tremendous burden of disease globally. Information is very scarce on the prevalence of Hepatitis B Virus (HBV) in Davangere. This study was carried out to assess the seroprevalence of Hepatitis B Surface Antigen (HBs Ag) among patients attending a tertiary care centre in Davangere. Method: This is a retrospective study in which patients tested for HBV using HBsAg as a marker were reviewed over six months. A total of 1602 patients comprising of 982 males and 620 females in the age groups 15 to 65 years (yrs) were tested for HBsAg from March 2011 to August 2011 using a commercial Enzyme Linked Immunosorbent Assay (ELISA) test kit. HBsAg seroprevalence rate of patients was calculated and stratified by age and sex. Statistical analysis was performed using Chi-Square test and Confidence Intervals (CI). Results: The study showed that, out of 1602 patients tested, 58 (3.62 %) were HBsAg positive (95% CI: 2.7-4.5). Higher prevalence of HBsAg was found among males (4.79%) than females (1.77%) (p = 0.002). The age specific prevalence rate rose from 1.85% (95% CI: 0.4-3.2) in patients aged 15-25 yrs to a maximum of 5.74% (95% CI: 3.09-8.39) in patients aged 46-55 yrs and decreased in older age groups (p < 0.001). The peaks were detected in male patients aged 46-55 yrs and in females aged 36-45 yrs. Jaundice was present in 8.62% (5/58) of HBsAg positive patients. Conclusion: It was found that variables such as age and sex were significantly associated with HBsAg positivity. HBsAg seropositivity in our study population was significant enough to implement strict community-based preventive measures and improved strategies for vaccination programmes.

KEY WORDS: Seroprevalence; HBsAg; ELISA; Patients; Davangere.

INTRODUCTION: Hepatitis B Virus (HBV) infection is a major global health problem with a high morbidity and mortality. It is estimated that about 2 billion people are infected with HBV worldwide; of which more than 350 million have chronic HBV and some one million die from HBV-related diseases annually. Nearly three-quarters of the global HBV infection pool is in Asian countries. India, with a carrier rate of 3%, contributes nearly 10% of the HBV carriers in the world. The presence of HBsAg in serum indicates active HBV infection, either acute or chronic. The latter observation is the basis for the present use of HBsAg test in many laboratories as well as blood banks, the diagnosis of which in any individual has important management implications, including appropriate counselling, monitoring and/or treating and vaccinating family or at-risk contacts. Keeping in view of such findings, many nations since 1969 initiated screening of blood donors for HBsAg as mandatory and this practice has resulted in the diminution of post transfusion hepatitis by 25%.

In order to check the seriousness of this problem in and around Davangere, the present work was undertaken.
MATERIAL AND METHODS: STUDY DESIGN: A six months retrospective study was conducted in the Department of Microbiology, S S Institute of Medical Sciences and Research Centre (SSIMS&RC), Davangere from March 2011 to August 2011. SSIMS&RC is a 910 bedded, well equipped tertiary care centre located aside NH-4 Bypass road on the outer part of Davangere city. The centre delivers super speciality care, emergency services in addition to the routine medical and surgical facilities to Davangere district and adjoining areas of neighbouring districts in Karnataka state, India.

STUDY POPULATION: Study subjects included patients tested for HBV using HBsAg as a marker during the study period. A total of 1602 patients comprising 982 males and 620 females in the age groups 15-65 yrs who presented to various Out Patient Departments (OPDs) and Inpatients of SSIMS&RC were tested for HBsAg. Identification of the subjects was done using laboratory records. Demographic details such as age, sex, medical history and the test results were noted.

SAMPLE COLLECTION AND TEST PROCEDURE: Fresh serum was obtained by centrifuging naturally clotted blood. The sera samples were assayed for HBsAg by commercially available Enzyme-Linked Immunosorbent Assay (ELISA) kit (ERBA LISA HEPATITIS B, TRANSASIA BIOMEDICALS LTD., DAMAN, INDIA). Manufacturer’s instructions were strictly followed to determine HBsAg seropositivity (reactive) or otherwise of each serum sample.

ERBA LISA HEPATITIS B is a solid phase immunoassay for the qualitative detection of HBsAg in human serum and plasma.

Addition of HBsAg containing human serum or plasma will form a stable complex with the bound antibody present in the well and with subsequently added anti-HBs Ag – Horse Radish Peroxidase (HRPO). Upon adding the substrate, anti-HBsAg-HRPO will split it to yield a blue coloured product. This indicates a positive test and in a negative test, there will be no significant colour formation. On adding Stopping solution, blue colour changes to yellow which is read using an ELISA reader. The HBsAg reactive (positive) sample was retested as duplicate.

STATISTICAL ANALYSIS: The data was analysed using appropriate statistical tests like Chi-Square test and Confidence Intervals. A p value<0.05 was considered statistically significant.

RESULTS: The overall prevalence of HBsAg among patients aged 15-65 yrs was 3.62 % (N=1602, n=58) (95% CI: 2.7-4.5). The prevalence of HBsAg in males was 4.79 % (47/982) compared to 1.77 % (11/620) in females. The difference was found to be statistically significant (p=0.002) (Table 1).

HBsAg seropositivity increased with age, 1.85 % (7/379) in 15-25 yrs age group to 5.74 % (17/296) in 46-55 yrs age group. The difference was found to be statistically of high significance (p<0.001) (Table 1). The study showed maximum prevalence of HBsAg at 7.41 % (14/189) in male patients aged 46-55 yrs and 5.43 % (7/129) in female patients aged 36-45 yrs. For male patients, minimum HBsAg prevalence was seen in the age group 15-25 yrs, whereas no prevalence was found in the female patients of 26-35 yrs & 56-65 yrs Age group (Table 2).

DISCUSSION: In the present study, HBsAg seroprevalence rate of 3.62 % was reported indicating HBV infection is high in this area ⁷. The result is also in close agreement with the historical data from
a number of similar hospital based studies involving antenatal mothers and blood donors from Delhi (3.70%) \(^8\), Jaipur (3.1%) \(^9\), Jodhpur (3.44%) \(^10\), Vellore (3.8%) \(^11\). However, the frequency of HBsAg seropositivity was found to be lower than that reported in Pune (4.17%) \(^12\), Tamil Nadu (24.5%) \(^13\), Madras (7.0%) \(^14\), whilst the rate was known to be higher compared to that in Bombay \(^15\), Ludhiana (0.66%) \(^16\), Chandigarh (1.0%) \(^17\), Lucknow (2.2%) \(^18\).

Similarly, a study conducted in Amritsar, revealed a 28% HBsAg prevalence among chronic liver disease patients. Another community based sero-epidemiological survey that addresses the immunization strategies against HBV infection in Bangalore, reported HBsAg carrier rate of 4.2% from the general population \(^19\), \(^20\). Prevalence of hepatitis B varies from country to country, city to city and even village to village and can change with time. It depends upon a complex interplay of behavioral, environmental and host factors. In general, it is lowest in countries or area with high standards of living (eg: Australia, North America, North Europe) and highest in countries or areas where socio economic level is lower (eg: China, South East Asia, South America) \(^21\), \(^22\).

Analysis of sex related seroprevalence showed 4.79% HBsAg positivity in males and 1.77% in females (statistically highly significant, \(p=0.002\)). This finding of the present study is in concern with other studies, which demonstrated a higher prevalence of HBsAg among the male population \(^23\), \(^24\), \(^25\), \(^24\). The overall high prevalence in the patients was mainly due to high prevalence of HBV infection in male patients, who also predominated the female patients.

Men are more exposed to the risk factors; the males are more educated and employed and work outside their homes or in agricultural lands, while women are mostly involved in household activities. Males are commonly involved in the practices leading to the transmission of infections like blood transfusion and visiting barber’s shop and so are more prone to get the infection at a much frequent rate as compared to their female counterparts in the society \(^25\). Another important contributing factor responsible for a higher prevalence among male patients of the present study is that the plasma disappearance rate for HBsAg in males is lower than females \(^26\).

In this study, positivity for HBsAg increased with age, revealing a significant trend of HBV sero positivity with relation to age (\(p<0.001\)). This is in accordance with earlier observations \(^27\), \(^28\), \(^29\). This could be due to the use of unsafe therapeutic practices including vaccinations by the use of unsterilized kits. Further the trend of immunization against hepatitis B has been introduced in the recent few years. Therefore, it is possible that the older age group population having a higher HBsAg prevalence in the present study might be due to lack of immunization against the disease in their times \(^22\). Alternatively, it may indicate a cohort phenomenon with HBV infection being in retreat \(^28\). The present study showed a significant drop in prevalence in patients aged 55 years and above for both the sexes. It could be due to higher immunity in them as a result of previous exposure to the HBV \(^27\).

Majority of the HBV seropositive patients (53/58) in our study showed no history of jaundice. In 5 patients however, jaundice was present which may or may not be due to hepatitis B. Such a result may not be unexpected due to the fact that most of the persons become HBsAg carriers after asymptomatic infection or after a mild disease which do not produce clinical jaundice \(^20\).

This study is not without limitations. Our data do not include newborns and younger children, the prevalence in who would have given added information on the modes of HBV transmission. We have lost the information on known risk factors for hepatitis B and hence was unable to draw any inference as to cause for the higher HBsAg positivity.
CONCLUSION: In conclusion, high prevalence of HBsAg was determined in this study. Horizontal transmission related to poor injection practices and high risk sexual behaviour may be the important factors for HBV infection among the patients tested. Further studies would however be required to validate this assertion. There is a need for awareness creation through public health education about HBV transmission and prevention, including efforts to ensure all injections be administered with sterile syringes and needles and promoting safe sexual practices. In addition, few long term studies are warranted in future on epidemiologic features of HBV to obtain a database to formulate control measures and develop vaccination strategy.

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REFERENCES:

Table 1. Relationship between HBsAg seropositivity and selected variables among patients attending a tertiary care centre, Davangere.

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>HBsAg</th>
<th>95%CI*</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n/N</td>
<td>%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>47/982</td>
<td>4.79%</td>
<td>3.45-6.12</td>
</tr>
<tr>
<td>Female</td>
<td>11/620</td>
<td>1.77%</td>
<td>0.74-2.81</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15-25</td>
<td>07/379</td>
<td>1.85%</td>
<td>0.49-3.2</td>
</tr>
<tr>
<td>26-35</td>
<td>11/379</td>
<td>2.90%</td>
<td>1.21-4.59</td>
</tr>
<tr>
<td>36-45</td>
<td>16/320</td>
<td>5.00%</td>
<td>2.61-7.39</td>
</tr>
<tr>
<td>46-55</td>
<td>17/296</td>
<td>5.74%</td>
<td>3.09-8.39</td>
</tr>
<tr>
<td>56-65</td>
<td>07/228</td>
<td>3.07%</td>
<td>0.83-5.31</td>
</tr>
</tbody>
</table>

* Confidence Interval, N=Total patients, n=HBsAg Positive patients

Table 2. HBsAg sero positivity in association with sex and age among patients attending a tertiary care centre, Davangere.

<table>
<thead>
<tr>
<th>Age (Years)</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>n/N</td>
<td>%</td>
<td>95%CI</td>
</tr>
<tr>
<td>15-25</td>
<td>6/199</td>
<td>3.02</td>
</tr>
<tr>
<td>26-35</td>
<td>11/247</td>
<td>4.45</td>
</tr>
<tr>
<td>36-45</td>
<td>9/191</td>
<td>4.71</td>
</tr>
<tr>
<td>56-65</td>
<td>7/156</td>
<td>4.49</td>
</tr>
</tbody>
</table>

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