Age wise seroprevalence of hepatitis viral markers in acute infectious hepatitis patients at a tertiary care centre in India

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Abstract
Introduction: Age wise seroprevalence of acute viral hepatitis still remains debatable. The burden of the disease could be reduced to a considerable extent, if seroprevalence of different viruses are known for various age groups.

Objectives: To determine the age wise seroprevalence of hepatitis viral markers in clinically suspected infectious hepatitis at a tertiary care hospital in urban India

Methodology: This prospective study was conducted over a period of 1 year from January -December 2008. The study arm had 600 patients with clinically suspected acute infectious hepatitis while control arm had 200 age and sex matched subjects. Both groups were divided into 0-10, 11-20, 21-30, 31-40 and >40 years age groups. Serum samples were tested for IgM anti HAV, IgM anti HEV, IgM anti HCV and HBsAg using commercially available enzyme linked immunosorbent assay kits.

Results: Highest seropositivity of anti-HAV IgM was in 0-10 years of age for the study arm. HAV seroprevalence decreased with increasing age. Highest seropositivity of HBsAg was in 20-30 years of age group for the study group (7.6%) followed by 11-20 years (4.5%), 0-10 years (2.8%) and >40 years (2.5%). Highest seropositivity to IgM anti HCV was seen in 11-20 years (9%) in the study arm followed by 21-30 years (6.1%), 0-10 years (5.1%) and >40 years (3.7%) of age. Highest seropositivity of anti-HEV IgM was in the 21-30 years (10.7%) in the study arm followed by 31-40 years (2.9%), 11-20 years (2.7%) and 0-10 years (0.9%).

Conclusion: Both HAV and HEV are significant causes of enterically transmitted acute viral hepatitis. HAV is the major cause of acute viral hepatitis in childhood. Hepatitis B virus is the major cause of acute viral hepatitis in adults. HCV is a common cause of acute hepatitis in young adults. HCV infections remain under reported while the actual disease burden is much higher.

Keywords: Seroprevalence, Age wise, HBsAg, HCV, HAV, HEV

Introduction
Viral hepatitis is defined as inflammation of liver due to infection with hepatotropic viruses.[1] The hepatotropic viruses are divided into enteral and parenteral groups based on their mode of transmission. Hepatitis A and E viruses are enterically transmitted by feco-oral route and do not have a chronic carrier state. Hepatitis B, C and D virus are parenterally transmitted with both acute and chronic illness manifestations. They persist in a chronic carrier state as a reservoir. The disease spectrum can be chronic hepatitis, cirrhosis and hepatocellular carcinoma.[2]

Hepatitis A virus (HAV) and hepatitis E virus (HEV) are common in communities where sanitation and food safety are compromised. Hepatitis B virus (HBV) and Hepatitis C virus (HCV) infections are seen among the recipients of blood, organs and tissues. Health care providers who sustain accidental needle stick injuries while caring for the infected patients are also exposed.[3] Acute hepatitis is characterized by jaundice, extreme fatigue, nausea, vomiting and abdominal pain.[4] HAV is a small unenveloped symmetrical RNA virus and shares many of the characteristics of the picornavirus.[5] Hepatitis B virus (HBV), a member of the hepadnavirus group is double-stranded DNA virus and replicates unusually by reverse transcription. HBV is endemic globally with hyperendemicity in many parts of the world.[5] HCV is an enveloped single-stranded RNA virus which appears to be phylogenetically related to flavivirus. HCV is associated with chronic liver disease and primary liver cancer.[5] HEV, the cause of enterically-transmitted non-A, non-B hepatitis, is another non-enveloped, single-stranded RNA virus, which shares many biophysical and biochemical features with caliciviruses.[5] Hepatitis D virus (HDV) is a small enveloped RNA virus and its transmission is similar to that of HBV.[6]

Hepatitis infections can be prevented by avoiding exposure to viruses and with vaccines. However, vaccines are available only for hepatitis A and B prevention.[7] The age wise seroprevalence of acute viral hepatitis still remains debatable in developing and developed countries and there are few prevalence studies from India. As most of the cases of enterically and parenterally transmitted viral hepatitis are preventable, the burden of the disease could be reduced to a considerable extent, if their seroprevalence is known age wise. Thus this study was undertaken to determine...
age wise seroprevalence of hepatitis viral markers in acute infectious hepatitis cases.

**Methodology**

We conducted this prospective study from January 2008 to December 2008 at Lady Hardinge Medical College, New Delhi. The study arm consisted of 600 patients with clinically suspected acute infectious hepatitis attending the outpatient department of various specialties in Smt. Sucheta Kriplani and Kalawati Saran Hospital, Delhi both attached to Lady Hardinge Medical College.

Inclusion criteria were recent onset of jaundice (<6 months), defined by serum bilirubin level >2.5 mg/dl and/or increase in serum transaminases >5 times the upper limit of normal and fever in absence of chronic liver disease or past history of jaundice.

Chronic liver disease or a history of jaundice of six months duration or alcoholic liver disease/acute fatty liver of hepatitis/cholestasis was taken as exclusion criteria.

200 age and sex matched patients were taken in control arm with no clinical evidence of acute infectious hepatitis. Routine samples received in serology section were analyzed. The blood samples from patients suspected of acute infectious hepatitis were centrifuged and serum was obtained. Sera obtained were frozen at −70°C, till the viral markers were tested. The serum samples taken from subjects (study and control arm) were tested for IgM anti HAV, IgM anti HEV, IgM anti HCV and HBsAg using commercially available enzyme linked immunosorbent assay kits (ELISA; Biokit, Barcelona, Spain).

Statistical analysis was done with SPSS version 10.0 (SPSS Inc., Chicago, Illinois). The means of continuous variables were compared using the Students t-test and categorical variables were compared using the Chi-square test and the Fishers Exact test, as appropriate. p < 0.05 was taken as significant.

**Results**

There were 362 male and 238 female patients in the study arm with male to female ratio of 1.5:1. The control arm (n = 200) comprised of 121 male and 79 female subjects. Both the study and control arm were further divided age wise groups, i.e., 0-10 years, 11-20, 21-30 years, 31-40 years and >40 years. The percentage of male subjects were not different between case and control group (p = 0.125). The mean age was 20.2 and 19.65 years respectively for study and control arms. The mean age of study and control arms was not statistically different (p= 0.46).

The total infective pathology was detected in 128 (21.3%) out of 600 samples suspected of acute infectious hepatitis in the study group while in 17 (8.5%) of the 200 samples included in control arm.

In the study arm, the overall seroprevalence for IgM anti HAV was 8.3% as compared to 2% in the control arm. The difference was statistically significant (p<0.05). The overall seroprevalence of HBsAg was 4% and 2.5% respectively for study and control arm. The overall seroprevalence of IgM anti HCV in the study arm was 5.5% while that in the control arm was 1.5%, the difference was statistically significant (p<0.05). The overall seroprevalence of IgM anti HEV in the study arm was 3.5% as compared to 2.5% in the control arm. The difference was statistically not significant (p>0.05).

Highest seropositivity of anti-HAV IgM was seen in 0-10 years of age group in the study arm (16.8%). HAV seropositivity was 7.2% in 11-20 years and 4.6% for 21-30 years age groups. When compared to controls the anti-HAV IgM seropositivity was significantly higher in the study arm in 0-10 years of age group (p=0.002). There was decreased HAV seroprevalence with increasing age.

On observing age wise seropositivity for HBV it was found that highest seropositivity of HBsAg was in 20-30 years (7.6%) in the study arm. The seropositivity for HBsAg was 4.5% for 11-20 years, 2.8% for 0-10 years and 2.5% for age >40 years. The seropositivity in study and control arm was not different across all the age groups (p > 0.05).

IgM anti HCV seropositivity was highest in 11-20 years in the study arm (9%). This was followed by 21-30 years (6.1%), 0-10 years (5.1%) and >40 years (3.7%). Highest seropositivity to IgM anti HCV was seen in 0-10 years (2.8%) in the control arm. This was followed by 11-20 years (2.7%), the difference in seropositivity in the study and control arm was statistically insignificant in all the age groups (p > 0.05).

Highest seropositivity in the study arm for anti-HEV IgM was seen in the 21-30 years age group (10.7%). This was followed by 31-40 years (2.9%), 11-20 years (2.7%) and 0-10 years (0.9%). In control arm group highest seropositivity of HEV was seen in the 31-40 years (4.5%) age group. This was followed by 0-10 years (2.8%), 11-20 years (2.7%) and 21-30 years (2.3%). Anti-HEV IgM seropositivity was significantly higher in the study arm in 21-30 years of age group then that of controls (10.7% vs. 2.3%; p=0.042).
Table 1: Age and Sex Distribution in Study and Control Groups

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Study Group (n=600)</th>
<th>Control Group (n=200)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male (%)</td>
<td>Female (%)</td>
</tr>
<tr>
<td>0-10 yrs</td>
<td>133 (62.1)</td>
<td>81 (37.8)</td>
</tr>
<tr>
<td>11-20 yrs</td>
<td>62 (56.3)</td>
<td>48 (43.6)</td>
</tr>
<tr>
<td>21-30 yrs</td>
<td>87 (66.9)</td>
<td>43 (33)</td>
</tr>
<tr>
<td>31-40 yrs</td>
<td>34 (50.7)</td>
<td>33 (49.2)</td>
</tr>
<tr>
<td>&gt;40 yrs</td>
<td>46 (58.2)</td>
<td>33 (41.7)</td>
</tr>
<tr>
<td>Total</td>
<td>362 (60.3)</td>
<td>238 (39.6)</td>
</tr>
</tbody>
</table>

M:F 1.5:1

Table 2: Age wise Seropositivity of HAV in Study and Control Group

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Study Group (n=600)</th>
<th>Control Group (n=200)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sample tested</td>
<td>Sample positive (%)</td>
<td>Sample tested</td>
</tr>
<tr>
<td>0-10 yrs</td>
<td>214</td>
<td>36 (16.8)</td>
<td>71</td>
</tr>
<tr>
<td>11-20 yrs</td>
<td>110</td>
<td>8 (7.2)</td>
<td>36</td>
</tr>
<tr>
<td>21-30 yrs</td>
<td>130</td>
<td>6 (4.6)</td>
<td>43</td>
</tr>
<tr>
<td>31-40 yrs</td>
<td>67</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td>&gt;40 yrs</td>
<td>71</td>
<td>0</td>
<td>28</td>
</tr>
<tr>
<td>Total</td>
<td>600</td>
<td>50 (8.3)</td>
<td>200</td>
</tr>
</tbody>
</table>

* p value < 0.05 = significant; p value > 0.05 = not significant

Table 3: Age wise Seropositivity of HBV in Study and Control Group

<table>
<thead>
<tr>
<th>Age group</th>
<th>Study Group (n=600)</th>
<th>Control Group (n=200)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sample tested</td>
<td>Sample positive (%)</td>
<td>Sample tested</td>
</tr>
<tr>
<td>0-10 yrs</td>
<td>214</td>
<td>6 (2.8)</td>
<td>71</td>
</tr>
<tr>
<td>11-20 yrs</td>
<td>110</td>
<td>5 (4.5)</td>
<td>36</td>
</tr>
<tr>
<td>21-30 yrs</td>
<td>130</td>
<td>10 (7.6)</td>
<td>43</td>
</tr>
<tr>
<td>31-40 yrs</td>
<td>67</td>
<td>1 (1.4)</td>
<td>22</td>
</tr>
<tr>
<td>&gt;40 yrs</td>
<td>79</td>
<td>2 (2.5)</td>
<td>28</td>
</tr>
<tr>
<td>Total</td>
<td>600</td>
<td>24 (4)</td>
<td>200</td>
</tr>
</tbody>
</table>

* p value < 0.05 = significant; p value > 0.05 = not significant

Table 4: Age wise Seropositivity of HCV in Study and Control Group

<table>
<thead>
<tr>
<th>Age group</th>
<th>Study Group (n=600)</th>
<th>Control Group (n=200)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sample tested</td>
<td>Sample positive (%)</td>
<td>Sample tested</td>
</tr>
<tr>
<td>0-10 yrs</td>
<td>214</td>
<td>11 (5.1)</td>
<td>71</td>
</tr>
<tr>
<td>11-20 yrs</td>
<td>110</td>
<td>10 (9.0)</td>
<td>36</td>
</tr>
<tr>
<td>21-30 yrs</td>
<td>130</td>
<td>8 (6.1)</td>
<td>43</td>
</tr>
<tr>
<td>31-40 yrs</td>
<td>67</td>
<td>1 (1.4)</td>
<td>22</td>
</tr>
<tr>
<td>&gt;40 yrs</td>
<td>79</td>
<td>3 (3.7)</td>
<td>28</td>
</tr>
<tr>
<td>Total</td>
<td>600</td>
<td>33 (5.5)</td>
<td>200</td>
</tr>
</tbody>
</table>

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Acute viral hepatitis is a global public health concern associated with substantial mortality and morbidity. Divergent opinions exist over the seroprevalence rates of different viral markers in different age and sex groups.\[6-13\]

On studying the trends of HAV infection in different age groups, the seropositivity of HAV decreased with age and none of the cases were positive in >30 years of age groups. HAV was implicated to be the commonest cause of enterically transmitted acute viral hepatitis in children in our study.

Similar trends in age wise seropositivity of Hepatitis A virus were seen by Poddar et al (Chandigarh) who reviewed 197 children in < 14 years of age group with acute viral hepatitis and found the prevalence of IgM anti HAV as 64.5%.\[13\] Kaur et al (New Delhi) analyzed age wise seropositivity of hepatitis A virus and found that percentage positivity of IgM anti HAV varied between children (3.1%) and adults (1.7%).\[11\]

Such trends of high incidence of HAV in <10 yrs is attributed to the fact that hepatitis A is predominantly an infection of childhood. HAV infection is spread predominantly by direct person to person contact by the feco-oral route or by the ingestion of contaminated food and water. It is highly contagious with a secondary attack rate of 15% to 20% and spreads rapidly between individuals in prolonged close contact, in schools, institutions and children camps.\[14\] The high prevalence rates in children is attributed to their susceptibility to infection due to poor hygiene, over crowding and poor sanitary conditions, when there is abundant shedding of HAV virus in stools. In a developing country like India, where HAV is highly endemic, infection is common in the childhood.\[15\]

In the control arm, highest seropositivity was 5.5% seen in 11-20 years age group. This was followed by 0-10 year’s age group with seropositivity rates of 2.8%. Epidemiological studies from Delhi found that the peak age of HAV seroprevalence is shifting from first decade of life to second and third decade.\[16,17\]

Mathur et al (ICMR, New Delhi) proposed that with the improvement in socio-economic conditions and its consequences, early childhood exposure to the virus has been delayed and hence there has been a gradual shift in the age of acquiring infection from early childhood to adulthood in different parts of the world.\[17\] Concomitantly there was an increase in symptomatic cases and in severity including liver failure.

HAV infection is a vaccine preventable disease but in India, vaccination against HAV is still not included in the universal immunization program. Hence, it is emphasized that vaccination strategies for HAV should be strengthened.

### Trends of Hepatitis B Virus in different age groups:

HBsAg seropositivity was highest in 21-30 years of age group in the study arm (7.6%). This was followed by seropositivity rate of 4.5% in 11-20 years of age group (%), 2.8% in 0-10 years, 2.5% in >40 years and 1.4% for 31-40 years.

Kurien T et al (Chennai) studied the age specific prevalence of hepatitis B infection, and recorded highest prevalence (32.7%) among clinical cases of jaundice in 15-20 yrs age groups.\[16\] Poor injection practices and high risk sexual behavior were found to be risk factors for transmission in the community. Jais et al (New Delhi) analyzed 2493 serum samples of clinical cases of jaundice to determine age wise seroprevalence of Hepatitis B infection. The overall seropositivity was 10.15% and HBsAg prevalence was highest in patients of 21-30 years age group (14.35%).\[19\] Kaur et al (New Delhi) reviewed 306 patients with acute viral hepatitis and found a large proportion of adults as HBsAg positive (18.1%) as compared to children.\[11\]

It was seen in our study that seropositivity of hepatitis B increased gradually with age. This was probably due to cumulative increase in the risk of exposure with increasing age. The high prevalence rates of HBsAg in 21-30 years group is due to increased sexual activity, promiscuity and intravenous drug abuse among the young adults. Blood transfusions, tattooing could be other contributory factors. HBV is transmitted through percutaneous and parenteral contact with the infected blood, body fluids etc.\[19\]

On the other hand, some authors have demonstrated high prevalence rates of HBsAg in <10

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**Table 5: Age wise Seropositivity of HEV in Study and Control Group**

<table>
<thead>
<tr>
<th>Age group</th>
<th>Study Group (n=600)</th>
<th>Control Group (n=200)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sample tested</td>
<td>Sample positive (%)</td>
<td>Sample tested</td>
</tr>
<tr>
<td>0-10 yrs</td>
<td>214</td>
<td>2 (0.9)</td>
<td>71</td>
</tr>
<tr>
<td>11-20 yrs</td>
<td>110</td>
<td>3 (2.7)</td>
<td>36</td>
</tr>
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<td>21-30 yrs</td>
<td>130</td>
<td>14 (10.7)</td>
<td>43</td>
</tr>
<tr>
<td>31-40 yrs</td>
<td>67</td>
<td>2 (2.9)</td>
<td>22</td>
</tr>
<tr>
<td>&gt; 40 yrs</td>
<td>79</td>
<td>0</td>
<td>28</td>
</tr>
<tr>
<td>Total</td>
<td>600</td>
<td>21 (3.5)</td>
<td>200</td>
</tr>
</tbody>
</table>

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**Discussion**

Acute viral hepatitis is a global public health concern associated with substantial mortality and morbidity. Divergent opinions exist over the seroprevalence rates of different viral markers in different age and sex groups.\[6-13\]

On studying the trends of HAV infection in different age groups, the seropositivity of HAV decreased with age and none of the cases were positive in >30 years of age groups. HAV was implicated to be the commonest cause of enterically transmitted acute viral hepatitis in children in our study.

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On the other hand, some authors have demonstrated high prevalence rates of HBsAg in <10
years of age. Various studies suggested that exposure to virus occurs early in age, perhaps even before the first year of life and vertical transmission from infected mothers may play a major role in viral transmission. Thus the high prevalence rate of HBsAg in <10 yrs of age group could be due to high perinatal transmission rates of HBV. Many studies have reported increasing prevalence of HBsAg in pregnant women ranging from 3.74%-10%. The rising prevalence of HBV in pregnant women is responsible for high perinatal transmission rates. In our study, the seroprevalence rate of HBV in <10 years was 2.8% both in the study and control group.

**HCV trends in various age groups:** HCV seropositivity was highest in 11-20 years of age group (9%). Declining seropositivity rates were observed for 21-30 years (6.1%), 0-10 years (5.1%) and >40 years (3.5%). Various studies have demonstrated high seroprevalence of IgM anti HCV among the adult population. Bhattacharya et al (Puducherry) found seroprevalence rate of 8.5% in 661 patients with acute viral hepatitis within 20-39 years of age group. Devi et al, (Manipur) reviewed 30 patients of acute hepatitis and found highest seropositivity in 30-40 years age group (44.6%). Prevalence rate are higher in young adults between 15-40 years due to continuing risk of exposure. HCV is one of the commonest causes of parenteral and sporadic non A non B hepatitis. HCV causes highest proportion of transfusion associated hepatitis. Certain populations groups like injecting drug users, recipients of unscreened blood and blood products, patients in hemodialysis centers, organ transplant patients and individuals with increased promiscuity tend to have increased probability of acquiring HCV.

Contaminated and inadequately sterilized syringes and needles are responsible for many outbreaks. Adults are more at risk of exposure due to unsafe procedures. Moreover, tattooing procedures undertaken by young adults is an added risk factor.

The highest seropositivity in the control group was 2.8% seen in 0-10 year’s age group. This was followed by 2.7% seropositivity among 11-20 years of age group. The differences in trends of HCV infection could be attributed to largest number of patients from these age groups. Thalassemia and hemoglobinopathy screening from pediatric department at our hospital contributed to a large share of samples. Unsafe injecting practices are a major risk factor for transmission of HCV. Vertical transmission is another mode of HCV transmission. Stringent screening of blood products introduced during the early 1990’s had minimized this mode of HCV acquisition leaving vertical transmission as the predominant mode of infection in children.

**Trends of Hepatitis E virus in different age groups:** Highest seropositivity of IgM anti HEV among the study arm was seen in 21-30 years of age group (10.7%). This was followed by 31-40 years (2.9%), 11-20 years (2.7%) and 0-10 years (0.9%). In the control arm, the maximum seropositivity was seen in 31-40 years (4.5%) followed by 0-10 years (2.8%), 11-20 years (2.7%) and 21-30 years (2.3%).

Misra et al (Karnataka) studied 569 hospitalized patients suspected of acute viral hepatitis and recorded high seroprevalence rates in adults than in adolescents and children i.e. 20.5% in adults as compared to 12% in children. Male preponderance was noted in the seropositive cases. The reason for higher seropositivity rates of HEV with increased age could be due to the fact that symptomatic HEV infection is commoner in 15-40 years age group and symptoms are less often seen in children. HEV remains a frequent childhood infection with most of infections being asymptomatic in children under 9 years of age in endemic hepatitis. Low standards of sanitation promote the transmission of the virus by feco-oral route.

Similar trends were recorded by Tandon (2001) et al who showed that HEV was more common in adults as compared with children.

Singh N et al (Rajasthan) reviewed 148 patients with acute viral hepatitis and recorded high seropositivity in adults (18.75%) as compared to children (8.33%). Kaur et al (New Delhi) reviewed 306 patients with acute viral hepatitis and found that HEV was more often seen in adults of 21-30 years (32.2%) with low seropositivity in children.

Hepatitis E is usually a self limiting disease with low rate of fulminant hepatic failure. HEV has a particularly fulminant course in pregnancy. Pregnant women particularly those in second and third trimesters are more frequently affected. In addition, among pregnant women, especially those infected in the third trimester, the disease is more severe with high mortality rates. Vertical transmission of HEV infections from mother to infant is also known. However out of 15 pregnant women in study none was positive for HEV.

**Conclusions**

Both HAV and HEV are important causes of enterically transmitted viral hepatitis. Thus, it is recommended that measures for public awareness regarding the spread of HAV and HEV, better sanitation facilities, safe drinking water and proper sewage treatment should be undertaken to limit their spread. HAV is the major cause of acute viral hepatitis in childhood. Vaccine for HAV is available but still not included universally in all immunization programs. Hence, it is recommended that vaccination programs should be intensified for HAV.

Both HBV and HCV are important causes of parenterally transmitted viral hepatitis. HBV is the major cause of acute viral hepatitis in adults. Therefore, public awareness regarding safe injection practices and safe sex practices should be undertaken to limits its
spread. Vaccination programs should be strengthened with inclusion of HBV vaccine in all universal immunization programs.

HCV is a common cause of acute hepatitis in young adults. Unsafe injection practices, tattooing and transfusion of unscreened blood are major reasons of transmission of HCV in this age. HCV infections remain under reported making actual disease burden much higher. As till date there is no vaccine for transmission of HCV in this age. HCV infections with inclusion of HBV vaccine in all universal immunization programs. Vaccination programs should be strengthened with inclusion of HBV vaccine in all universal immunization programs.

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