Study of Hepatitis E virus IgM and IgG Antibodies- Hospital Based Approach

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ABSTRACT
Background: Hepatitis E is an important public-health concern as a major cause of enterically transmitted hepatitis worldwide, spread primarily by faecal contaminated drinking water and is responsible for over 50% of cases of acute viral hepatitis in endemic countries.

Aims & objectives:
2. To assess Prevalence of Hepatitis E in various Socio-economic strata.
3. To determine various age and sex specific prevalence of Hepatitis E.

Material & Methods: Total 200 blood samples of patient suspected of acute viral hepatitis were collected and tested for anti HEV IgM and IgG antibody by ELISA method.

Observation and Result: Out of 200 patients, 49 patients (39%) showed anti HEV IgM antibodies, while 52 patients (41%) were positive for IgG antibodies. 25 patients (20%) were positive for both IgM and IgG antibodies. The anti HEV IgM antibodies were more in the 16-30 years (57.20%), while anti HEV IgG antibodies were present in equal number (38.5%) in the 16-30 and 31-45 years. Hepatitis E virus IgG and IgM antibodies positivity rate was more common in Males compared to females.

Conclusion:
1. Incidence of HEV infection is maximum in young adults (15-30 years) 42% than in paediatric and old age groups.
2. Males (62%) are more seen to be infected with HEV than females (38%).

INTRODUCTION
Hepatitis E is an enterically transmitted self-limiting infection that is transmitted by the oral-faecal route primarily due to an unhygienic lifestyle, mainly consumption of contaminated food and water. This form of non-A, non-B hepatitis came to be known as enterically transmitted non-A non-B hepatitis or epidemic non-A non-B hepatitis. [1,2] Hepatitis E virus (HEV) is an RNA virus.[3] Its genome consists of a single-stranded, positive-sense RNA of approximately 7.2 kb size.[4] Morphologically, HEV shows resemblance to Norwalk virus but its sequence most closely resembles to that of Rubella virus. The incubation period of HEV ranges from 3 to 9 weeks. HEV is present in the blood, bile secretions and faces of the patients.[5] The disease primarily affects young adults between the ages of 15 and 40 and reportedly has a mortality rate of up to 25% in pregnant women.[6] Symptomatic HEV infection is common in young adults whereas it is asymptomatic in children. The clinical presentation of hepatitis E is comparable to hepatitis A as there is very close similarity between both these types of hepatitis.[7] In Asia, HEV infection is more commonly seen in adults and children, irrespective of the sex, and the monsoon season acts as one of the most important risk factor in the spread of the disease.[8] In Pakistan, HEV infection remains highly endemic, mainly affecting the adult population.[9] A number of mini epidemics have been reported in 1995 that were all considered to be due to faecal-contaminated water supply.[10] This hospital-based study was carried out in referred patients with no serological evidence of hepatitis B and C infection. The immunoglobulin (Ig) M and G (IgG) against HEV respectively were used as markers of the acute and the convalescent phase of HEV infection.[11]

MATERIALS AND METHODS:
The study was conducted on 200 patients admitted in tertiary care hospital, Ahmedabad. These patients were presented with following signs and symptoms.

Criteria for selection of patient:
(A) Clinical:
Fever, mild chills, anorexia, abdominal pain, nausea, Vomit-
ing, Malaise diarrhoea, Yellow sclera, yellow discolouration of urine, Myalgia, Pruritus, Weight loss, Headache, Back pain, Ar-thralgia, palmer erythema, spider naive.

(B) Biochemical:
Serum Alanine Aminotransferase (ALT or SGPT) ->35IU/ML
Serum Aspartate Aminotransferase (AST or SGOT) ->35IU/ML
Serum bilirubin: -> 1.2 mg/dl
HBs Ag: Non Reactive
HCV: Non Reactive
• No previous history of acute or chronic liver disease;
• Exclusion of other common causes of liver injury, including medications, alcohol, and hepatotoxins; congestive heart failure, metastatic cancer, and other infections.

Collection of Sample:
3 to 5 ml of blood is collected by vein puncture with disposable needle & syringe from each patient. Each blood sample is centrifuged at a speed of 3000 rpm for 20 minutes. All the serum samples were stored at -20 C until needed for analysis. The presence of IgM and IgG anti-HEV was determined by using commercially available enzyme immunosorbent assay (ELISA) kits according to manufacturer’s instructions.

Test for IgM:
ELISA for HEV IgM antibody detection was done by using DIA. PRO Diagnostic kit. The presence of IgM anti-HEV was determined using ELISA using the recombinant ORF2 and ORF3 antigens according to the manufacturer’s instructions.

Test for IgG:
ELISA for HEV IgG antibody detection was done by using MP Diagnostics kit.

OBSERVATION AND RESULT:
Two hundred patients admitted in tertiary care hospital that presented with symptoms of acute viral hepatitis and satisfied the clinical, biochemical criteria for participation were included in this study. Presence of IgM and IgG antibodies were evaluated among these patients. The distribution of cases in relation to
age, sex, socio-economic condition, clinical presentation and presence of IgM & IgG antibodies were as follows.

Out of 200 maximum patients are between 16-30 years of age group (42%) followed by 31-60 years (30%). Majority of the patients in this study were from lower socio economical class (58%).

Most of the patients were presented with fever, jaundice, anorexia, pale colored urine (85-95%), malaise, abdominal pain, hepatomegaly, nausea, vomiting, diarrhoea, (60-80%). many patients also had arthralgia, itching and rash.

Liver enzymatic level of patients with Ig M positive patients showed SGPT value five times more than that of normal value.

### Table 1: Distribution of patients according to Anti HEV IgM & IgG antibodies

<table>
<thead>
<tr>
<th>Antibodies</th>
<th>No of positive patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM</td>
<td>49</td>
<td>39%</td>
</tr>
<tr>
<td>IgG</td>
<td>52</td>
<td>41%</td>
</tr>
<tr>
<td>BOTH</td>
<td>25</td>
<td>20%</td>
</tr>
</tbody>
</table>

Out of 200 patients, 49 patients (39%) showed anti HEV IgM antibodies, while 52 Patients (41%) were positive for IgG antibodies. 25 patients (20%) were positive for both IgM and IgG antibodies.

Hepatitis E virus IgG and IgM antibodies positivity rate was more common in Males compare to females.

### Table 2: Age wise Distribution of Patients positive for Anti HEV IgM & IgG antibodies.

<table>
<thead>
<tr>
<th>Age groups (years)</th>
<th>Anti HEV IgM positive</th>
<th>Anti HEV IgG positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-15</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>16-30</td>
<td>28</td>
<td>20</td>
</tr>
<tr>
<td>31-45</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>46-60</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>&gt;60</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

The anti HEV IgM antibodies were more in the 16-30 years (57.20%) and 31-45 years (20.4%) less number of HEV IgM antibodies seen in the 0-15 years (6.1%) and 46-60 years (16.3%). while HEV IgG antibodies were present in equal number (38.5%) in the 16-30 and 31-45 years.

### Discussion

Present study was carried out on 200 patients came to the tertiary care hospital, Ahmedabad with clinical presentation that resembles to that of acute viral hepatitis with high SGPT, SGOT and raised bilirubin. All patients were negative for HBsAg, HCV. IgM Anti HEV and IgG Anti HEV was performed in all two hundred patients.

Out of 200 patients, 49 (39%) patients were positive for IgM antibodies, 52 (41%) patients were positive for IgG antibodies. 25 (20%) patients were positive for IgM & IgG antibodies. Present study is compared with the studies.

### Table 3: Comparison of presence of Anti HEV IgM & IgG antibodies between Present study and Ramesh Roop Rai et al

<table>
<thead>
<tr>
<th>Age group in years</th>
<th>Present study</th>
<th>Ramesh Roop Rai et al</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>0-15</td>
<td>11</td>
<td>08</td>
</tr>
<tr>
<td>16-30</td>
<td>51</td>
<td>33</td>
</tr>
<tr>
<td>31-45</td>
<td>40</td>
<td>21</td>
</tr>
<tr>
<td>46-60</td>
<td>19</td>
<td>10</td>
</tr>
</tbody>
</table>

The present study group can be compared with the study conducted in western India by Ramesh Roop Rai in 2008. Most of the patients are between 16-30 years (42%, 58.9%) age group followed by 31-45 years (30%, 18%).

Adults in the age-group of 15-30 years being predominantly affected. Children below 10 years were spared. This could be because anicteric hepatitis or subclinical infection is common in children less than 9 years of age in endemic hepatitis. An alternative explanation could be that HEV is maintained in the community as a sporadic infection; thus, HEV is acquired early in life, making infants and children immune to another attack.

### Table 4: Comparison of presence of Anti HEV IgM & IgG antibodies in present study and Ramesh Roop Rai et al

<table>
<thead>
<tr>
<th>Study</th>
<th>Anti HEV IgM</th>
<th>Present Study</th>
<th>Ramesh Roop Rai et al</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM</td>
<td>39%</td>
<td>71.4%</td>
<td>69.09%</td>
</tr>
<tr>
<td>IgG</td>
<td>41%</td>
<td>77.1%</td>
<td>76.26%</td>
</tr>
<tr>
<td>Both</td>
<td>20%</td>
<td>48.5%</td>
<td>25.45%</td>
</tr>
</tbody>
</table>

The magnitudes and temporal relationships of the IgM and IgG anti-HEV responses can be compared with study by Ramesh roop rai et al conducted in western India. As both shows percentage of IgG anti HEV antibodies more than Ig M anti HEV antibodies suggest endemcity and subclinical nature of HEV infection. Zhang J et al study was conducted in china which also shows high prevalence and endemicity of HEV infection. Anti-IgM antibody positivity indicates that these sporadic cases represent recent infection with HEV. It is possible that 20% of IgM and IgG cases represent the transition to the anti-HEV IgG antibodies during the course of infection. However, the lower number cases with anti-HEV IgM antibodies among the acute phase hepatitis patients could be due to a rapid decrease in IgM levels.

### Summary and Conclusion

- In the present study, 200 cases of acute viral hepatitis were studied for HEV infection.
- Incidence of HEV infection is maximum in young adults (15-30 years) 42% than in paediatric and old age groups.
- Males (62%) are more seen to be infected with HEV than females (38%).
- Government hospitals like ours, catering mostly to people who are economically downtrodden, and belonged lower socio economical class showed HEV infection (58%) maximally than middle and upper class. These patients belongs to the overcrowded area, which reflects the poor sanitation conditions and low standard of lifestyle-all these things contributes to the transmission of infection.
- Most of the patients were presented with fever, jaundice, anorexia, pale coloured urine (85-95%), malaise, abdominal pain, hepatomegaly, nausea, vomiting, diarrhoea, (60-80%). many patients also had arthralgia, itching and rash.
- Liver enzymatic level of patients with IgM positive patients showed SGPT value five times more than that of normal value.
- Out of 200 patients, 49 patients (39%) showed anti HEV IgM antibodies, while 52 patients (41%) were positive for IgG antibodies. 25 patients (20%) were positive for both IgM and IgG antibodies.
- IgM anti-HEV is a reliable and sensitive marker for diagnosis of recent HEV infection, but specimens should be collected within 4 weeks after disease onset to avoid false-negative results.
REFERENCE