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ORIGINAL ARTICLE

HEV prevalence, risk factors and outcomes in a tertiary care hospital of Uttar Pradesh: a retrospective observational study

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ABSTRACT

Background and Objectives: Acute hepatitis is characterized by inflammation of the liver parenchyma or hepatocellular injury, leading to impaired liver function. Hepatitis E virus (HEV) is a major cause of acute viral hepatitis in Asia, including India. This study aimed to investigate the various etiologies of viral hepatitis and analyse the demographic profile and clinical outcomes of HEV infection.

Materials and Methods: This retrospective observational study was conducted at the VRDL, Department of Microbiology, JNMCH, Aligarh, and included 1,168 patients presenting with deranged liver function tests and clinical signs of hepatitis between August 2022 and March 2024. Serum samples were tested for HAV (IgM), HBV (HBsAg), HCV (IgM and IgG), and HEV (IgM) using ELISA (Dia Pro, Italy). HEV IgM-positive patients were followed up using a predesigned proforma to assess clinical outcomes and their association with demographic factors.

Results: Out of 1,168 samples screened for viral hepatitis markers, 753 (64%) tested positive for Hepatitis A, B, C, or E. HAV was the most common (554 cases, 73.5%), followed by HEV (125 cases, 16.6%). These HEV IgM positive samples were further subjected to RT-PCR, and 34 were positive and 91 negative among them. Most HEV-positive cases were aged 16-30 years, with a mean age of 19.3 ± 12.1 years. A history of consuming unfiltered water was reported in 78% of HEV cases. The highest number of HEV cases were from Jeevangarh (21.6%) and Jamalpur (14.4%), followed by Dodhpur (8.8%) and Nagla Mohalla (8%). Fever (96%) was the most common symptom, followed by jaundice (86%) and abdominal pain (84%). Key risk factors included residing in rural or semi-urban areas, use of public toilets, and overcrowding. Among the 125 HEV IgM-positive patients, only two fatalities were reported; the remaining recovered within 3 to 6 months.

Conclusion: HEV is a major cause of acute hepatitis in our region, with sporadic cases year-round and outbreaks peaking during the monsoon and post-monsoon seasons. Most cases originated from areas with poor sanitation and hygiene.

Keywords: Hepatitis E; Fever; Liver function test; Jaundice; Drinking water

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INTRODUCTION

Viruses are the most common infectious cause of acute hepatitis. Among viruses, hepatotropic viruses like hepatitis A, B, C, D and E are the most important causes. Hepatitis E virus (HEV) is a RNA virus, which belongs to the Hepeviridae family and the genus hepevirus. It is naked, non-segmented, positive sense and single-stranded. The genome of HEV has three open reading frames (1). It has four genotypes 1-4. Genotype 1 and 2 are more common and are involved in human infection whereas genotypes 3 and 4 are zoonotic. HEV genotype 1 and 2 are transmitted via the fecal-oral route, whereas HEV genotype 3 and 4 spread by consumption of pork and wild boar meat that has not been cooked properly (1).

HEV infection is most commonly seen in the age group of 15 to 45. After a patient gets infected with HEV infection, clinical manifestation starts after a period of 2 to 8 weeks, which is considered as the incubation period of HEV. The patient usually presents with wide spectrum of clinical manifestations which are often self-limiting. The clinical manifestation can be subclinical/acute hepatitis to fulminant hepatitis. However, in pregnant women and immunocompromised individuals, HEV has severe clinical outcomes. A patient can present in either the early phase (pre-icteric phase) or late phase (icteric phase). Symptoms in pre-icteric or first phase last for 10 days and present with influenza like symptoms (fever, myalgia, thrombocytopenia), nausea, vomiting, abdominal discomfort and tenderness. Symptoms in icteric or first phase persist for 15-40 days and present with icterus and dark urine. Virus clearance happens in this phase. Laboratory investigations reveal raised liver enzymes and tests which detect antibody production are also positive during this phase (2). Every year there are an estimated 20 million HEV infections worldwide, leading to an estimated 3.3 million symptomatic cases of hepatitis E. WHO estimates that hepatitis E caused approximately 44 000 deaths in 2015, accounting for 3.3% of the mortality due to viral hepatitis (3).

There are two different clinical and epidemiological patterns observed in HEV infection; infection in high endemicity area and infection in low endemicity area. There are differences in disease frequency, virus genotype, route of transmission, affected population and disease characteristics (4). In areas of high endemicity there are large outbreaks and the responsible genotypes are 1 and 2. Young people are mostly affected with male predominance. Transmission is through fecal-oral route (5). In areas of low endemicity a small number of cases occur with sporadic acute hepatitis, with genotype 3 being mostly involved with few cases of genotype 4. Elderly population with other co-morbidities are usually affected. Animals act as reservoir and consumption of undercooked meat and close contact with animals play a role in transmission of disease (6). Developed countries like Western Europe, United States, Canada, Australia, New Zealand, Japan, Taiwan and Hong-Kong fall in low endemic area for HEV infection (7). High endemicity area are usually developing countries like the Indian subcontinent, the Middle East, South and Central Asia and Northern and Western Africa (6).

Various risk factors in the development of HEV infection include: people living in areas where an outbreak has occurred, overcrowding, refugee camps, natural disasters, travelling to HEV endemic areas, individuals with chronic liver disease or immunocompromised state, and animal exposure (8). HEV infection is diagnosed using patient's serum sample or feces, which is collected between 1st to 4th weeks after onset of illness. The sample is tested for anti HEV- IgM and anti HEV-IgG antibody as acute and chronic phase markers, respectively. Various immunological and molecular tests are available for HEV diagnosis. Immunological methods include: Enzyme Immuno Assay (EIA), Immunofluorescence Microscopy (IFE), Western Blot. Molecular methods include: Immune Electron microscopy, Viral Isolation, and Nucleic acid amplification test (2).

MATERIALS AND METHODS

A Retrospective observational study was carried out in the Department of Microbiology, J.N. Medical College Hospital, AMU, Aligarh on patients presenting with signs and symptoms of hepatitis during the period August 2022 to March 2024. As the prevalence of HEV was found to be 9% from a study in India (9), sample size was calculated (10), and considered to be 125.

Study group. This study was performed on specimens received from patients presenting to different departments of JNMCH, ALIGARH with signs and symptoms suggestive of acute hepatitis. 1168 were

enrolled in the study. Deranged liver function test (LFT) was defined as any biochemical parameter of LFT that is 1.5 times above the reference range.

Inclusion criteria. 1168 patients were enrolled in the study with a history of fever and jaundice, along with deranged LFT and patients giving consent.

Exclusion criteria. Patients with liver failure, obstructive Jaundice, haemolytic jaundice, autoimmune hepatitis, non- alcoholic fatty liver disease, history of alcohol intake and immunosuppressant drugs, and patients not giving consent. The cases were screened for HAV, HBV, HCV, HDV, and HEV using ELISA kits. All patients underwent complete physical examination and detailed clinical history was elicited from them using a predesigned proforma. Patients were followed up for 6 months to see the outcome of the disease and its association with different demographic characters. The study was approved by the institutional ethical committee of J. N Medical College, AMU, Aligarh.

Immunoassays. Blood was withdrawn by venipuncture. Blood was allowed to clot for 20 minutes and serum was separated by centrifugation at 12000 rpm for 5 minutes then it was stored in Eppendorf tubes at -20 degree Celsius, preferably at -80 degree Celsius, until tests were performed. A total of 1168 samples were screened for viral hepatitis markers. The screening was done using enzyme-linked immunosorbent assay (ELISA), HAV (IgM) and HEV (IgM) by ELISA (DIA.PRO kit, Italy), HBV (HBsAg) and HCV (IgM and IgG) by ELISA (Tulip diagnostics kit). All the tests were performed according to manufacturer instruction. Tests results are interpreted as ratio of the sample OD450nm/630nm and the cut-off value (S/Co) accordingly <1.0 negative, 1.0 to 1.2 equivocal and >1.2 positive. IgM positive samples for HEV were further studied.

Statistical analysis. Statistical analysis was done using the SPSS software version 20. p-value of <0.05 was considered as significant.

RESULTS

Out of a total of 1168 samples screened for viral hepatitis markers, 753 (64%) samples were positive for either Hepatitis A, B, C or E. 415 (36%) samples were negative for all type of Viral Hepatitis. Out of 753 Hepatitis positive samples, 554 (73.5%, with 95% CI of 70.3%, 76.7%) were positive for HAV followed by HEV for which 125 (16.6%, with 95% CI of 13.9%, 19.3%) samples were positive, while 46 (6.1%, with 95% CI of 4.4%, 7.8%) and 28 (3.7%, with 95% CI of 2.4%, 5%) samples were positive for HBV and HCV respectively as shown in Fig. 1. 70% of the cases were treated on OPD basis. Among the HEV positive samples, maximum number of patients were male 73 (58%) followed by females 52 (42%). Male to female ratio was 1.4:1. Out of the 125 cases, majority were in the age group of 16-30 years followed by 0-15 years. The mean age of HEV positive samples was 19.30 + 12.1 years.

HEV in developing countries tend to occur throughout the year with maximum number of cases, seen during the monsoon season as seen in Fig. 2. Maximum number of cases were from the Jeevangarh (21.6%) and Jamalpur (14.40%) areas of Aligarh, followed by Dodhpur (8.8%) and Nagla Mohalla (8%) as seen in Fig. 3.

The source of drinking water also turned out to be a major risk factor for HEV infection. Majority of patients (78%) had history of consuming dirty unfil-

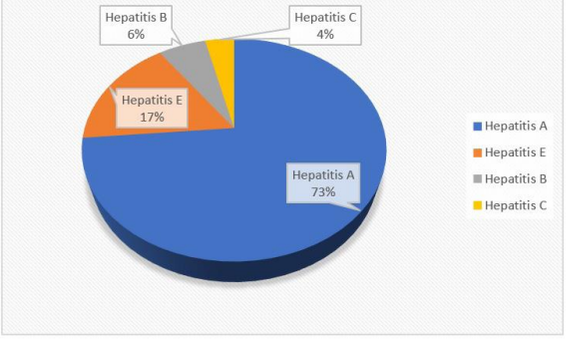

Fig. 1. Etiology of acute viral hepatitis in Aligarh region (n= 753)

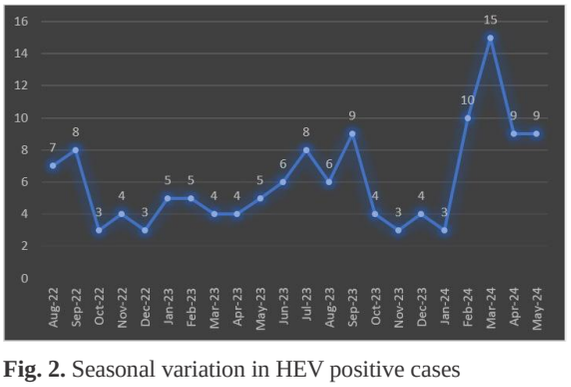

Fig. 2. Seasonal variation in HEV positive cases

Fig. 3. Geographical distribution of HEV positive cases

tered water regularly as compared to 22% patients who drank filtered water. Majority of the patients presented during the 1st (42.4%) week and 4th (26.4%) week of illness. Fever was present in 96% cases and was the most common symptom among cases positive for Hepatitis E, followed by jaundice (86%) and abdominal pain (84%). Among other symptoms, loss of appetite, myalgia and pruritus were also reported in few cases, among which, loss of appetite being most common as shown in Fig. 4.

Among all the risk factors, dwelling in rural and semi-urban areas (78.4%, odds ratio 4) and use of public toilets (73.6%, odds ratio 4) turned out to be major risk factors followed by overcrowding (58.4%, odds ratio 2) as shown in Fig. 5.

Table 1 shows the LFT in HEV positive males and females. The mean ALT, ALP and AST was more for females than males. P-value was 0.049 for AST which is significant. The mean bilirubin for males was more than females.

Out of total 52 females in the study, 9 (17%) females were pregnant who were positive for Hepatitis E on IgM ELISA. Among these 9 females, 6 were primigravida and 3 were multigravida. The mean age of pregnant female was 26yrs and range was 21-32 yrs. 7 females were in their 2nd trimester followed by 2 females in their 3rd trimester. All the HEV positive pregnant females delivered at term except 2 (22.2%). Mean birth weight was 2.8 kg. A total of 4 (44.5%) neonates developed neonatal jaundice but none required hospital admission. Out of 9 neonates, 3 (33%) had low birth weight < 2.5 kg whereas 9 (67%) had birth weight > 2.5 kg. There was no maternal or fetal mortality in our study. Out of 125 patients only 2 patients died. Rest of patients recovered completely within span of 3 to 6 months. Table 2 shows the LFT in HEV positive pregnant and non-pregnant females.

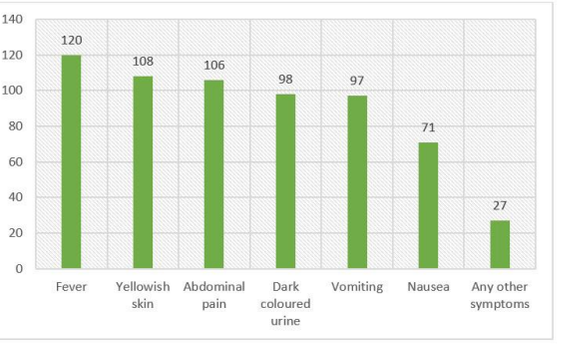

Fig. 4. Clinical symptoms associated with HEV positive patients (n=125)

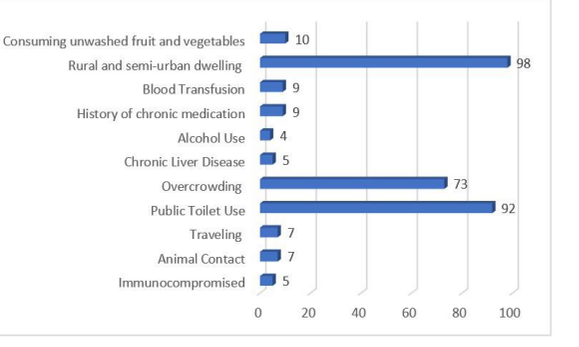

Fig. 5. Risk factors associated with patients positive for HEV (n=125)

Table 1. Comparison of liver function test in HEV positive males and females

Category	Mean AST	Mean ALT	Mean ALP	Mean TBI	Mean DBI
Male	751.97	1055.56	341.59	5.035	3.153
Female	1206.85	1219.76	502.92	4.339	2.578
p-value	0.049	0.455	0.305	0.29	0.31

Table 2. Comparison of liver function test in HEV positive pregnant and non-pregnant females

Category	Mean AST	Mean ALT	Mean ALP	Mean TBI	Mean DBI
Pregnant Female	1302.41	583.21	264.57	5.205	2.844
Non-Pregnant Female	1186.86	1353	552.81	4.158	2.523
p-value	0.393	0.05	0.605	0.691	0.937

DISCUSSION

HEV is a leading cause of acute viral hepatitis worldwide. It is usually self-limiting, even in immunocompromised individuals. The global seroprevalence of HEV is reported to be 1.4% (11). The seroprevalence of HEV in the Southeast Asian region is approximately 21% (12). In our study, the prevalence of HEV was found to be 16.60% with 95% CI of 13.9%, 19.3%. The variation in HEV prevalence can be attributed to differences in cultural practices, socioeconomic conditions, climate, and sanitation standards across regions.

Out of 753 positive samples for viral hepatitis, 37 samples showed co-infection. Among this the highest co-infection was seen with HAV and HEV 31 (4.1%), which could be due to the fact that both have same mode of transmission. A study from north India reported dual infection rate of 5.9% (13). HBV and HEV 4 (0.5%) and least with HCV and HEV 2 (0.25%).

These 125 HEV IgM positive samples were further subjected to RT-PCR, among which 34 were positive and 91 were negative. Single sample for IgM antibody can lead to false negative results as antibodies may not be detectable in very early stage of infection. HEV RNA detection has a higher specificity in diagnosing disease in early infection when virus is in multiplying phase and antibodies are not formed. In immunocompromised patient PCR is important as they may not produce detectable antibodies.

In our cohort, 73 (58%) males and 52 (42%) females were infected with HEV. Other studies from North India have also reported male predominance, with male prevalence rates of 62.3%, 65.5%, and 34.2%, respectively. This male predominance may be due to greater exposure to contaminated food and water outside the home, more frequent use of public toilets, and differences in health-seeking behavior between males and females.

The majority of HEV-positive cases in our study (51%) belonged to the 16-30-year age group. Similarly, a large-scale study by Murhekar et al. (2018) found that the majority of cases occurred in the 20-29 year age group (14). Although HEV infection can occur in children, it often goes undiagnosed due to mild or asymptomatic presentation. In developing countries, due to repeated clinical and subclinical infections, most individuals develop immunity to HEV by the age of 40 (15).

In terms of geographical distribution, the majority of HEV cases in our study came from Jeevangarh (22%), Jamalpur (14.5%), Dodhpur (9%), and Nagla Mohalla (8%)—all regions of Aligarh known for poor sanitation and hygiene conditions. These areas lack access to safe drinking water and have a low socioeconomic status. The presence of roadside food stalls and limited educational awareness further contribute to the fecal-oral transmission of HEV.

The incubation period of HEV ranges from 2 to 6 weeks. Common symptoms include fever, nausea, generalized weakness, abdominal pain, anorexia, pruritus, and vomiting. In our study, fever (96%) was the most commonly reported symptom, followed by jaundice (86%) and abdominal pain (85%). Similar symptom profiles have been reported in previous studies (16).

Among all the risk factors evaluated, dwelling in rural and semi-urban areas (78.4%, odds ratio 4) and use of public toilets (73.6%, odds ratio 4) turned out to be major risk factors followed by overcrowding (58.4%, odds ratio 2). Practices such as open defecation, use of unclean public toilets, dinking untreated contaminated water and inadequate hand hygiene significantly increase the risk of HEV infection.

A higher prevalence of HEV was observed among individuals with no formal education, while those with higher education levels had lower infection rates. Lower educational attainment is often associated with lower socioeconomic status and reduced awareness of hygiene and preventive practices, leading to increased vulnerability to infection.

Rural and semi-urban populations had higher HEV prevalence compared to urban areas, likely due to poor sanitation infrastructure and overcrowding. These findings underscore the need to promote hygienic practices like washing hands with soap and water after using toilets and before preparing meals or eating, particularly among vulnerable groups such as pregnant women and young children.

Measures such as boiling water or using chlorine tablets to deactivate the virus, storage of the treated water in covered and clean containers to avoid hand contact, and cleaning latrines to interrupt fecal-oral route of transmission, help in preventing both HAV and HEV infection as both have same route of transmission.

In our study, pregnant women had higher mean AST and bilirubin (both direct and indirect) levels compared to non-pregnant women. HEV infection during pregnancy is associated with increased risk of fulminant hepatitis and poor outcomes. Viral hepatitis generally causes elevated liver enzymes, and several studies have demonstrated that HEV can re-

sult in severe liver dysfunction and fulminant hepatic failure, especially in pregnancy (17). It is, therefore, important to screen pregnant women with deranged liver function for HEV infection.

Regarding clinical outcomes, of the 125 HEV IgM-positive patients, only two deaths were reported. The remaining patients recovered fully within 3 to 6 months. The two fatalities occurred in children under 10 years of age, both of whom had congenital heart disease, and the deaths were attributed to complications unrelated to HEV infection. Hecolin is the only licensed vaccine available for HEV.

Limitations. A limitation of our study was the inability to collect follow-up serum samples to confirm seroconversion from IgM-positive to negative, which would have allowed for more precise evaluation of disease progression and recovery.

CONCLUSION

HEV turned out to be an important etiological factor in causing acute hepatitis in our region, although HAV was the most common one. Sporadic cases of HEV occur throughout the year but outbreaks are most common during monsoon and post-monsoon season as seen in our study. The areas from where majority of cases presented had poor sanitation and hygiene conditions.

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