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Prevalence of Hepatitis A Virus (HAV) and Hepatitis E Virus (HEV) among Febrile Patients with Gastrointestinal Symptoms: A Prospective Study in A Tertiary Care Hospital

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ABSTRACT

Introduction: The prevalence of enteric hepatotropic viruses, such as hepatitis A virus (HAV) and hepatitis E virus (HEV), is influenced by the level of sanitation in a given area. **Aim:** This study aimed to determine the prevalence of HAV and HEV in febrile patients presenting with symptoms of diarrhoea and persistent vomiting. **Methods:** A prospective study was conducted at a tertiary care hospital, where 328 febrile patients were included. Blood samples were collected and serum was separated and analysed for specific HAV and HEV antibodies using ELISA (Enzyme Linked Immuosorbent Assay). **Results:** Out of 328 patients, tested for HAV IgM 24(7.3%) and HEV IgM 04(1.2%) were tested positive. The higher prevalence was observed in females than in males for both HAV and HEV infection, and the cases were eminent during monsoon season. **Conclusion**: The present study, underscores the importance of the early diagnosis to prevent the outbreaks in communities.

Keywords: *HAV, HEV, ELISA, Enteric Hepatotropic Viruses.*

1. INTRODUCTION

Hepatitis A virus (HAV) (Picarnaviridae) and Hepatitis E virus (HEV) (Hepeviridae) are non-enveloped, positive-sense RNA viruses are associated with contaminated food and water borne infections with the incubation periods of 2 to 4 weeks and 2 to 10 week, respectively [2-4]. Hepatitis E was responsible for 44,000 deaths in 2015, accounting for 3.3% of all viral hepatitis-related fatalities, according to the World Health Organization (WHO), [5]. 10.4% of hepatitis E cases and 7.4% of hepatitis A cases tested positive in India, from 2011 to 2013 [6]. With 20 million cases reported annually, HEV is currently the leading cause of acute hepatitis worldwide resulting in an estimated 50,000 to 70,000 fatalities, with three million cases presenting symptoms [7]. HAV and HEV infections are more prevalent during the monsoon and post-monsoon seasons [8]. HEV is less resistant to environmental conditions such as temperature, compared to HAV [9]. HAV has six genotypes while HEV has four genotypes that infect humans. The distribution of both HAV and HEV genotypes worldwide is irregular and directly associate with the mode of transmission. Symptoms of HAV and HEV infections include fever, nausea, arthralgia, myalgia, and jaundice. While hepatitis A and E are often self-limiting, they can progress to fulminant hepatitis. Both HAV and HEV have the potential to cause severe illness, and in some cases, acute liver failure [10]. HEV infection generally has a more challenging clinical course compared to HAV infection, particularly in pregnant women infected during the second and third trimesters. Therefore, patients with HAV-HEV co-infection, including both children and adults, may experience significant complications and increased mortality due to acute liver failure [11].

Conventional and real-time PCR assays can be used to detect HEV and HAV RNA during acute infections. The initial antibody response is primarily IgM and usually persists for up to 6 months. Serological methods such as rapid immune chromatographic and ELISA tests can detect IgM levels at the onset of symptoms [12]. The present study highlights the prevalence of of HEV and HAV among febrile patients with waterborne infections attending a tertiary care centre.

2. MATERIAL AND METHODS

Study design and inclusion criteria

A prospective study was done to investigate febrile illness cases accompanied by severe diarrhoea and persistent vomiting, in India during the monsoon season. 328 serum samples were analysed of patients suspecting Hepatitis A virus (HAV) and Hepatitis E virus (HEV) infections with a fever, attending a tertiary care center. Individuals of all age groups who exhibited symptoms of febrile illness, such as fever, anorexia, vomiting, diarrhoea and acute clinical illness were included in the study. The clinical and demographic data of the patients were collected for the analysis.

Sample collection and ELISA

Serum samples were collected from the patients exhibiting the above symptoms. Clinical and demographic details of each patient were recorded and tabulated for further analysis. The collected serum samples were analysed to detect IgM antibodies for anti-HAV and anti-HEV using commercially available ELISA kits (RecombiLisa, CTK Biotech, California, USA). The ELISA kits were used according to the manufacturer's instructions. IgM Antibody Detection: The IgM antibodies for anti-HAV and anti-HEV were detected using the ELISA kits. The ELISA procedure is based on the IgM capture technique. Test specimens were incubated in a solid phase coated with monoclonal anti-human IgM antibody. Any unbound specimen was removed by a wash step. The specimens were then incubated with HRP conjugate, followed by another wash step. After the addition of TMB, the presence of the conjugate complex was indicated by a blue colour. The reaction was quenched by adding a stop solution, and the absorbance was measured at 450nm. The cut off values were calculated according to the manufacturer's instructions. Values above the calculated cut off were reported as positive, indicating the presence of IgM antibodies against both anti-HAV and anti-HEV. The ELISA assay readings were collected and analysed using descriptive statistics.

Ethical Considerations

Informed consent was obtained from each participant before sample collection and data recording. Patient confidentiality was maintained throughout the study. All the necessary approvals from the institutional ethics committee were obtained, in compliance with ethical guidelines.

3. RESULTS AND DISCUSSION:

Our study was conducted on a total of 328 serum samples collected during the monsoon season in South India, specifically from August to October 2021. These samples were collected from patients attending a tertiary care hospital. The prevalence of Hepatitis A virus (HAV) infection was found to be 7.3%, with a total of 20 females and 4 male testing positive (Table 1). Among the HAV-positive cases, 16 individuals also tested positive for Dengue IgM antibodies. propitiously, no mortality was reported among the participants. HAV infection was most common among people aged 21 to 30.

Table 1.Sero-prevalence of HEV infection

	HEV Positive	HEV Negative	HAV Positive	HAV Negative
Total Patients (328)	4	324	24	304
Males (184)	0	184	4	180
Females (144)	4	140	20	124

In terms of Hepatitis E virus (HEV) infection, only one female participant tested positive, resulting in a prevalence rate of 1.2%. Patients from all age groups, ranging from 10 to 60 years, were included in the study. The prevalence of HAV infection was 1.2% in males and 6.09% in females. On the other hand, the prevalence of HEV infection among females was 1.2%.

Out of the 328 patients included in the study, 220 resided in rural areas, while 108 were from urban areas. The 24 individuals who tested positive for HAV infection and the female patient with HEV infection were all from rural areas. The principal goal of our research was to evaluate the prevalence of Hepatitis A virus (HAV) and Hepatitis E virus (HEV) infections during the monsoon and post-monsoon seasons in the southern region of India. A high number of HAV and HEV cases were reported at the end of the monsoon season and the start of winter, with a significant increase in HEV cases

at the onset of the rainy season. The results are comparable with Al-Naaime et al. (2012), demonstrating the seasonal variations in the prevalence of HAV and HEV infections [13]. The current study further indicates a 6% prevalence rate for HAV infection, with a higher incidence among females than males (6.09% vs. 1.2%, respectively). The prevalence of HEV infection was low, with only four female participants testing positive. These results are consistent with previous investigations in India, where HAV and HEV infections prevail during the monsoon season, particularly in rural areas [14, 15]. Our study discovered that the age group most impacted by HAV infection was 20-30. This trend has been observed in previous research, where HAV infection is more common among individuals above 20 years[13, 16]. Furthermore, we found that the prevalence of HAV and HEV infections was higher in females than males, although this disagrees with some previous studies where males had higher prevalence rates [17]. Interestingly, we encountered co-infection with Dengue IgM antibodies in 10 of the 24 HAV-positive individuals. Although these patients had an elevated body temperature, no mortality was observed. This finding is pertinent, as co-infection with numerous viruses can complicate diagnosis and treatment, resulting in poorer outcomes[18].

Further studies are required to assess the impact of co-infection on illness severity and clinical consequences, emphasizing the need for investigations to be added in patients with protracted fever, particularly during rainy seasons, to avoid fatal outcomes. The higher prevalence of HAV infection among females in the study is consistent with earlier studies conducted in India [15], attributed to poor sanitation and hygiene practices. Furthermore, there is a need to focus on treating viral infections and prevention and control measures due to the high incidence of HAV infection in the younger generation. The low HEV infection frequency could be due to a small sample size or a specific geographic area. Nevertheless, the variations in routes of transmission and risk factors demonstrated in earlier studies reported in India [14] were consistent. The high positivity among rural regions underlines the need for targeted interventions to prevent and control these ailments. Enhancing sanitation and hygiene practices, extending access to secure drinking water, and encouraging immunization can help decrease HAV and HEV infection incidence in these regions [15].

4. CONCLUSION

The invaluable insights provided by the present prospective study in South India included the epidemiology of these viral infections. A prevalence of 7.3% for HAV and 1.2% for HEV infections was seen among patients who showed up with fever, severe diarrhoea, and persistent vomiting. Infections of HAV were more common than HEV in our study. A higher frequency of HAV infection was noted among females and people aged 20 to 30. Furthermore, co-infection with the Dengue virus was found in a subset of HAV-positive individuals, validating the importance of investigating multiple infections in patients with persistent fever, especially during the monsoon season. The disparity between hygienic practices and access to sanitation facilities in urban and rural settings is revealed by a higher prevalence of HAV and HEV infections in the present study. Our study highlights the need for safe drinking water facilities, improved hygienic practices, and targeted interventions in marginalized communities. Overall, the present study adds to our understanding of the epidemiology of HAV and HEV infections during the monsoon season in South India and may help develop public health strategies and interventions to reduce the burden of viral hepatitis in high-risk populations. Further studies with larger sample sizes and comprehensive clinical data are needed to improve our understanding of these infections and guide practical prevention actions.

5. STATEMENTS AND DECLARATIONS FUNDING

No funding was received for the present project.

6. COMPETING INTERESTS

The authors have no Competing Interests to declare.

7. AUTHOR CONTRIBUTION

Habeeb Ali Baig and Srinath mote contributed to study concept and design. Data collection and tests were performed by Habeeb Ali Baig, Abdelrhman Elhaj, Musa Elhag, Sarah Ali. Data analysis and manuscript was prepared by Sadhineni Srinath Patel and Waseema Sultana. The revision of the manuscript was done by Srinath mote. All the authors read and approved the final manuscript.

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