Detection of Genotype 1a and 1f of Hepatitis E Virus in Patients Treated at Tertiary Care Hospitals in Central India

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Keywords

Hepatitis E virus · Central India · Genotype 1a and 1f

Abstract

Background and Aims: An acute hepatitis, caused by hepatitis E virus (HEV), is a significant global health concern, especially in developing countries. HEV has 1 serotype and 8 genotypes, which are further divided into subtypes. Despite the availability of sporadic cases and outbreak data from India, specific information regarding HEV epidemiological data is lacking from central India. This study was conducted to understand epidemiological and molecular features of HEV in central India. **Methods:** In this 3-year study conducted from July 2012 to June 2015, IgM ELISA was used for the diagnosis of suspected HEV cases. For identifying the genotype, nRT PCR was conducted and the PCR products were sequenced and analysed. Clinical and demographic data were analysed using statistical tools to highlight the trends. **Result:** Out of 1,369 suspected cases, 341 (24.9%) were positive for HEV. The positivity was significantly higher in males (69.2%) and in the age group of >15–45 years (72.5%). The HEV cases peaked during the summer. Subtypes 1a and 1f of genotype 1 were detected in the area during the study period. **Conclusion:** HEV is a major aetiological agent of viral

hepatitis in central India with adults and males at higher risk of infection. Two subtypes of the virus were detected in the region. Continuous serological surveillance and molecular monitoring will help to understand the epidemiology of HEV infection, outbreak mitigation and aid in providing treatment.

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Introduction

Hepatitis E virus (HEV) infection causes a considerable public health burden and is responsible for large outbreaks of acute hepatitis in different parts of the world, particularly in developing countries. It accounts for around 20 million infections and over 3 million acute cases worldwide with an approximate 56,600 deaths per year [1]. India is considered an endemic zone for HEV. Studies on different geographic areas have revealed the prevalence of acute HEV cases in different time periods as ranging from 9.4 to 71% from western, southern, eastern, and northern India [2]. Although sporadic cases are detected throughout the year, the outbreaks and high positivity of HEV generally occur during the monsoon and summer [3, 4].

HEV infection in children goes undiagnosed as the disease is mostly asymptomatic, but is evident in young adults (15–40 years), and in a few cases infection may result in fulminant hepatitis (acute liver failure) and death. In Asian countries, a case fatality rate (CFR) of 1–2% is reported in the general population, whilst a case fatality rate of about 20% is reported in pregnant women, especially during the third trimester [5, 6].

HEV, a member of the family *Hepeviridae* within the genus *Hepevirus* [3], is a non-enveloped, spherical virus of about 27–34 nm in size with positive-sense RNA and a length of 7.2 kb [5, 7–9]. Its RNA genome possesses short untranslated regions at either end and 3 open reading frames (ORF). The ORF1 encodes polyprotein predicted to have enzymatic activities of viral replication, ORF2 is responsible for the synthesis of capsid protein, and ORF3 expresses a small phosphoprotein [3]. The virus has a single serotype and based on nucleotide sequences, it is classified into 8 genotypes which are further classified into subtypes [10]. Genotypes 1 and 2 have exclusively been found to cause infections in humans, whereas genotype 3 and 4 are zoonotic in nature and are more frequently detected in animals such as pigs, boars, and deer [11–13]. Genotypes 5–8 are detected in wild boar and camel, and limited sequences of these genotypes are available [10]. The circulation of genotype 1 has been observed in Africa and Asia; genotype 2 is found in Mexico and in some African countries; genotype 3 is distributed throughout world and genotype 4 has also been detected in Asia [11–13].

The genotype and subtype of an infecting virus are known to play a role in determining the severity of disease. Moreover, the mode of transmission, zoonosis, high-risk age groups, chronicity of infection, and outbreak-causing abilities also vary among the genotypes [14]. Therefore, the molecular characterization of HEV is important. The genotype and subtype of an HEV virus can be determined by studying the regions under ORF1 and ORF2, or based on full genome sequences. The ORF1 contains the most hypervariable region of the HEV genome, thereby it is preferred for genotyping and subtyping [10, 15, 16]. Genotypes 1a and 1c were reported to have been circulating in south, north, and west India [11, 15, 16]. Moreover, the circulation of genotype 4 among pigs was also documented in southern and western Indian states [17–19]. However, information regarding epidemiology and circulating HEV genotypes from central India is lacking. This study was therefore planned to identify these genotypes and estimate the prevalence of HEV in tertiary care hospitals from central India.

Materials and Methods

Samples

This is a routine data-based cross sectional study, conducted for a period of 3 years, from July 2012 to June 2015. The virology laboratory of ICMR-National Institute for Research in Tribal Health is part of ICMR's VRDL project and caters for the needs of viral diagnosis in central India. Treating physicians at the hospitals in the region are encouraged to refer samples to this laboratory for diagnosis. The clinical and demographic details were collected in a predesigned format. Patients with more than one symptom of acute hepatitis, such as jaundice, fever, dark urine, malaise, anorexia, nausea, etc., and who gave consent to participate were enrolled in the study. The sample size was calculated by considering a prevalence of 15% with a 95% CI and 80% power. We arrived at a minimum sample size of 1,260 after adding samples to adjust for the non-response error.

Diagnosis

Blood samples of 2 mL were collected from all the suspected HEV cases. The serum was separated by centrifugation at 4 °C and tested immediately by ELISA for HEV IgM antibodies (MP Diagnostics, Germany) following the manufacturer's protocol. The results were conveyed to those concerned for further treatment. Part of each sample was preserved at –70 °C for further analysis.

Nucleotide Sequencing and Genotyping

To identify the genotype, 98 samples collected during the early phase of infection (<15 days of onset of symptoms) were screened. Furthermore, care was taken that they were evenly distributed over the period of 3 years. These samples were subjected to RNA extraction using a QIAamp viral RNA mini kit (QIAGEN, Hilden, Germany) following the manufacturer's protocol. From the extracted RNA, the partial RdRp region of ORF1 was amplified following the protocol described by Arankalle et al. [16] using SuperScript III with platinum *Taq*HiFi (Invitrogen, Carlsbad, CA, USA). Out of 98, 28 showed satisfactory amplicons. The resulting PCR products (535 bp) were sequenced as described previously [20]. The sequences were submitted to the GenBank.

The resulting sequences were aligned with the 29 reference sequences of major genotypes and sub-genotypes downloaded from the GenBank database using Clustal W software. A phylogenetic tree was constructed with MEGA5.05 software using kimura-2-parameter model with the neighbour-joining algorithm by comparing 29 sequences downloaded from GenBank with 22 GenBank-submitted sequences from this study. The sequence of a novel genotype detected in the rat was used as an out-group. The reliability of the phylogenetic tree was assessed by bootstrap resampling (1,000 pseudoreplicates).

Statistical Analysis

The clinical and demographic details collected in the laboratory request form were entered into MS office Excel 2010 and analysed to understand the clinical and epidemiological features. Statistical analyses were performed using SPSS version 20.0. The non-numerical categorical variables were coded numerically and analysed using χ^2 or as appropriate. Odds ratios (OR) with 95% CI were calculated for each association. *p* values <0.05 were considered statistically significant.

Fig. 1. Percent positivity of HEV infection during the study period (July 2012 to June 2015). Maximum cases were detected during the summer months (March to June) each year.

Table 1. Age and sex distribution of the suspected and confirmed HEV cases

Age, years	Males positive/tested, n (%)	Females positive/tested, n (%)	Total positive/tested, n (%)	OR (95% CI)
0–15	30/191 (15.70)	19/140 (13.57)	49/331 (14.8)	Reference
>15–25	67/186 (36.02)	41/148 (27.70)	108/334 (32.3)	2.75* (1.88–4.02)
>25–45	98/310 (31.61)	37/166 (22.28)	135/476 (28.3)	2.27* (1.58–3.27)
>45–60	32/114 (28.07)	5/47 (10.63)	37/161 (22.9)	1.71 (1.06–2.76)
>60	5/37 (13.51)	1/16 (6.25)	6/53 (11.3)	0.73 (0.29–1.81)
Total ¹	232/838 (27.68)	103/517 (19.92)	335/1,355 (24.7)	

* $p < 0.0001$.

¹ The demographic data of 14 suspected and 6 positive cases was missing in the clinical information form.

Ethical Statement

The study was approved by the institutional ethics committee (No. RMRCT/Ethics committee/2725/2011) and the patient data were kept confidential and anonymous.

Results

A total of 1,369 samples were received from 29 districts of central India (Madhya Pradesh and Chhattisgarh) during the study period, of which 341 (24.9%) from 20 districts were found to be positive for HEV infection. The age of the suspected cases ranged from 3 months to 80 years. About 76% of the samples ($n = 1,024$) were from the >15 years age group. Out of 341 HEV-positive cases, clinical and demographic data of 335 were available, of

which 49 (14.6%) were in the age group ≤ 15 years, 108 (32.2%) were aged >15–25 years, 135 (40.3%) were in the age group >25–45 years, 37 (11%) were aged >45–60 years, and 6 cases (1.8%) were in the age group of 60 years and above. The majority (72.5%) of HEV-positive cases were in the age group 15–45 years. The difference in positivity between children (<15 years; 49/335; 14.6%) and adults (>15 years; 286/335; 85.4%) was statistically significant ($p < 0.0001$), and adolescents and adults in the age group >15–45 years had almost double the risk of infection (OR 2.46, 95% CI 1.75–3.46; $p < 0.0001$). Similarly, males (232; 69.2%) had a greater risk of infection than females (103; 30.7%; OR 1.53, 95% CI 1.18–2.00; $p = 0.0014$; Table 1). Two gravida were positive and both recovered and were discharged.

Fig. 2. Phylogenetic tree of HEV detected in central India from 2012 to 2015. The phylogenetic tree was generated using 22 sequences from this study (marked with ♦) of 513 bases of ORF1 aligned with 29 reference sequences. The neighbour-joining tree was constructed and reliability of nodes was tested by 1,000 bootstrap replicate test. A novel genotype detected in rats was used as an out-group. The sequences are named as GenBank Acc. No./place of detection/ country/year of detection. The sub-genotypes and genotypes are marked on the right-hand side.

Most of the patients had multiple clinical signs of hepatitis. Clinically, 87.4% patients presented with yellowish discoloration of the sclera and/or skin, 65% reported having dark urine, 82.7% patients had fever, whereas vomiting, nausea, and liver tenderness were seen in 60, 64.3, and 40.7% of the patients, respectively. Thirty-seven percent of the patients complained of abdominal pain. Hepatomegaly was observed in 50% of the patients, while 75% had a higher serum bilirubin level than normal.

HEV infection was detected throughout the year during the study period. The cases mainly peaked during the summer from March to June. Comparison of positivity among the seasons revealed that a significantly higher number of cases (171/341) were detected during summer (OR 2.5, 95% CI 1.8–3.5, $p < 0.0001$) in comparison to winter from November to February (71/341), and during monsoon from July to October (99/341; Fig. 1).

Out of 31 samples that showed the presence of RNA, 28 were subjected to sequencing, of which 22 gave satisfactory sequencing results that were submitted to GenBank (Acc. No. KT071749–53, KT259339–55).

The phylogenetic tree generated revealed that all 22 HEV sequences from central India clustered with reported genotype 1. The phylogenetic tree suggested that the strains were more closely related to the strains reported from Nepal, India, and Pakistan. The 22 sequences were further grouped into 2 subtypes (Fig. 2). Group 1 comprised 4 sequences that belonged to subtype 1f (Acc. No. KT259344, KT259345, KT259349, KT259355), while the remaining clustered in the second group and belonged to 1a. HEV belonging to 1f had an identity of 98% at the nucleotide level and 100% at the amino acid level to the Indian strain (GenBank Acc. No. JF443723), and the sequences of viruses of 1a were closely related (98% at the nucleotide level and 100% at the amino acid level) to the Nepalese strain (GenBank Acc. No. KM921812).

Discussion

Acute HEV infection is a major public health problem across the globe. India is endemic for this disease; sporadic cases and outbreaks with positivity ranging from 9 to 71% have been reported from different parts of India [2, 21]. This is the first study from central India that documents a prevalence of 24.9% of HEV infection in the suspected cases, indicating that HEV is a leading aetiological agent among hepatotropic viruses. Several other studies have also reported similar observations from other parts of India [2, 5]. During the study period of 3 years,

samples from villages, towns, and cities of 20 districts were found to be positive, indicating widespread cases of HEV in the region.

The highest proportion of HEV cases (72.5%) was detected in the age group of >15–45 years. Our findings are similar to other documented studies [2, 3, 5, 15, 21]. It is well known that the symptoms of HEV infection are more common and apparent in adults, whereas children have asymptomatic infections [3, 22]. Significantly more males were detected as HEV positive in the present study. Similar findings have been reported from endemic areas of the world such as Bangladesh, China [23–25], and other states and cities of India such as Tamil Nadu [26], Bangalore, and Mysore from Karnataka [5, 23]. Although the higher positivity in males is attributed to their greater risk of exposure to contaminated water [3], the possibility of harsh symptoms owing to biological reasons cannot be ruled out.

Clinically, most of the patients presented with jaundice followed by fever. Hepatomegaly was the most common finding during abdominal examination. The biochemical test revealed that about 75% had high (more than 1.8 mg/dL) serum bilirubin levels. These are the most common clinical features of HEV infection.

Although HEV-positive cases were detected throughout the year, the highest percent positivity among HEV cases each year was during the summer months (March to June). Similar findings were reported from different regions [3, 15]. It is suggested that seasonal patterns vary according to geographical area [27]. Generally, HEV cases rise during the monsoon and post-monsoon periods owing to the flooding of rivers and water canals that result in the contamination of the water sources by open drainage and sewage systems [4, 23]. On the other hand, during summer, due to negative pressure in the supply lines, they may become contaminated by faecal matter from closely running drainage or sewage systems, resulting in an HEV upsurge [3]. The high positivity rates in summer in central India is probably the result of the latter situation.

The phylogenetic analysis revealed that all the 22 sequences clustered into genotype 1 belonged to subtypes 1a and 1f. The subtyping based on complete genome sequences reveals that HEV is divided into 8 genotypes, of which 1 and 2 are mainly confined to humans and 3–8 are predominantly zoonotic in nature [4, 10, 24]. However, very limited sequence information on sequences of genotype 5–8 is available [10]. Genotypes 1 and 2 are mostly reported from endemic regions [3, 28], and genotype 1 is more frequently associated with large outbreaks than other genotypes [4, 22]. Genotypes and sub-genotypes are

also known to affect the transmission, chronicity, and severity of the disease [29]. Previous reports suggested that genotype 1 and 4 are circulating among humans and pigs, respectively, in India [17–19, 28, 30]. Sub-genotype 1a and 1c have been detected from humans in India [11, 15, 16]. To our knowledge, this is first study showing the presence of subtypes 1a and 1f in central India. More in-depth molecular studies using the ORF2 region and complete genome sequences will help recognize the subtypes of HEV present in this region. Further hospital-based studies coupled with molecular characterization will help elucidate the relation between genotypes/subtypes and the severity of HEV infection.

Being laboratory based, this study has a few limitations, such as inadequate clinical, biochemical, mortality, and pregnancy details of the patients, and limited data sequencing, etc. Nonetheless, since we tested more than

1,300 samples from 29 districts of central India, this study has sufficient data depicting the epidemiological picture, along with molecular characterization of HEV detected in central India.

In conclusion, our findings establish that central India is an endemic zone of HEV infection and 2 sub-genotypes of genotype 1 (1a and 1f) are present in the region. This study will provide baseline information for future studies. An unremitting molecular surveillance along with serological diagnosis will help in understanding the intricacies of HEV epidemiology.

Disclosure Statement

The authors have no conflicts of interest to declare .

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