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Investigation of suspected viral hepatitis outbreaks in North West India☆,☆☆

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ABSTRACT

Hepatitis E (HEV) infection is diagnosed on the basis of serum anti-HEV IgM detection. In outbreaks, early diagnostic method is important for prompt control measures. This study compared 3 diagnostic methods in 60 serum samples collected in suspected HEV outbreaks. The suitability of saliva samples for antibody detection was also evaluated in 21 paired serum saliva samples. The anti-HEV IgM, HEV-Ag, and HEV-RNA were detected in serum samples of 52 (86.66%), 16 (26.66%), and 18 (30%) patients, respectively. The concordance between serum and saliva IgM was found to be 76.91%. The positivity of PCR and HEV-Ag detection was 100% within 1 week of illness which declined to 5–10% thereafter. The outbreak was attributed to HEV genotype 1, subtype 1a, and the clinical and environmental strains clustered together. HEV-antigen and RNA were an early diagnostic marker with 96.66% concordance. Saliva samples can be used as an alternative in outbreak setting.

1. Introduction

Viral hepatitis E, a waterborne hepatitis, has extensive epidemic potential, though focal outbreaks and sporadic cases are also frequently observed. Overall, the WHO (2014) has reported approximately 20 million hepatitis E infections, more than 3 million acute cases, and nearly 56,600 deaths due to hepatitis E. The causative agent was identified as a novel putative hepatitis E virus (HEV) during a massive waterborne epidemic of hepatitis which occurred in Delhi and caused 30,000 cases during 1955–1956 (Ramalingaswami and Purcell, 1988). This virus contributes to 30–70% of sporadic hepatitis cases in the Indian subcontinent (Aggarwal, 2011). Drinking water contaminated with sewage is the commonest mode of HEV transmission in developing nations like India. The virus is endemic in North India with documented hepatitis E outbreaks being reported from Mandi-Gobindgarh in the year 2005 (Bali et al., 2008; Kumar et al., 2006; Prinja et al., 2008) and Lalru in the year 2010 (Majumdar et al., 2013a). HEV is now regarded as the major cause of sporadic as well as epidemic hepatitis, which is no longer restricted to Asia and the developing countries. Increasing number of cases is being documented from developed world such as United States and Europe (Clemente-Casares et al., 2003; Echevarría, 2014; Huang et al., 2002; Meng et al., 1997; Riveiro-Barciela et al., 2012).

The present study reports a waterborne outbreak of viral hepatitis which occurred almost simultaneously in Nawanshahar (Punjab) and Palsora a suburb of Chandigarh in March–April, 2012. The detection of early warning signals, well-timed investigations, and application of specific control measures are important to control the outbreak. Hence, the present study compared the anti-HEV IgM, HEV-antigen, and HEV-RNA detection for the early diagnosis of a suspected HEV outbreak. The usefulness of collecting noninvasive clinical samples like saliva for diagnosis of HEV was also evaluated. Further, phylogenetic analysis of the viral strains from human and environmental samples was carried out for tracing the source of infection.

2. Materials and methods

2.1. Study design

In the month of March–April 2012, the Department of Virology, Postgraduate Institute of Medical Education and Research, Chandigarh, was informed about a suspected outbreak of enterically transmitted viral hepatitis from 2 different places, Nawanshahar in Punjab and Palsora, a village near Chandigarh, North India. An active surveillance of the affected region was carried out, and approximately 3 mL of blood samples was collected from 27 icteric patients from Nawanshahar and 33

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patients from Palsora aseptically by trained health care personnel after obtaining a written informed consent. Additionally, 21 matched saliva samples were also collected from Nawanshahar patients. All the samples were transported in cold chain to the virology laboratory. Since the clinical samples were received during an outbreak for diagnostic purposes, prior ethical clearance could not be obtained. However, the study was retrospectively approved by the Institute Ethics Committee of Postgraduate Institute of Medical Education and Research, Chandigarh, as per national guidelines.

2.2. Descriptive epidemiology

Nawanshahar district of Punjab and Palsora village of Chandigarh are situated at a distance of 93.8 km in the northwest part of India at a latitude of 31°N and longitude of 76°E with moderate rainfall and highest and lowest temperatures being 40 °C and 25 °C, respectively, in the months of March–April, when the outbreak occurred. Both the townships are in suburban areas with low socioeconomic living conditions, and at both places, sewage work was undertaken before the outbreak. An investigation team visited houses and collected information about cases, drinking water quality, source of water supply, and drainage system. The existing blueprint of the water supply pipelines and drains was also examined.

2.3. Description of the patient population

The mean age of patients affected was 30.48 ± 14.58 years (95% confidence interval [CI]: 26.72–34.25), and the male:female ratio was 1.6:1. The predominant clinical manifestations were yellow urine (95%), fatigue (85%), yellow eye (81.66%), anorexia (75%), vomiting (65%), fever (61.66%), pain abdomen (58.33%), pruritis (30%), and arthalgia (21.66%), respectively. Liver enzymes were found to be raised, mean levels of aspartate aminotransferase (618.6 ± 115.8 IU/mL), alanine aminotransferase (694 ± 131.4 IU/mL), and mean bilirubin level was found to be 6.288 ± 3.975 mg/dL. Pregnant women with jaundice were not reported in these 2 outbreaks.

2.4. Antibody detection

Sixty serum samples were tested for the following viral markers: anti-hepatitis A virus (HAV) IgM (ImmunoComb® II HAV Ab, Orgenics, Israel) and anti-HEV IgM (ImmunoVision, Springdale, AR) using commercially available enzyme-linked immunosorbent assay (ELISA) kits as per the manufacturer's instructions. Anti-HEV IgM detection system used a combination of recombinant HEV ORF-2 and ORF-3 peptide. The manufacturer of this kit has documented a sensitivity and specificity of >99% as per the kit insert. Neat saliva samples from 21 patients were diluted to 1:4 dilutions in sample diluent (i.e., 100 μL saliva + 400 μL sample diluent) and tested for the detection of anti-HEV IgM using commercially available ELISA kit (ImmunoVision).

2.5. HEV-antigen detection

Sixty serum samples were tested using a commercially available HEV-antigen ELISA kit (Wantai, Beijing, China). This is a solid phase sandwich ELISA, in which the microwell strips were precoated with rabbit anti-HEV antibodies directed against ORF-2 protein. The absorbance was measured at 450 nm. According to the manufacturer's information provided, HEV-Ag cutoff = 0.12 + NC (the mean absorbance value for 3 negative controls) was calculated. Sample with absorbance higher than cutoff values were considered positive.

2.6. RNA extraction and reverse transcription–polymerase chain reaction (RT-PCR)

RNA extraction was carried out from all the 60 serum samples using QIAamp Viral RNA Mini Kit (Qiagen, Germantown, MD). Briefly, 140 μL of serum was used for RNA extraction as per the manufacturer's guideline which was finally eluted in 50 μL of elution buffer. A 10-μL volume of the RNA was reverse transcribed to cDNA on the same day using M-MuLV RT and random hexameric primers (Fermentas, Hanover, MD). Nested PCR protocol was used for amplification using primers targeting the ORF 1 gene as previously described (Majumdar et al., 2013b). The amplicon of 343 bp was visualized by 2% agarose gel electrophoresis following ethidium bromide staining. With each run, 1 positive and negative control was included. Positive control was a recombinant plasmid (pIRES-neo-ORF1), a kind gift from Dr Shahid Jameel, ICGEB, New Delhi. Representative 6 clinical strains from Nawanshahar and 5 from Palsora, sewage samples from both the places, and tap water sample from Palsora were sequenced for confirmation of the etiological agent.

2.7. Sewage and tap water sampling

Samples of untreated sewage were collected from sewage pumping stations of Nawanshahar and Palsora. A sterile glass bottle was lowered into the flowing water to collect approximately 1 L of sewage and transported to the laboratory in cold chain. Further, an early morning, free-flowing 1-L tap water sample was collected in a sterile glass bottle from a final distribution tap in Palsora. The tap water was muddy, and visible particulate materials were appreciated. Adequate personal safety precautions were taken while collecting and processing the samples. Materials used for sample collection and processing were decontaminated by autoclaving.

2.8. Sewage and tap water sample processing

Briefly, 40-mL sewage sample was ultracentrifuged (110,000 × g for 1 h at 4 °C) to pellet down both the viral particles and suspended material. The sediment was eluted using 4 mL 0.25 N glycine buffer, pH 9.5. Suspended solids were then separated by centrifugation at 12,000 × g for 15 min. Viruses were finally pelleted down using a second ultracentrifugation step (110,000 × g for 1 h at 4 °C) and resuspended in 0.1-mL phosphate-buffered saline (Clemente-Casares et al., 2003). From the virus concentrate, RNA was extracted using QIAamp Viral RNA Mini Kit (Qiagen), cDNA was synthesized using M-MuLV reverse transcriptase enzyme (Fermentas), and HEV ORF1 gene was amplified as described above followed by sequencing and phylogenetic analysis.

2.9. Sequence analysis and phylogenetic tree construction

HEV-specific RNA-dependent RNA polymerase (RdRp) gene was amplified from 11 clinical (6 from Nawanshahar and 5 from Palsora) and 3 environmental samples, i.e., sewage samples collected from Palsora, Nawanshahar, and the tap water of Palsora were sequenced. ABI chromatogram files were viewed using Finch TV 1.4.0, following sequence drafting with bioedit 7.0.9 software. For the confirmation of these sequences, database search was implemented using BLAST program available at NCBI Web site. For sequence comparison, standard representative strain sequences were retrieved from GenBank as previously described (Majumdar et al., 2015). Clustal X 2.0.11 program was used for multiple sequence alignment followed by Molecular Evolutionary Genetics Analysis (Tamura et al., 2007) for phylogenetic tree construction.