Molecular characterization of hepatitis A virus circulating in Uttar Pradesh, India: A hospital-based study

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Background & objectives: Hepatitis A is prevalent worldwide and is among the leading cause of acute viral hepatitis in India. Major geographical differences in endemicity of hepatitis A are closely related to hygienic and sanitary conditions and other indicators of the level of socio-economic development. The present study was aimed to know the seropositivity prevalence and predominant circulating strain of HAV in a north India.

Methods: Patients with acute viral hepatitis were enrolled. Blood samples were collected over a period of one year from June 2016 to May 2017. Serum samples were tested for anti-immunoglobulin M (IgM) HAV antibodies. The seropositive samples were analyzed for HAV-RNA by real-time reverse transcription-polymerase chain reaction (RT-PCR). Samples detected on molecular assay were subjected to conventional semi-nested RT-PCR for VP1 gene. Further sequencing of amplified RT-PCR products was done, and data were analyzed.

Results: A total of 1615 patients were enrolled, and serum samples were collected and tested. The male:female ratio was 1.3:1 with a mean age of 24.31±17.02 yr (range 0-83 yr). Among these, 128 (7.93%) were positive for anti-HAV IgM antibodies; 41.63 per cent of seropositive patients were in their childhood or early adolescent age group. Of all seropositive samples, 59 (46.09%) were positive for HAV RNA. Genotyping sequencing of 10 representative strains was carried out, and the circulating genotype was found to be IIA. The nucleotide sequences showed homology among the strains.

Interpretation & conclusions: Our results showed that hepatitis A was a common disease in children with IIA as a circulating genotype in this region. In approximately 50 per cent of cases, HAV RNA could be detected. Higher number of HAV IgM-seropositive cases was observed during monsoon period.

Key words: AVH - genotype - HAV - hepatitis A - endemic - India

Hepatitis A virus (HAV) is transmitted through faeco-oral route by contaminated water and food. Approximately 1.5 million clinical cases of hepatitis A occur worldwide annually¹. The incidence rate is strongly related to socio-economic indicators and access to safe drinking water. Approximately 85

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per cent of individuals who are infected with HAV recover fully clinically and biochemically within three months, and nearly, all have complete recovery by six months. It has been seen that severe manifestations are more common in young adults requiring hospitalization with overall case fatality rate of 0.3 per cent. HAV is a non-enveloped 27 nm, heat, acid- and ether-resistant RNA virus in the hepatovirus genus of the Picornaviridae family. Its virion contains four capsid polypeptides, designated as VP1 to VP4, which are cleaved post-translationally from the polyprotein product of approximately 7500 nucleotide genome. It has single serotype with six genotypes. Three HAV genotypes, I, II and III, divided into subtypes A and B, infect humans. Genotype I is prevalent in Europe and North and South America, and genotype III is endemic in Asia.

HAV is considered to be endemic in India. According to National Centre for Disease Control, India, HAV is responsible for about 10-30 per cent of acute hepatitis cases in individuals with acute liver failure in India. The Indian population has shown an upward shift in the average age at the first HAV infection, among the socio-economically developed population resulting in pockets of susceptible populations. Outbreaks of HAV have been reported, mainly affecting young adults from different parts of the country, e.g., in Delhi, Kerala and Shimla. Genotypes I and III of HAV are the predominant strains circulating in India. The present hospital-based study was aimed to know the seropositivity and predominant circulating strain of HAV in Uttar Pradesh (UP), north India.

Material & Methods

Consecutively, all cases with clinical presentation of acute viral hepatitis (AVH) (WHO case definition), referred to the Virology Laboratory, department of Microbiology, King George’s Medical University, Lucknow, UP, India, during June 2016-May 2017, were enrolled in this observational study. The protocol was approved by the Institutional Ethics Committee and written informed consent was obtained from each participant. Patients of all age groups and both sexes were included. From each patient, blood sample (5 ml) was collected. Blood samples were centrifuged; serum was collected and tested for anti-HAV immunoglobulin M (IgM) using ELISA kit (DIA Pro Diagnostic Bioprobes Srl., Italy). The remaining sample was stored at –70°C. All anti-HAV IgM positive samples were subjected to HAV real-time reverse transcription-polymerase chain reaction (RT-PCR) assay using a protocol described by Costafreda et al. For molecular profiling, HAV RNA-positive serum samples were subjected to conventional semi-nested RT-PCR targeting 518 base pair (bp) fragment encompassing the VP1 region which was further subjected to sequencing using a protocol described by Tallo et al. As our study was focussed on phylogenetic analysis of HAV only, the other faeco-orally transmitted viruses were not tested.

All the amplified products were purified and sequenced using BigDye Terminator Cycle-Sequencing Kit (Applied Biosystems, USA) on ABI 3130 genetic Analyzer (Applied Biosystems, USA). Nucleotide sequences were edited and subjected to GenBank using Basic Local Alignment Search Tool (BLAST) programme (www.ncbi.nlm.nih.gov/BLAST) for comparing with all the available similar sequences. Further, phylogenetic analysis was carried out, and tree was constructed by maximum likelihood method using MEGA7 software with the neighbour-joining method from a Kimura 2-parameter distance matrix. Genotype was determined using reference sequences belonging to different HAV genotypes.

Results & Discussion

A total of 1615 AVH cases were enrolled over a one-year period. The mean age of the participants was 24.31±17.02 yr (range 0-83 yr) and male:female ratio was 1.3:1. Anti-HAV IgM antibodies seropositivity was 7.9 (128/1615) per cent (Table). The mean age of seropositive cases was 9.3±9.4 yr. About 41 per cent of seropositive patients were in their childhood or early adolescent age group. Of the 128 seropositive samples, HAV RNA was detected in 59 (46.09%) samples. The IgM HAV antibodies percentage positivity in AVH cases referred from Sarawasti (22.2%), Unnao (17%) and Gorakhpur (12.5%) districts was high. The seasonal distribution of enrolled cases showed that AVH occurred throughout the year, though both the number of AVH cases and the HAV IgM seropositive cases increased during monsoon period between June and August (Fig. 1). Of the 59 HAV RNA-positive samples, only 10 high viraemia samples could be amplified for VP1 gene by
Peaks in total AV cases admitted and IgM HAV seropositivity occurred during the monsoon season (June-August). This pattern of upsurge of HAV cases in monsoon has been reported in earlier studies and suggests a possibility related to contamination of drinking water during periods of heavy rain. Since the study was done on referred samples, it could not represent true picture as far as district-wise positivity was concerned. More studies on larger sample sizes may be conducted. Moreover, HAV was not detected in several districts; this may be because of less number/adult samples.

Molecular epidemiology of HAV is important to understand the strains circulating in various geographical regions and tracing the source of contamination in an outbreak situation. On molecular profiling genotype IIIA was found to be the prevalent circulating genotype in UP. Genotype III has been reported as the predominant genotype (70%) followed by genotype IA (30%) from Delhi, north India. Co-circulation and co-infections with subgenotypes IIIA and IB have been reported from Pune, western India. HAV genotype IIIA has been reported as an aetiological agent of various other waterborne outbreaks from northern, southern and western India. The genetic diversity in VP1 gene of this region (2-4%) suggests diversity in circulating genotype IIIA in India and requires further analysis of other gene targets. This study of the mutational analysis of HAV RNA from different parts of the country would determine the characteristics and source of infection and also provide information on trends and transmission pattern.

In conclusion, HAV infection was found common in children in the region. Genotype IIIA was found to be circulating genotype. The mutations at VP1 region warrant further analysis.
Fig. 1. Seasonal distribution of the total sample tested and confirmed hepatitis A virus case.

Fig. 2. Molecular phylogenetic analysis of 10 representative hepatitis A virus (HAV) strains by maximum likelihood method. Each strain is labelled by GeneBank accession number followed by country. The strains from this study are marked with (▲). The respective genotypes are mentioned with accession number and country. The evolutionary distances were computed using the Maximum Composite Likelihood method and the tree was drawn to scale, with branch lengths measured in the number of substitutions per site. Evolutionary analyses were conducted in MEGA7.
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Conflict of Interest: None.

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