

Brief Original Article

Epidemiological and molecular investigation of a hepatitis A outbreak in Tamil Nadu, Southern India

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Abstract

Introduction: Hepatitis A virus causes an acute infection mainly in young children. The present study was carried out to characterize the nature of hepatitis A virus (HAV) involved in an outbreak of jaundice in children.

Methodology: Serum and stool samples from five children were sampled from among 26 clinically diagnosed jaundice cases. HAV IgM ELISA and PCR were used for confirmatory diagnosis and molecular characterization by direct amplicon sequencing and analysis.

Results: All the serum samples collected from the symptomatic cases were found to be positive for Anti-HAV IgM ELISA as were all the serum samples and stool samples using semi-nested PCR. Phylogenetic analysis revealed that the HAV involved in the outbreak belonged to genotype IIIA.

Conclusions: The infection was caused by HAV genotype IIIA. Improved access to clean drinking water, sanitation around drinking water sources and routine chlorination of drinking water in poor and developing countries are needed, as well as childhood HAV vaccination under regular immunization programs in endemic countries.

Key words: Hepatitis; outbreak; HAV; India; sequencing; genotype.

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Introduction

Viral hepatitis or jaundice outbreaks are caused by hepatitis viruses A, B, C and E. Hepatitis outbreaks are classified based on the causative agent when at least one case is laboratory-confirmed and the others are epidemiologically linked. Cases are categorized as hepatitis A, B, C, E, or unspecified, if the causative agent could not be identified [1].

Hepatitis A is generally an acute, self-limiting liver infection transmitted through the faeco-oral route, caused by a picornavirus, the hepatitis A virus (HAV) belonging to genus *Hepatovirus*. It causes 10 million infections worldwide each year [2,3], and is estimated to have caused approximately 11,000 deaths in 2015 [4]. Most children acquire an asymptomatic infection in childhood and thereby develop immunity naturally. Infection in adulthood is generally severe, especially in childhood naïve populations [5]. The Integrated Disease Surveillance Program (IDSP), Govt. of India, has published viral hepatitis surveillance data for the

period 2011-13 [1]. Among the total 163 outbreaks with known etiology, 78 (48%) were caused by hepatitis E, 54 (33%) by hepatitis A, 19 (12%) by both hepatitis A and E, and 12 (7%) by hepatitis B or hepatitis C. Contaminated drinking water was the source of most outbreaks.

In the case of hepatitis-A virus (HAV) infection, a confirmed case is “a case that meets the clinical criteria for acute hepatitis and is IgM anti-HAV positive, OR “a case that has hepatitis A virus RNA detected by NAAT (such as PCR or genotyping), OR “a case that meets the clinical criteria and occurs in a person who had contact (e.g. household or sexual) with a laboratory-confirmed hepatitis A case 15-50 days prior to onset of symptoms” [6].

In this study, we report an outbreak of hepatitis A virus from a village in Tamil Nadu which occurred during April-May, 2018, confirmed by serological and molecular testing. Identification of etiology and deciphering molecular epidemiology in cases or

outbreaks of viral hepatitis helps in identifying the probable source of infection and selecting control measures to be initiated.

Methodology

Case history and collection of samples

An outbreak of hepatitis/jaundice was reported at Vairapuram colony, Villupuram District, Tamil Nadu during April-May, 2018. There were 288 households in the colony with a population of 1,219. The number of children in the village in the age group 0-19 years was 474. Apart from symptomatic cases presented to the hospital, an active case search was done for the entire population of the village. A total of 26 children between 2-15 years of age were affected, exhibiting the combination of these symptoms: fever, anorexia, pain in abdomen, yellowish discoloration of eyes, and dark coloured urine. Jaundice was a common symptom found in all the children and was present in all the clinical cases. Representative serum and stool samples from five children with jaundice were collected for serological and molecular studies.

Serology

Serum samples were processed for Anti-HAV IgM ELISA (Biomerieux, Marcy l'Etoile, France), Anti-HEV IgM ELISA (Biomerieux, Marcy l'Etoile, France), HBsAg ELISA (J. Mitra and Co, New Delhi, India) and Anti-HCV ELISA (J. Mitra and Co, New Delhi, India) as per the kit manufacturer's instructions.

RNA extraction and reverse transcription-nested polymerase chain reaction (RT-nested PCR)

Total nucleic acid was extracted from 200 µL of serum samples using High Pure Viral Nucleic Acid Extraction Kit (Roche, Penzberg, Germany). RNA from stool samples was extracted using QIAamp® Viral RNA Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. Reverse transcription (RT) for first-strand synthesis was carried

out using a PrimeScript™ RT reagent kit (Takara Bio Inc, Shiga, Japan). Briefly, 20 µL reaction mixture contained 4 µL of PrimeScript buffer, 1 µL of PrimeScript RT enzyme mix I, 1 µL of random hexamer primers (100 µM), 4 µL of nuclease-free water and 10 µL of RNA sample. Reverse transcription was carried out at 42°C for 15 minutes, and inactivation at 85°C for 5 seconds.

HAV detection and genotyping was performed by semi-nested PCR (keeping the reverse primer identical) and sequencing-phylogenetic analysis approach using the second-round PCR product covering the VP1/2A region. PCR was performed with TopTaq Master Mix Kit (Qiagen, Hilden, Germany). One µL of cDNA was used as the template for the first round, and 1 µL of double diluted PCR product was used as the template in the second round of PCR. Details of primers and amplification conditions used are given in Table 1.

Sequencing and genetic analysis

The amplified second-round PCR products were purified using a GeneJET PCR Purification Kit (Thermo Fisher Scientific, Waltham, USA) and sequenced through a commercial Sanger sequencing service. Nucleotide alignment and phylogenetic analysis were performed using Molecular Evolutionary Genetics Analysis software version 7.0. (MEGA 7.0). The phylogenetic tree was constructed by Neighbor-Joining method with 1000 bootstrap replicates. Along with the outbreak sample HAV sequences, hospital-derived non-outbreak HAV positive sample sequences (n = 5, year 2018) were also included for phylogenetic analysis.

Results

A total of 26 suspected viral hepatitis cases were identified. Incidence rate by age and sex are given in Table 2, showing that the most common age group was 2-5 years, and males were more affected. No cases were observed in children above the age of 15. The incidence

Table 1. Primers and amplification conditions used for semi-nested PCR for detecting HAV.

PCR	Primer name	Sequence (5'→3')	Amplification conditions	Amplicon size (bp)
I round	HAV_2F	RGAGTATACTGCCATAGGA	94°C for 3 minutes, 35 cycles of 94°C for 30 seconds; 55°C for 20 seconds and 72°C for 75 seconds, 72°C for 5 minutes	1203
	HAV_2R	TGAGATTTTTGCTTGTGAA		
II round	HAV_JF	CTGGTYTCTATTCAGATTGC	94°C for 3 minutes, 35 cycles of 94°C for 30 seconds; 55°C for 20 seconds and 72°C for 25 seconds, 72°C for 3 minutes	365
	HAV_2R	TGAGATTTTTGCTTGTGAA		

Table 2. Age group and gender distribution of HAV-positive cases.

Age group	Male	Female	Total incidence	Incidence (%)
0 - 1 years	0/14	0/11	0/25	0%
2- 5 years	10/53	7/58	17/111	15.31%
6 - 10 years	7/57	1/53	8/110	7.27%
11 - 15 years	0/60	1/56	1/116	0.86%
16 – 19 years	0/58	0/54	0/112	0%
Total	17/242	9/232	26/474	5.48%
Mortality	0	0	0	

rate in children (0-19 years) was 5.48% (26/474). An epidemic curve with the number of symptomatic jaundice cases clinically diagnosed at the primary health centre or identified during active case search has been plotted and is shown in Figure 1. The maximum number of cases was recorded in week 15; there was a reduction after the initiation of control measures. No new cases were recorded after week 21.

All the symptomatic cases used a common water supply from an overhead tank receiving water from a well with night soil-contaminated surroundings. No other common source of water or food was identified. Also, the water samples from overhead tank and well were found to be positive for coliforms, as reported by a State public health team. All the serum samples

Figure 1. The epidemic curve showing numbers of symptomatic jaundice cases in the outbreak.

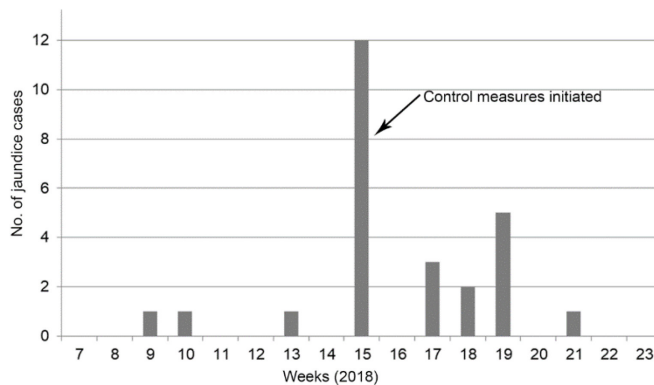
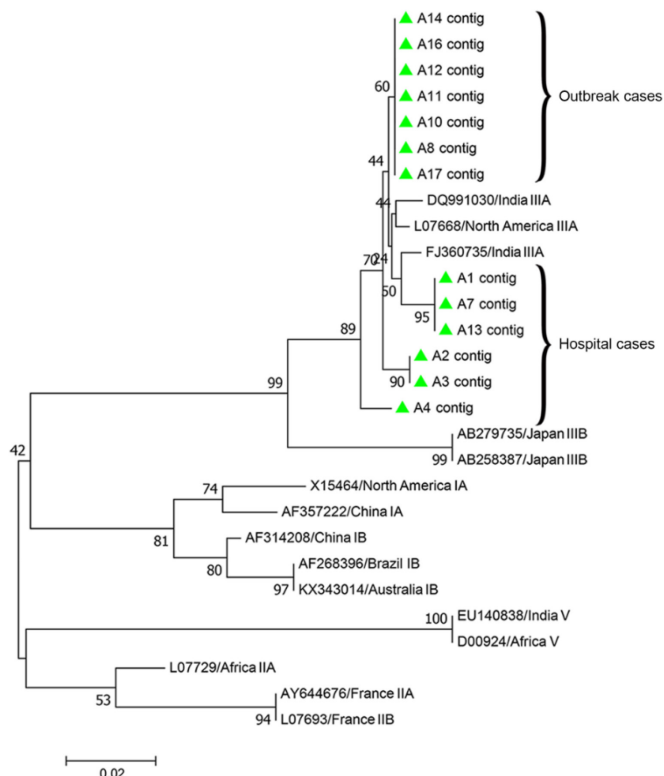


Figure 2. Phylogenetic analysis targeting the VP1-2A junction region, with sequences obtained in the present study indicated by ▲. Reference strains are described as follows: GenBank accession number/Country of origin/Genotype.



collected from the symptomatic cases were positive for Anti-HAV IgM ELISA and e for HAV RNA using semi-nested PCR. Serum samples were negative for HEV, HBV and HCV by ELISA. Phylogenetic analysis using VP1-2A region nucleotide sequence showed that the HAV that caused the outbreak belonged to genotype IIIA (Figure 2), which is a common genotype involved in hospital-detected HAV infections and outbreaks in India [7-12]. The nucleotide sequences obtained in this study have been deposited in the GenBank database under the following accession numbers: MH431620-MH431625.

Discussion

The cause of the acute hepatitis outbreak studied here was identified as HAV through serological and molecular methods. Data from this outbreak supports earlier data that the majority of HAV related cases are from the pediatric age group [8,13-16]. In India, contaminated drinking water has been identified as the source of most outbreaks caused by hepatitis A and E [1,5,7,8,17,18]. The main control measures initiated to contain this outbreak included disinfection of the overhead water tank and the well, community education for safe disposal of night soil of the affected children and boiling water for drinking, mass cleaning, removal of garbage, and disinfection of the affected areas.

Proposed recommendations to prevent future outbreaks include personal and environmental hygiene, regular chlorination of overhead tanks, and construction of household toilets. In the current investigation, although HAV could not be directly detected in the drinking water, the corroborative evidence of reduced cases after drinking water chlorination, and the case occurrence within the area with one common source of water from an overhead tank which contained coliforms, were suggestive of drinking water contamination and faeco-oral transmission between households and neighborhood contacts. The children from the affected area attend school with children from other areas of the village, but there were no jaundice cases identified in other areas, ruling out possible water or food contamination in the school.

The HAV strains isolated worldwide belong to one serotype and are divided into six genotypes (I-VI). The common HAV genotype in humans in India is genotype III A [8]; however, genotypes IA and IB [9] have also been reported. The genotype of HAV in the current outbreak was identified as IIIA, which is the predominantly circulating genotype. Hospital-derived non-outbreak HAV positive samples also belonged to genotype IIIA but with a few nucleotide changes. Nucleotide sequences obtained from outbreak samples (serum and feces) showed 100% nucleotide homology.

Unlike India, in developed countries like the United States of America (USA) hepatitis A infection is associated with international travel outside North America or exposure to an individual who had undergone international travel [19]. Hepatitis A surveillance in suspected areas or regions is of paramount importance to 1) detect and provide data to control outbreaks; 2) identify contacts of cases/patients who require post-exposure prophylaxis; 3) characterize changes in the epidemiology of infected populations and risk factors, and 4) guide vaccination policies and other prevention efforts [19]. Rapid identification and prompt reporting of cases of hepatitis A are important, because post-exposure prophylaxis, administered within two weeks after exposure, is known to be highly efficacious and can prevent the development of symptomatic illness in exposed persons as well as preventing subsequent transmission to others.

In a multicentric HAV seroprevalence study conducted in non-vaccinated children in India [20], the seropositivity in the age group 1.5-6 years was 30.3%, whereas in the group of 6-10 years it was 50.3%. In the current study, 65% (17/26) of the symptomatic cases with jaundice occurred in the age group 1-5 years. A tertiary care hospital-based study in India reported that

94% of all symptomatic hepatitis A cases occurred in children under 15 years of age [8]. This clearly shows that the vulnerable age group in which symptomatic cases occur may not have sero-protection, and suggests that immunoprophylaxis may be especially useful in early childhood. Since the introduction of effective vaccines in the USA in 1995, hepatitis A rates there declined by > 96% from 1995 to 2014 [19]. The recommendation of improvements in water quality and sanitation has not yet been achieved in India [1], and the recommendation to include hepatitis A vaccine in childhood immunization programs to reduce the public health burden of hepatitis has not yet been initiated. Both inactivated and live attenuated HAV vaccines are licensed for use in India, but are yet to be included in the regular immunization program. Further large scale studies on epidemiology, assessment of overall hepatitis A load among viral hepatitis cases, and the relevance of HAV genotype in clinical outcome may give useful information to make a decision on inclusion of HAV and its genotype(s) in the routine immunization program in India.

Conclusion

Contaminated drinking water was suspected as the source of a hepatitis outbreak among children in one village; control measures targeting the water source reduced the incidence of new cases. The outbreak was caused by HAV of genotype IIIA. This study reinforces the need for improved access to clean drinking water, sanitation around drinking water sources, and routine chlorination of drinking water in poor and developing countries, as well as the need for childhood HAV vaccination within the routine immunization program in endemic countries.

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