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MOLECULAR CHARACTERIZATION OF HEPATITIS A VIRUS FROM SPORADIC AND EPIDEMIC CASES OF JAUNDICE IN TAMIL NADU, INDIA

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ABSTRACT

Hepatitis A is an enterically transmitted viral disease, highly prevalent in India and mainly occurs as a sporadic childhood disease. Focal outbreaks have also been reported among children and adults in India. This study was initiated to find out the occurrence of sporadic and epidemic cases of jaundice caused by HAV in Tamil Nadu. Both sporadic and epidemic cases of HAV were subjected to molecular characterization to document the prevalent HAV genotype in Tamil Nadu.

A total of 584 sporadic cases and 23 cases from a jaundice outbreak in Perambalur District, Tamil Nadu during Jan 2009 were included in the present study. Sera were tested for anti-HAV IgM by ELISA. Twenty-two sporadic and 23 epidemic samples positive for HAV were subjected to RT-PCR and RNA positive samples were further processed for sequencing and phylogenetic analysis.

Three hundred and seven (52.6%) sporadic cases and all the 23(100%) epidemic jaundice cases were positive for HAV. Among the sporadic cases, 239(77.8%) were children less than 10 years of age. However, RT-PCR was positive only in four sporadic and two epidemic cases. Sequencing and phylogenetic analysis revealed that both sporadic and epidemic form of HAV infection was caused by HAV genotype IIIA.

HAV was found to be the leading cause of jaundice especially among children in Tamil Nadu. Genotype IIIA was the prevalent HAV genotype both in the epidemic and sporadic cases of jaundice in the southern state in India, which has the potential to cause large scale epidemics in the community. Environmental hygiene, sanitation and provision of safe and protected drinking water have to be improved to avoid jaundice epidemics in Tamil Nadu. An effective control strategy including new vaccination policies for the children need to be emphasized to reduce the HAV related disease burden in the community.

Keywords: HAV, Jaundice, Sporadic, Epidemic, Genotype, Phylogenetic analysis

INTRODUCTION

Hepatitis A is an enterically transmitted viral disease of global public health importance (1). HAV is one of the common causative agents for acute hepatitis and jaundice, particularly in developing countries where 20-25% of clinical jaundice is caused by HAV infection (2). In developed countries, HAV transmitted by contaminated food or water through faeco-oral route has caused epidemics among adults (3). It is also common among unvaccinated travelers to endemic countries (4) and intravenous drug users (5). However, in most of the developing countries including India, it is hyper-endemic and the infection is acquired

early in life, most of the cases are anicteric, with 90% exposure by the age of 5 years and universal exposure by adolescence (6).

Based on the seroepidemiological survey conducted in 1982, 1992 and 1998, a change in epidemiology of hepatitis A in India was documented and the possibility of hepatitis A outbreak in the near future was predicted (7-10). The presence of HAV can be determined using IgM antibodies with the help of RT-PCR (11). In the last few years, small outbreaks of hepatitis A in children have been reported from North India (12), Western India (13) and South India (14, 15). Out of the 7 genotypes (I-VII) genotype IIIA was predominantly reported from these HAV outbreaks in India, but so far not documented from Tamil Nadu.

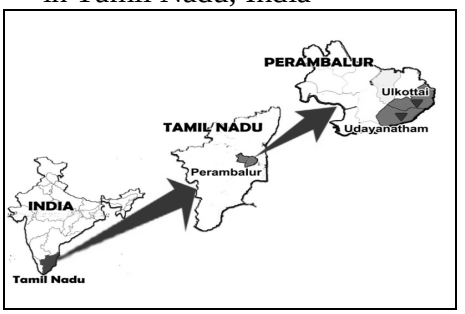
In view of the changing epidemiology of HAV in India, this present study was carried out to investigate the HAV infection among sporadic and epidemic cases of jaundice caused by HAV and also to identify the HAV genotype circulating in Tamil Nadu state of India.

MATERIALS AND METHODS:

Study Design and Area: A descriptive cross-sectional study was undertaken to investigate 584 clinically diagnosed sporadic cases of jaundice in patients from Government hospitals of several districts of Tamil Nadu during 2008-2009,representing all the four regions of the state and having a wide geographical distribution.

Apart from the sporadic cases, this study also includes cases from an epidemic of jaundice reported on January 6, 2009 from two adjacent villages namely, Angarayanallur and Silal of Devamangalam Health Sub Centre, Ulkottai Primary Health Centre, Perambalur District in Tamil Nadu situated in Southern India (Figure – 1).

Figure – 1: Map showing location of the area of jaundice out break in Tamil Nadu, India



The outbreak was investigated by the State Rapid Response Team. Door-to-door surveillance campaign was adopted to detect the clinical cases of jaundice. A total of 39 cases with symptoms of acute clinical jaundice were detected from both the villages.

Collection of Samples:

Clinical Samples: Out of 604 blood samples investigated in this study, 584 were sporadic cases from all over Tamil Nadu and 23 were epidemic cases of jaundice from Peambalur District. Relevant clinical, epidemiological and environmental details were recorded in a pre-set proforma. Serum samples were stored at minus 20ºC until tested.

Environmental Samples: A total of 15 water samples were also collected from various drinking water sources available in both the affected villages and subjected to bacteriological analysis for confirmation of faecal contamination with the help of PA Coliform Kit (HiMedia, India).

Serology: All the serum samples were screened for the presence of IgM antibodies against hepatitis A and hepatitis E viruses by ImmuLISA Kit supplied by M/s Orgenics Ltd, Israel.

RT-PCR: A total of 50 serum samples (27 sporadic cases and 23 epidemic cases)

positive for anti-HAV IgM were subjected to RT-PCR (16). RNA was extracted using QIAamp viral RNA mini kit (Qiagen, USA) according to the kit instructions. Primers representing the conserved 5'non coding region(5'-NCR) of HAV genome were used: outer forward- 5'GGC TAC GGG TGA AAC CTC TT 3'; outer reverse – 5'CCA ATT TTG CAA CTT CATG 3' ; inner forward – 5' TAA CAG CGG CGG ATA TTG GTG 3' and inner reverse 5' GGT CAA GGC CAC TCC CAAC 3'. Primers were obtained from Bangalore Genei, Bangalore, India. The amplified products were visualized by agarose gel electrophoresis. Out of the 50 serum samples amplified, six samples were positive for HAV RNA of which two were epidemic samples and four were sporadic samples of HAV infection (Table – 1).

Table – 1: Jaundice samples positive for RNA amplification (n=6)

S. No	Sample Code	Nature of Jaundice	District	Region in Tamil Nadu
1	PER11	Epidemic	Perambalur	Central
2	PER18	Epidemic	Perambalur	Central
3	CHE207	Sporadic	Chennai	North
4	TVL4	Sporadic	Tiruvallore	North
5	TUT24	Sporadic	Tuticorin	South
6	KRI2	Sporadic	Krishnagiri	West

Sequencing: PCR products were column purified (QIA gel purification kit, Qiagen, USA) and sequenced using Big Dye Terminator cycle sequencing ready reaction kit (Applied Biosystems, USA) in an automatic sequencer (ABI PRISM 310 Genetic Analyser, Applied Biosystems, USA).

Phylogenetic analysis: The phylogenetic analysis of HAV-RNA were performed using the Clustal X. Accession numbers and designations of the sequences employed for analysis in the present study are given in Table-2.

Table – 2: Accession Numbers and designation of the sequences employed

Genotype	Genotype for the comparison samples
IA	X75215(GBM)
IB	M20273(MBB)
IIB	AY644670(SLF88)
IIIA	FJ360730
IIIB	AB258387
IV	M59286(CY145)
V	D00924(AGM27)

RESULTS

Sporadic cases: Among the 584 sporadic jaundice cases investigated, a total of 348 cases (56.6%) were children and adolescents of less than 19 years of age and 236 cases (43.4%) were adults aged 20 or more years. Among the jaundiced patients, 364 were male (62.3%) and 220 were female (37.7%). In children and adolescents there was no substantial difference in distribution of male and female cases. But in adults, there was a threefold rise in the occurrence of jaundice in males when compared to females.

A total of 262 cases (44.9%) were from the Northern region, followed by 121(20.7 %) from the Southern region, 101(17.3%) from the Western region and 95(16.3%) from the Eastern region representing the entire geographic area of Tamil Nadu. Out of 584 sporadic cases of jaundice investigated, 307 cases (56.6%) were positive for HAV infection of which 239 HAV positives (77.8%) were children below 10 years of age (Table - 3).

Epidemic cases: The population size of Angarayanallur was 1639 (Male 807, Female 832) and 367 (Male 182, Female 185) in Silal village in the jaundice affected area of Perambalur district. Altogether 346 houses were surveyed in both the villages during the outbreak. During house-to-house surveillance, 39

cases with symptoms of acute jaundice were detected of which 28 cases (78.8%) were from Singarayanallur and 11 cases (28.2%) from Silal village. 25 cases (64.1%) were male and 14 cases (35.9%) were female. Serum samples were collected from 23 suspected cases with clinical jaundice.

Table – 3: Age wise distribution of sporadic cases of HAV infection in Tamil Nadu

S. No	Age Group (Yrs)	Nos. Positive	%
1	0 – 1	1	0.3%
2	1 – 5	118	38.4%
3	6 – 10	120	39.1%
4	11 – 19	41	13.4%
5	20 – 59	26	8.5%
6	60 & above	1	0.3%
	Total	307	

Among the 39 jaundice cases, 28 cases (71.8%) were children below 10 years of age, 11 cases (28.2%) were adolescents in the age group of 11-19 years. Adult cases were not found in this outbreak (Table – 4). All the 23 serum samples were positive for anti-HAV IgM and negative for anti-HEV IgM.

Table–4: Age Wise Distribution of Epidemic Cases of HAV infection in Perambalur District, Tamil Nadu

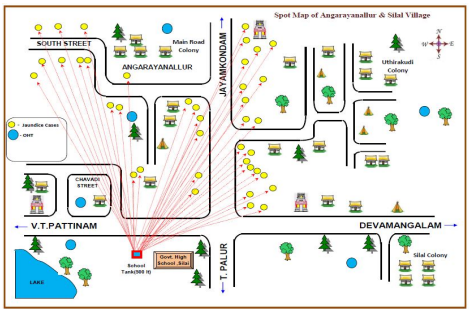
S.No	Age Group	No of Cases	%
1	0 – 1	0	-
2	1 – 5	9	23.1%
3	6 – 10	19	48.7%
4	11 – 19	11	28.2%
5	20 – 59	0	-
6	60 & above	0	-
	Total	39	

Epidemiological Findings: Drinking water supply for both the villages of Angarayanallur and Silal were supplied by Over Head Tanks (OHT) and distributed through public taps. In addition, hand pumps were also installed in certain places of the villages. There are 5 OHTs in Angarayanallur village and 6 OHTs in Silal village.

In Silal village, out of the 6 OHTs four were supplied by bore well water and the remaining two were supplied by Udayarpalayam Combined Water Supply Scheme (CWSS) of Tamil Nadu Water Supply and Drainage Board (TWAD) from Kollidam River, a branch of River Cauvery.

Spot map was drawn to visualize the clustering of jaundice cases in a particular locality to find out the probable source of infection but cases were widely distributed in many places of the village (Figure - 2).

Figure–2: Spot Map Showing Distribution of Jaundice Cases and Source of Infection in Silal and Angarayanallur Village, Perambalur District, Tamil Nadu



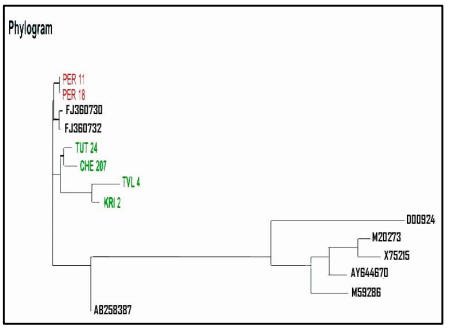
Silal village has a Government High School located at the southern part of the village. The school has a small drinking water tank with a capacity of 500 ls, which received direct water supply from Udayarpalayam Combined Water Supply Scheme through a branch of the main water line supplied to the main OHT with a capacity of 60,000 ls.

Water samples collected from all the OHTs and other water sources at the time of outbreak was found to be of good

quality without any faecal contamination, except the one located within the school premises. This was found to be the source of infection for causing this outbreak of jaundice caused by HAV. This conclusion was drawn due to the fact that the jaundice cases reported in this outbreak were from this school where the suspected contaminated source of water was identified. Isolation of HAV RNA was not attempted from the water samples in this study.

Genotyping of HAV isolates: A total of 6 samples were positive for HAV RNA by RT-PCR out of which 4 were sporadic cases (TUT24, CHE207, TVL4 and KRI12) and 2 were epidemic cases (PER11 and PER18) reported from the Perambalur District. All the six positive samples were subjected to sequencing and the resulted sequences were further subjected to phylogenetic analysis based on 5' NCR region sequences of HAV isolates using Clustal X (Figure- 3).

Figure -3: Phylogenetic analysis based on 5'NCR region sequences of HAV isolates. The epidemic strains are marked red and sporadic cases marked green



The sequences were further subjected to blast analysis and similar sequences were obtained. The similarity analysis clearly indicated that both sporadic and epidemic cases of jaundice caused by HAV isolates in Tamil Nadu were found to be Genotype IIIA as they show more similarity to FJ360730 of Genotype IIIA.

Further analysis showed presence of mutations among the various isolates. The epidemic strains of HAV (PER 11 and PER 18) did not show much variation and hence were exhibited in the same clade whereas minor genetic variation was evident among the strains of sporadic cases exhibited in different clades.

DISCUSSION:

Acute Viral hepatitis is a major public health problem in India, which is hyper endemic for HAV and HEV. Seroprevalence studies reveal that 90%-100% of the population acquires anti-HAV antibody, becoming immune by adolescence (17). This study also shows that HAV infection was found to be the leading cause of jaundice in Tamil Nadu in 2009.

In the present study, out of the 307 HAV positive cases, 238 cases (78%) were in the age group of less than 10 years. In adolescents, there is a gradual decline in HAV infection among the jaundice cases tested with only 13.4% of jaundice cases aged 11-19 years. In adults and aged persons, there is a drastic decline of HAV infection, wherein the seropositivity was 8.5 % in adults in the age group of 20 – 59 years and 0.3 % in the age group of 60 years and above.

Globally, with improvement in socio-economic conditions and its consequences and the availability of effective vaccines, early childhood exposure to hepatitis A virus has decreased. Hence, it has been suggested that a gradual shift in the age of acquiring the infection from early childhood to adulthood in different parts of the world (18). The peak age of seroprevalence is shifting from the 1st decade of life to the 2nd and 3rd decades. This shift in age of acquiring HAV infection from childhood

to older age groups is termed as "Epidemiological Shift".

Whether the concept of epidemiological shift proposed in HAV infection will be applicable to the Indian scenario is debatable. In a country like India, the factors that influence the epidemiology of HAV infection were the extensive variations and heterogeneity in the determinants of acquiring anti-HAV antibodies, diverse economic and social classes and differences in living conditions of populations within the same geographic regions.

In a study conducted in Chennai, Tamil Nadu during 2002, a lower seroprevalence of HAV was observed in 0-2 years of age group (31.6%) when compared to 2 – 6 years (83.1%) and 6–12 years (94.1%) (19). Recently, a profile of Hepatitis A virus infection reported from Chennai found 22.5% seen in 1-5 years age group, 61.6% in 5-10 years and 15.9% in the age group of 10-15 years (20). Both these studies were reported from institutions located in Chennai and not truly representing the status of HAV infection in Tamil Nadu.

The lowest HAV seroprevalence rates among children in India have consistently been reported from Kerala between 4.5% to 10.3% in children below 5 years of age (21, 22). This is in contrast to studies reported from various regions of the Country which have shown the seroprevalence to be between 60-80% in children below 5 years (23, 24, 25).

In the present study, such epidemiological shift has not been observed in Tamil Nadu and HAV infection was predominantly seen in children below the age of 10 years. Interestingly, the findings of the present study did not show a decline trend in childhood HAV seroprevalence rates as predicted, but in contrast it has shown a slightly increased susceptibility to HAV infection in adolescents.

On the other hand, a seroepidemiological survey conducted in North India concluded that at least 90% of Indian children acquire protective antibodies against HAV by the age of 10 years (26). But in the present study conducted in Tamil Nadu (South India), out of 68 jaundice cases in the age group of 11-19 years, 41 cases (60.3%) were found to be positive for HAV infection. This clearly suggests, the preponderance of HAV infection occur not only in children less than 10 years of age, but adolescents in the age group of 11-19 years could also be a target group to get the infection. HAV infection in neonates and infants of less than 1 year was also found to be very low (0.3%) in the present study. However, drawing conclusion with sample size of six cases in this particular age group may not be significant.

As far as epidemic cases of HAV are concerned, an outbreak of hepatitis A occurred in 2005 from Kottayam District of Kerala, in which young adults were mainly involved (16; 27). In another study from Shimla, Himachal Pradesh, North India, the episode was not only confined to children less than 10 years of age (30.9%) but adolescents (54.5%) and adults (14.5%) were also affected (28).

In contrast to the HAV outbreaks occurred in the neighboring state of Kerala and also from Northern India, HAV outbreak reported from Perambalur District of Tamil Nadu in this study found that 28 cases (71.8%) were below 10 years of age and 11 cases (28.2%) were adolescents all attending the school . HAV infection was not seen in adults and there was no intra-familial spread. Similar finding was also reported earlier in an outbreak of hepatitis A virus in an urban slum in Vellore, Tamil Nadu in which all

the cases were in children less than 10 years of age (15).

In HAV disease transmission occurs through faeco-oral route and contamination of drinking water plays a major role in causing infection in the community. This is also evident that the epidemic of HAV reported in this study from Perambalur District of Tamil Nadu, contaminated water supply in the school was found to be the source of infection causing outbreak of jaundice among school children.

Molecular Characterisation of HAV: So far, there has been no report on the circulating genotype of HAV in Tami Nadu. Moreover, genotype variability of sporadic and epidemic strains of HAV in Tamil Nadu has not been studied and reported. Hence molecular characterization of HAV from sporadic and epidemic cases of jaundice was investigated to find out the circulating HAV genotype in Tamil Nadu. The molecular characterization revealed Genotype IIIA in both sporadic and epidemic cases of jaundice.

This finding corroborates with the findings of other HAV outbreaks reported from other parts of India. In a large outbreak of HAV in Kottayam, Kerala, Genotype IIIA was detected in all the samples (16). In a HAV outbreak among children in Pune, Western India, genotype IIIA was identified (13). In another study from the same area, co-circulation of sub genotypes of IIIA and IB was evident and causing co-infections among patients with jaundice (29). Prevalence of genotype IA and IIIA of HAV among jaundice cases were also reported in Northern India (30).

Based on the phylogenetic analysis obtained from this study it was evident that the HAV strains isolated from both sporadic and outbreak cases of jaundice with HAV infection belong to Genotype III A as they show more similarity to FJ 306730. Based on limited genetic analysis genotype III A is likely to be the predominant circulating strain. Further analysis showed presence of mutations among the various isolates. The isolates from the epidemic cases (PER11, PER18) did not show much variation and hence were exhibited in the same clade whereas minor genetic variation was evident among the strains of sporadic cases and were exhibited in different clade. This indicates the existence of minor genetic variations among the HAV isolates in different regions of Tamil Nadu.

CONCLUSION:

The present study was undertaken with an eye on the public health perspective to find out HAV infection among jaundice patients so as to draw a meaningful conclusion on the disease burden caused by HAV infection in the community in Tamil Nadu. The existing disease surveillance system in Tamil Nadu has picked up the early warning signals of jaundice outbreak in Perambalur District, which has facilitated the district public health machinery to initiate prompt action to prevent and control further spread of the disease in the community. Continuous surveillance on the provision of safe drinking water in public places and schools would help to reduce the disease burden caused by water borne diseases to a great extent in a developing country like India.

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