Epidemic hepatitis E: serological evidence for lack of intrafamilial spread

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Introduction: Hepatitis E presents as epidemic as well as sporadic disease. Fecal contamination of drinking water results in epidemics of hepatitis E. The extent of intrafamilial spread needs to be assessed employing serological assays. **Aims:** To understand the dynamics of intrafamilial spread of the disease. **Methods:** The study was conducted using blood samples collected during the 1988 and 1989 epidemics of viral hepatitis in Kudal and Atit villages of Maharashtra state; the epidemics were subsequently shown to be due to hepatitis E virus (HEV). The one-time collection carried out at the end of the Kudal epidemic was from 184 apparently healthy individuals irrespective of family history of jaundice during the epidemic. In the Atit epidemic, 153 family contacts of 49 IgM anti-HEV positive patients were bled. An additional 151 blood samples were collected from apparently healthy individuals irrespective of family history of jaundice during the epidemic. One month later, blood samples were collected from 64 of the 153 family contacts. Relevant history was recorded each time. All serum samples were tested for ALT levels and for IgM and IgG antibodies to hepatitis E virus employing ELISA. **Results:** IgM anti-HEV positivity among persons with family history of jaundice was not different from those without such a history (8/62 [12.9%] and 11/122 [9%] at Kudal; 9/57 [15.8%] and 22/94 [23.4%] at Atit; $p>0.1$). Excluding IgG anti-HEV positive samples from the analysis also yielded non-significant results. Of the 32 follow-up samples collected from family contacts without IgG or IgM antibodies to HEV in the initial blood sample, 31 remained IgM and IgG anti-HEV negative at the end of 1 month. One of the family contacts was found to be IgG anti-HEV positive in the second blood sample. The disease was not related to the index case. **Conclusion:** Intrafamilial spread of HEV is negligible. [*Indian J Gastroenterol* 2000;19:24-28]

Key words: Hepatitis E virus, family contacts, subclinical infection

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Contamination of drinking water with fecal material containing hepatitis E virus (HEV) results in epidemics of the disease. Several such epidemics have been reported from India[1] as well as other developing countries.[2-7] A large number of sporadic acute viral hepatitis patients from India also suffer from hepatitis E.[8] Though high mortality among pregnant women is a characteristic feature of epidemics of hepatitis E, non-pregnant women and men also succumb to sporadic hepatitis E.[9]

For management of contacts of hepatitis E cases, it is necessary to understand the dynamics of intrafamilial spread of the disease. We examined the extent of intrafamilial spread of the virus during epidemics of hepatitis which were subsequently shown to be due to HEV.

Methods

Epidemics

The villages of Kudal and Atit in Satara district of the state of Maharashtra experienced epidemics of hepatitis E during May-July 1988 and January-March 1989, respectively. Unfiltered piped water was supplied to the villages. Turbidity of the drinking water was noted 6 and 7 weeks prior to the first case during the epidemics. On receiving complaints from the villagers, water from the main sources was tested by the local health authorities, and raised *Escherichia coli* content was observed. Following this, hyper-chlorination of the main source was carried out. Subsequent reports of water testing showed *E. coli* counts to be within normal limits.

In all, 92 and 130 clinical cases were recorded respectively in populations of 4335 (Kudal, attack rate 21.2/1000) and 3851 (Atit, attack rate 33.9/1000).

The Figure documents the epidemic curves and time points at which investigations were undertaken. At the request of the Director of Health Services, Maharashtra, visits were made to Kudal and Atit villages and acute-phase serum samples were collected from 54 and 51 patients, respectively for etiological diagnosis. Based on serological analysis the etiology was initially shown to be non-A, non-B hepatitis and later confirmed as HEV.

Field investigations

At the end of the Kudal epidemic, a one-time investigation was carried out (Fig). Blood samples were collected from 184 apparently healthy individuals. Family history of jaundice during the epidemic was elicited.

During the epidemic in Atit village, no serological tests were available and therefore rise in serum ALT levels and/or jaundice were considered as diagnostic

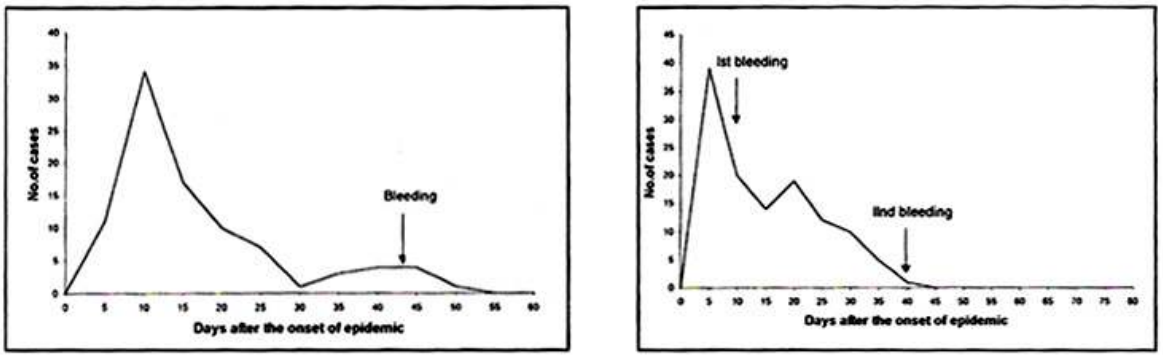

Fig: Epidemic curves and time-points of investigations undertaken during Kudal (left) and Atit epidemics

criteria for viral hepatitis. Following availability of ELISAs for the detection of IgM and IgG antibodies to hepatitis E virus,[1] retrospective serological testing was performed.

Forty-nine patients with hepatitis with IgM anti-HEV in the acute-phase serum sample were included as index cases. These cases as well as other villagers were informed about the possibility of anicteric infections within the family. All those who responded to a call for such an assessment were bled (Fig). These included 153 of 293 (52.2%) family contacts of the 49 index cases. Irrespective of family history of jaundice, 151 additional blood samples were collected from apparently healthy individuals consuming drinking water from the same contaminated source. Relevant history was recorded from all on a standard questionnaire.

One month later, blood samples were collected from 64 of the 153 family contacts bled initially. History of jaundice or associated symptoms was recorded. Two months after the initial bleeding, any history of symptoms associated with hepatitis was recorded from all the 153 study subjects. No blood sample was collected.

Serology

All serum samples were tested for ALT levels[10] as well as for IgM and IgG antibodies to hepatitis E virus with an ELISA which was based on coating of the solid phase with a $55X10^3$ MW protein, expressed in insect cells, from the open reading frame-2 (ORF-2) of HEV.[11] Cut-off value was calculated as mean optical density for negative controls X 3. IgG anti-HEV positive individuals were rebled during the follow up. All such paired samples from the same individual were titrated in ELISA in two-fold dilutions and in the same ELISA plate.

Our earlier studies employing the same ELISA have shown that about 90% of patients in the non-A, non-B hepatitis epidemic were IgM anti-HEV positive up to 3 weeks post-onset. A sharp decline in IgM anti-HEV positivity was noted from week 5 onwards.[1]

Long-term follow up of epidemic hepatitis E patients (IgM anti-HEV positive) documented that IgG anti-HEV antibodies persisted for at least 5 years.[12]

Statistical analysis

χ^2 square test was used for comparing proportions; Yates' correction was applied whenever appropriate. For comparisons of proportions involving smaller denominators, Fischer's exact test was used.

Results

Kudal epidemic

Eighty (43.5%) of the 184 blood samples collected from apparently healthy individuals were found to be IgG anti-HEV positive. The proportions of recent HEV infections (IgM anti-HEV positive) were similar among persons with family history of jaundice (8/62, 12.9%) and those without such a history (11/122, 9%). Excluding IgG anti-HEV positive cases from the analysis also yielded non-significant results (8/36 [22.2%] and 11/68 [16.2%], respectively).

IgM anti-HEV positivity was further analyzed age-wise. In children with or without family history of jaundice, IgM anti-HEV positivity was 2/20 (10%) and 1/57 (1.8%), respectively. Among adults, 6/42 (14.3%) and 10/65 (15.9%) with and without family history of jaundice respectively were IgM anti-HEV positive. The differences were not statistically significant. Thus, the proportion of recent HEV infections with respect to family history of jaundice was independent of age of the contact.

Atit epidemic

Table 1 gives details of the study population and serological status of the family contacts. Sixty of 153 family contacts were $\leq$ 15 years of age. IgG anti-HEV antibodies indicating prior exposure to HEV were present in 41 family contacts (26.8%), which included 3 children (5%).

IgM anti-HEV was present in 24 family contacts, including eight children. Three of these family contacts

Table 1: Atit Village: Study population, HEV sero-status and follow-up

First visit	153 family contacts			
First sample (during outbreak)	41 IgG anti-HEV +ve IgM anti-HEV -ve	24 IgM anti-HEV -ve	88 IgG anti-HEV -ve IgM anti-HEV -ve Hence susceptible	
Second visit Second sample (1 month after outbreak)	10 of 41 bled. None developed HEV infection. No rise in anti-HEV titers in paired sera	22 of 24 bled. All recent HEV infections	32 of 88 bled 31/32 remained IgM and IgG anti-HEV negative. Did not develop HEV infection	1/32 IgG anti-HEV positive. Raised ALT in first sample. Disease not related to index case
Third visit No blood sample, only questionnaire (2 months after outbreak)	None of 153 developed symptoms related to HEV			

had elevated serum ALT levels. One was a 65-year-old contact (aunt) of an index case with jaundice for two days. The other contact was the 28-year-old sister-in-law of an index case who had been jaundiced for 10 days. The third contact was an 11-year-old boy whose father had suffered from clinical hepatitis for 10 days. Thus, infection in all the 3 cases was found to be related to an earlier exposure to HEV and represented multiple infections in the same family.

A 4-year-old niece of an index case also had elevated ALT levels. She tested IgM anti-HAV positive. Subsequently, she was shown to be negative for IgM and IgG anti-HEV antibodies, thereby confirming that she was suffering from hepatitis A alone. None of these contacts developed jaundice or clinical symptoms during the 2-month follow-up period.

In order to identify HEV-susceptible individuals among the 153 persons bled initially, 41 IgG anti-HEV positive and 24 IgM anti-HEV positive family contacts were excluded. The remaining 88 individuals, who could be considered as susceptible to HEV (Table 1), included 42 children.

Of the 64 family contacts whose blood samples were collected again, 24 were children, i.e., 24/60 children and 40/93 adults could be re-bled one month after the initial bleeding. IgM anti-HEV positivity in the initial sample was recorded in 14 adults and 8 children. Ten additional adults were positive for IgG anti-HEV antibodies, indicating prior exposure to HEV. Thus, of the 64 individuals followed up at one month, 32 were shown to have circulating IgM or IgG anti-HEV antibodies at the time of initial bleeding itself. The remaining 32 individuals were negative for both the antibodies and therefore were classified as susceptible to HEV (Table 1).

Of the 32 susceptible individuals, 31 remained IgM and IgG anti-HEV negative at the end of 1 month. One of the family contacts (11/M) was found to be IgG anti-HEV positive in the second blood sample. He had elevated ALT levels at the time of initial bleeding and developed clinical disease subsequently. The index case for this family contact had clinical disease for 10 days when initial bleeding was undertaken. Thus, development of hepatitis E in this contact was not related to transmission from the index case.

Overall, none of the contacts demonstrated evidence of hepatitis E infection at the 1-month follow up. The mean (SD) post-onset day of clinical symptoms (POD) of the index case at the time of second sample collection was 41 (11) days. None of the ten IgG anti-HEV positive family contacts demonstrated a rise in anti-HEV IgG titers when samples collected one month apart from the same individual were tested in two-fold dilutions in the same ELISA plate.

At the end of the second month, symptoms related to hepatitis E were not noted in any of the 153 family contacts included initially in this study. No blood sample was collected at this time.

HEV transmission to spouse and other family contacts

Among the family contacts studied, 14 spouses were also bled. At the time of initial bleeding, 6 of these (42.8%) were found to be positive for IgM anti-HEV whereas 6 other spouses (42.8%) were positive for IgG anti-HEV antibodies. Of the other family contacts of the same 14 cases, IgM anti-HEV positivity was found in 29.7% (11/37). The difference was not significant.

Exclusion of IgG anti-HEV positive contacts (prior exposure and probably immune) from analysis yielded 6/8 and 11/28 IgM anti-HEV positivity in spouses and other family contacts, respectively. The difference was not statistically significant.

In order to ascertain if IgM anti-HEV positivity among the contacts was related to transmission from the index case or a common source, the POD of the index case at the time of the initial bleeding of the contacts was taken into consideration (incubation period of hepatitis E: 2-7 weeks). When the IgM anti-HEV positive contacts were bled after 21 days following onset of the index case, transmission from the index case was con-

Table 2: HEV seromarkers among large families*

Category	Family 1		Family 2		Family 3		Family 4	
	<15 y	>15 y	<15 y	>15 y	<15 y	>15 y	<15 y	>15 y
No. of contacts	3	10	7	6	1	9	5	4
IgM anti-HEV +ve	2	2	3	-	1	5	1	1
IgM anti-HEV -ve + IgG anti-HEV +ve	-	2	-	2	-	2	1	1
IgM anti-HEV -ve + IgG anti-HEV -ve**	1	6	4	4	-	2	3	2

*At time of initial bleeding

**None developed HEV infection during follow-up

sidered possible. At the time of initial bleeding of the IgM anti-HEV positive spouses, the index cases were having symptoms for 30, 30, 15, 15, 15 and 5 days. For other IgM anti-HEV positive contacts, the PODs of the index case were 30, 30, 30, 15, 12, 12, 12, 10, 5, 5 and 2. The probability of transmission from the index case to susceptible spouses (2/7, 28.5%) or other family contacts (3/28, 10.7%) was not statistically different. As 12/14 (85.7%) spouses were either IgM or IgG anti-HEV positive at the time of initial bleeding itself, subsequent follow up was not relevant.

Family size and HEV spread

Twenty-six families including 1-13 contacts reported multiple HEV infections ranging from 1-6 IgM anti-HEV positive cases. Single HEV infections (i.e., only the index case) were recorded in 23 families consisting of 1-4 family contacts. This study included 4 large families consisting of 9, 10, 13 and 13 contacts. None of the susceptible contacts (IgG and IgM anti-HEV negative) developed IgM or IgG anti-HEV antibodies or clinical disease during subsequent follow up (Table 2).

Serological analysis of additional samples collected

IgM anti-HEV positivity was 15.8% (9/57) and 23.4% (22/94) among those in Atit with and without family history of jaundice, respectively. The difference was not statistically significant. Excluding IgG anti-HEV positive cases from analysis also yielded non-significant results (9/34 [26.5%] and 22/55 [40%], respectively).

Discussion

With the availability of serological tests for IgM anti-HEV, in countries like India a large number of sporadic acute viral hepatitis cases would be diagnosed as hepatitis E. The immediate concern of the treating physicians and patients would be the risk of intrafamilial spread of the virus. Epidemics of hepatitis E are not followed by distinct secondary peaks, indicating absence of frequent person-to-person transmission. The present study shows that intrafamilial spread of HEV is insignificant.

Though the present investigation was executed in 1988-89, retrospective serological analysis was carried out only recently. Not surprisingly, since hepatitis E is an endemic enteric disease,[9,13] 40.8% of adult family contacts and 5% of child family contacts were found to be IgG anti-HEV positive, indicating prior exposure to the virus. Based on anti-HEV status of the contacts' initial sample, the contacts were classified as "susceptible" or immune to HEV infection. Thus, care was taken to evaluate "true" susceptibles for possibility of intrafamilial transmission of HEV. Our data show that such transmission is negligible, confirming previous reports based on questionnaire surveys without serological analysis.[14,15]

During investigation of an epidemic of hepatitis E in Khadakwasla village in 1989, a house-to-house questionnaire survey of the entire village was conducted by us two months after the last case was reported.[14] Evidence of probable secondary infection (time interval between index and secondary cases >30 days) could be obtained in 4 families (2%). Similar results were obtained by Aggarwal and Naik[15] during their study of a large epidemic of hepatitis in Kanpur city (1991) involving 79,000 cases. The possibility of secondary spread (time interval between index and secondary cases 2-7 weeks) was noted for 8/111 cases (7.2%) surveyed. If the criteria for secondary case (time interval 2-7 weeks) is kept constant for both studies, the proportion of probable secondary cases during epidemics of hepatitis E in rural (Khadakwasla, 2%) and urban (Kanpur, 4.5%) settings is small and similar.

The prevalence of IgG and IgM anti-HEV antibodies among contacts of sporadic hepatitis E cases from Pune (25/66) was not different from contacts of sporadic non-E hepatitis cases (73/117; our unpublished data). Though follow-up blood samples or history of clinical hepatitis were not available from these contacts, we conclude that extensive intrafamilial spread of HEV does not occur in the sporadic situation.

The conclusion of Khuroo and Dar of frequent intrafamilial spread of hepatitis E among contacts of sporadic cases of the disease was based on ALT rises[16] (18/62 among contacts versus 0/14 among controls). It may also be attributed to selection bias and small sample size.

Peak clinical attack rates among young adults in the age group of 15-40 years have been a characteristic feature of epidemics of hepatitis E. This was suspected to be due to (i) higher subclinical infections among children and/or (ii) lesser exposure of the pediatric population to the virus. Following development of sen-

sitive serologic assays for anti-HEV antibodies, we found that exposure to HEV was indeed significantly higher among those who were above 15 years of age.[13] One of the possible but unlikely reasons for higher exposure to HEV could be sexual transmission of the virus. During the present study, we investigated transmission of HEV from 14 index patients to their spouses and other familial contacts. By excluding (i) IgG anti-HEV positive contacts as evidenced by the first sample and (ii) contacts positive for IgM anti-HEV within 21 days of onset of clinical symptoms in the index patients, transmission of HEV to susceptible spouses (2/8) was not significantly different from that seen in other family contacts (3/28). Based on interview of 385 couples during the epidemic of hepatitis E at Kanpur which included 26 cases of hepatitis, Aggarwal and Naik concluded that sexual spread of HEV was negligible.[15]

Another study[16] based on sporadic hepatitis E patients (non-A, non-B, non-CMV, non-EBV, without serological analysis for HEV) also did not find a difference in the incidence of hepatitis E in sexual (3/8) and non-sexual (15/54) contacts. Though the number of spouses investigated remains small in all the studies reported so far, it appears that sexual transmission does not play a significant role in the transmission of HEV.

We conclude that intrafamilial spread of HEV is negligible. For effective control of this enteric infection, emphasis must be given to the supply of safe drinking water.

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