Published in final edited form as:

Eur J Clin Microbiol Infect Dis. 2014 May ; 33(5): 755–759. doi:10.1007/s10096-013-2007-5.

Duration of Viraemia in Chinese Acute Sporadic Hepatitis E

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Abstract

Purpose—Acute sporadic hepatitis E (ASHE) cases induced by hepatitis E virus genotype 4 (HEV-4) are increasing in China. Our study aimed to estimate the duration of HEV-4 viraemia in Chinese ASHE.

Methods—A total of 619 serum specimens from 499 ASHE patients were examined for the presence of HEV RNA. The association between viraemia detection and serum sampling time was compared between subtypes. The cumulative probability of HEV viraemia detection was determined using Kaplan-Meier survival analysis, and the viraemia duration was estimated.

Results—A total of 42.7% serum specimens were positive for HEV RNA and all the isolated strains were identified as genotype 4 and subsequently assigned to five subtypes. Among the patients infected with subtypes 4d and 4i, the time interval from the initiation of clinical symptoms to serum specimen sampling was shorter than that among the patients with subtypes 4a, 4b, and 4h. Kaplan-Meier analysis was conducted with 101 sequential specimens as well as with both 101 sequential specimens and 236 single negative specimens. The cumulative probability of HEV-4 viraemia detection was estimated to decline quickly to approximately 10% within 32 days after the initiation of clinical symptoms and then to decline very slowly to 5% by the 41st day and to zero by the 131st day.

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Conflict of interest: None.

Conclusions—The majority of ASHE cases maintain detectable HEV-4 viraemia within one month after onset, whereas a small portion of cases maintain long-term viraemia and may act as a reservoir for further transmission.

Keywords

Hepatitis E virus; Genotype 4; Viraemia; Phylogenetic analysis; China

Introduction

Acute hepatitis E is induced by hepatitis E virus (HEV) infection. Large outbreaks only occur in developing countries that are highly endemic for HEV [1], whereas sporadic cases are increasing in both developing countries [2,3] and some industrialised countries where HEV is non-endemic [4,5]. Many HEV infections typically develop self-limiting hepatitis without obvious clinical symptoms [6], and many clinical cases also recover rapidly upon treatment [7]. Therefore, it is difficult to determine the duration of transient HEV viraemia. In the epidemic in 2008 in Nellore, India, it was reported that the serum specimens positive for HEV RNA were all sampled within two weeks after the onset of clinical symptoms [8]. However, the viraemia time may be longer in acute sporadic hepatitis E (ASHE) cases. One study conducted in India observed that the maximum duration after the onset of the first symptom at which a serum sample tested positive was 45 days [7]. In another study in India, the viraemia was detected in 6.6% of samples by the seventh week from the onset of symptoms [9]. In China, the average duration of HEV viraemia has been reported to be 21 days after the onset of illness [10].

In China, HEV genotype 4 (HEV-4) is dominant, and thus far, seven subtypes of HEV-4 (4a, 4b, 4c, 4d, 4e, 4h, and 4i) have been identified in humans [11], but the duration of HEV-4 viraemia in Chinese ASHE is still understudied. Our study aimed to determine the association between serum sampling time and detection of viraemia, in addition to further estimating the viraemia duration in Chinese ASHE.

Materials and methods

Subjects

We collected new ASHE cases from hospitals. They were diagnosed via reactivity to anti-HEV IgM (Wantai Co., Ltd., Beijing, China) and non-reactivity to antibodies of hepatitis A and C viruses. Upon diagnosis, the first serum specimen of the patient was sampled. After that, one or more subsequent serum specimens were sampled. The patients' demographic and clinical information, including sex, age, diagnosis date, date of serum sampling, and date of self-reported initiation of clinical symptoms were also collected. Informed consent was obtained from all patients. Our study was approved by the institutional review board of the Fudan University School of Public Health (IRB 00002408 & FWA 00002399) under IRB #07-03-0074 and IRB #2010-03-0217.

From 2005 to 2011, we enrolled a total of 499 ASHE patients from local hospitals in Shanghai, Jiangsu, Zhejiang, Anhui in eastern China, as well as Guangxi in southwestern

China (Table 1). Out of all participating patients, 499 first serum specimens were sampled at diagnosis. Subsequently, for 53 patients, 120 subsequent specimens were sampled 1-13 weeks after diagnosis.

HEV RNA detection

HEV RNA was extracted from 100 µL of ASHE patient serum specimens. An 821 nucleotide (nt) sequence in HEV ORF1 (corresponding to 3961-4781 nt of the prototype Burmese HEV strain, GenBank accession no. M73218) was amplified with reverse transcription nested PCR, which has been previously described [12,13]. Duplicate assays were conducted for positive results to avoid contamination.

Determination of genotype and subtype

Positive PCR products were sequenced in both directions to resolve possible ambiguous nucleotides. The nucleotide sequences were used to determine genotypes and subtypes by comparison with known full-length HEV strains available in GenBank with MEGA v5.05 software (www.megasoftware.net). The HEV sequences detected and sequenced completely in our study were deposited in the GenBank database under accession nos. KC184491 - KC184695.

Statistical methods

A statistical analysis set is shown in Fig. 1. General statistics, including *t*-test results, one-way ANOVA, Chi-square test results, and nonparametric test results, were used where applicable. In our study, there were no cases where HEV RNA tested negative in a sample but positive in a subsequent sample. Thus, Kaplan-Meier survival analysis could be employed to estimate the viraemia duration. In the detection of HEV RNA in sequential serum specimens, the first “negative result” was regarded as an “event” in the survival analysis, and then subsequent “negative results” were excluded because an “event” occurs only once in the survival analysis. “Positive results” were regarded as “censored data” because we did not know when the samples exactly became negative according to HEV RNA assays. Based on the “observed survival time” (time interval from initiation of clinical symptoms to serum specimen sampling, which is defined as “lag time” in our study), the Kaplan-Meier method can illustrate the survival curve to determine the “real survival time” (duration of HEV viraemia). To meet the applicable conditions of Kaplan-Meier method, single negative specimens were also included (Fig. 1). A log-rank test was conducted to compare the duration between male and female cases. All analyses were performed by using SPSS 16.0 (SPSS Inc., USA).

Results

Viraemia detection in ASHE

Of the single and first sequential specimens sampled at diagnosis, 42.7% (213/499) were positive for HEV RNA. There were significant differences in sex, areas, and diagnosis months between the positive and negative patients, except in age (Table 1). Of 53 first specimens and 120 subsequent specimens, 30.1% (52/173) were positive for HEV RNA (Fig. 2).

HEV genotype and subtypes

In our study, 205 fragments of 821 nt were successfully sequenced and all classified as HEV-4. There were subtypes 4a (n=104), 4b (n=39), 4d (n=28), 4h (n=15), and 4i (n=19). The distribution of subtypes was not significantly correlated with age ($P=0.07$), sex ($P=0.21$), or diagnosis months ($P=0.38$), whereas spatial distribution was very different ($P<0.001$). In eastern China, subtype 4a was predominant (53.9%), whereas in southwestern China, subtype 4b was significantly dominant (93.3%).

Lag time and viraemia estimation

The positive specimens had a significantly shorter lag time than the negative specimens in the single and first sequential specimens ($P<0.001$) as well as in the sequential specimens ($P=0.002$) (Table 2). Subtypes 4d and 4i cases had shorter lag times compared with subtypes 4a, 4b, and 4h cases, but the differences were not significant ($P=0.06$).

The Kaplan-Meier method was employed to estimate the duration of viraemia (Fig. 1). The cumulative probability of detection declined to 10% (analysis A, 101 sequential specimens) and 11% (analysis B, 101 sequential specimens and 236 single negative specimens with recorded lag time) within 32 days after initiation of clinical symptoms (Fig. 3). The probability decreased to zero by the 43rd day (A) and decreased to 5% by the 41st day and to zero by the 131st day (B). The mean duration was 22.5 days (A) and 20.4 days (B). The 75, 50, and 25 percentiles were 15, 21, and 30 days (A) and 11, 17, and 26 days (B), respectively. There was no significant difference in the duration between males and females ($P=0.16$ in analysis A and $P=0.72$ in analysis B).

Discussion

In our study, the duration of viraemia and the trends were determined based on the association between serum sampling time and detection of viraemia using Kaplan-Meier survival analysis. Considering the small sample size of sequential specimens (173 sequential specimens from 53 ASHE patients vs. 446 single specimens) in the study, we estimated and compared the duration of HEV-4 viraemia using either only sequential specimens or both sequential specimens and single negative specimens. The Kaplan-Meier curves were similar, and the cumulative probability of detection declined quickly to approximately 10% within 32 days after the onset. However, in analysis B, with both sequential specimens and single negative specimens, the curve declined slowly after 41 days, suggesting the long persistence of viraemia only occurs in less than 5% of ASHE cases. Direct observation of sequential tests may result in imprecise estimation of HEV viraemia duration. In previous studies, the viraemia duration was estimated to be 3-7 weeks [7,9,10]; however, those studies could not provide detailed trends or adjustment for some abnormal values, which may have biased the results. Our results indicate a declining trend of viraemia detection with time after onset. Although lag time was estimated according to the patients' self-reporting, which may be somewhat inaccurate, our results were more precise because the Kaplan-Meier method can estimate survival time based on observational data, allowing for some error [14]. The data indicate that HEV-4 viraemia declined within one month after onset. Less than 5% of

HEV-4 viraemia cases lasted 1-4 months. The lengthy viraemia may increase the risk of the infected patient acting as a reservoir for further transmission.

In our study, only 43% of specimens were positive for HEV RNA. The detection rates of HEV-4 viraemia in ASHE cases vary greatly between 20 and 70% in China [15,16], depending on the test methods, inclusion criteria of ASHE patients, illness progress and lag time. Our results also depended on PCR assay sensitivity level. Given that we collected serum specimens sampled at diagnosis when the patients had not yet received medical treatment, our results were considered to be characteristic of real infection status.

In China, HEV-4 has been increasingly predominant [11]. Previous studies have reported that HEV-4 infections have been associated with increased transaminase levels compared with HEV genotype 3 infections [17,18], which may suggest longer and more severe liver lesions among HEV-4 infections and indicate a longer duration of viraemia. The HEV-4 subtypes identified in our study were consistent with the results in previous studies [11]. We found that subtypes 4d and 4i had shorter lag times than subtypes 4a, 4b, and 4h. However, we cannot determine and compare the duration of viraemia as we do not know when patients became HEV RNA negative for different subtypes. In addition, we identified limited subtypes and isolated few strains of some subtypes, which may have biased the result. Thus, further studies targeting the clinical difference in ASHE cases induced by diverse genotypes and subtypes are warranted.

Acknowledgments

We thank Ms. Bethanie Rammer for editing this manuscript.

Funding: This work was supported by the China National Natural Science Funds [grant number 81001264, 30872158], Shanghai Municipal Health Bureau [grant number 2010-177], and in part, the U.S. National Institute of Health / Fogarty International Centre [grant number D43TW008313].

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Fig. 1.

Statistical analysis set of the serum specimens collected from acute sporadic hepatitis E (ASHE) cases. General statistics were conducted with 499 ASHE cases. Kaplan-Meier analysis A was conducted with 101 sequential specimens (53 first specimens and 48 subsequent specimens), whereas analysis B was recorded with 101 sequential specimens and 236 single negative specimens with recorded lag time

Fig. 2.

Detection of hepatitis E virus genotype 4 RNA in 173 sequentially sampled serum specimens. The black and white dots represent positive and negative results for the HEV RNA test, respectively

Fig. 3.

Cumulative probability of viraemia detection of hepatitis E virus genotype 4 (HEV-4) using Kaplan-Meier survival analysis. The 75, 50, 25 percentiles and mean of cumulative probability were 15 (95% confidence interval: 13.0-17.0), 21 (18.7-23.3), 30 (26.7-33.3), and 22.5 (19.7-25.2) days (A) and 11 (9.8-12.2), 17 (15.2-18.8), 26 (23.7-28.3), and 20.4 (18.7-22.2) days (B)

Table 1
Detection of hepatitis E virus (HEV) RNA among acute sporadic hepatitis E patients

	HEV RNA positive	HEV RNA negative	<i>P</i> for difference
Age (average, year)	51.8±13.2	49.1±15.3	>0.05
Sex (No. cases)			0.001
Male	174 (46.9)*	197 (53.1)	
female	39 (30.5)	89 (69.5)	
Area (No. cases)			<0.001
Shanghai	70 (37.0)	119 (63.0)	
Jiangsu	64 (75.3)	21 (24.7)	
Zhejiang	10 (41.7)	14 (58.3)	
Anhui	54 (39.4)	83 (60.6)	
Guangxi	15 (23.4)	49 (76.6)	
Diagnosis month (No. cases)	-	-	0.032
Jan-Feb	58 (46.8)	66 (53.2)	
Mar-Apr	72 (47.4)	80 (52.6)	
May-Jun	27 (29.3)	65 (70.7)	
Jul-Aug	16 (32.7)	33 (67.3)	
Sep-Oct	18 (50.0)	18 (50.0)	
Nov-Dec	22 (47.8)	24 (52.2)	

* Numbers in the parentheses represent row percentages (%)

Table 2
Lag time of the specimens positive and negative for hepatitis E virus (HEV) RNA (days)

	No. cases *	25 percentile	50 percentile	75 percentile	mean
Single and first serum specimens					
HEV RNA positive	178	7	9	15	11.3
subtype 4a	78	7	10	16.3	12.2
subtype 4b	38	8	10.5	16	11.8
subtype 4d	25	6	8	11	8.8
subtype 4h	13	6.5	7	16	10.7
subtype 4i	17	6	9	12.5	8.8
HEV RNA negative	258	10	16	24	19
Sequential serum specimens					
HEV RNA positive	52	7.3	11	16	12.9
HEV RNA negative	49 **	10.5	16	23.5	18.7

* Not all the specimens were recorded with lag time.

** In the same patients' sequential specimens, repeated negative specimens were excluded.