ORIGINAL ARTICLE

A Seroprevalence Study of Viral Hepatitis E Infection in Human Immunodeficiency Virus Type 1 Infected Subjects in Malaysia

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Summary

Hepatitis E virus (HEV) is a RNA virus transmitted enterically. A study of anti-HEV antibodies in 145 human immunodeficiency virus type 1 (HIV-1) infected subjects found that 14.4% of them were reactive to anti-HEV antibodies. Anti-HEV IgG and anti-HEV IgM was detected in 10.3% and 4.1% of the subjects respectively. Prevalence of anti-HEV (either IgG or IgM) was similar across all adult ages (p=0.154), between the three ethnic groups (p=0.378), and across risk groups (p=0.120). The results showed that HEV infection in subjects recruited in this study was most likely transmitted via faecal-route.

Key Words: Hepatitis E virus, Human immunodeficiency virus infection, Heterosexual, Intravenous drug use, Faecal-oral route

Introduction

Hepatitis E virus (HEV) is an enterically transmitted pathogen and is epidemiologically distinct from hepatitis A virus (HAV). The HEV infection is not associated with chronic liver disease or other serious sequelae; the disease is usually mild except in pregnant women in the third trimester, who may suffer a high fatality rate from fulminant hepatic failure[1]. HEV belongs to the Caliciviridae family, the genome consists of 5'-non-coding region, non-structural region, structural region and 3'-non-coding region. It has a single stranded, positive-sense RNA genome of about 7.5kb with three open reading frames (ORFs). ORF1 encodes the non-structural polypeptides including helicases and RNA-dependent RNA polymerases, ORF2 encodes the structural protein of viral capsid and ORF3 encodes an immunoreactive peptide[2]. The low level of nucleotide variations and extensive antigenic cross reaction among HEV strains isolated from different parts of world indicated the existence of a single serotype[3]. To date there have been two major groups of HEV strains described: the Burmese and Mexican groups. There is variation between the genomes of these major groups allowing for the determination of the geographic origin of the strains[2].

HEV is responsible for epidemic and sporadic acute hepatitis especially in developing countries. Three major epidemics caused by HEV have been recorded in Kathmandu Valley in 1973, 1981 and 1987[4]. In the

Indian subcontinent, the outbreak of HEV infection in 1955 - 1956 affected 29000 people, and in 1975 - 76, another epidemic hepatitis associated with HEV was reported involving 2572 subjects[5,6]. Outbreaks of HEV also have been reported in Egypt[7], Sudan[8], Ethiopia[9], Vietnam[10] and Kuwait[11].

Sporadic cases of acute hepatitis in developing countries have been found to be associated with HEV. In Southern India, HEV is a major cause of acute hepatitis, which accounted for 32.5% of subjects as compared to only 12% with HAV[12]. The prevalence of antibody to HEV varies with the reporting countries. In China, 18.2% of healthy subjects were found to have anti-HEV IgG[13]. High prevalence of anti-HEV in children, blood donors and prisoners was reported in Chile[14]. In Hong Kong, 16.1% of 355 normal healthy subjects surveyed were positive for anti-HEV IgG; in the same study, 16.5 % of patients with acute hepatitis were found positive for anti-HEV IgG and 5.8% of them were also positive for anti-HEV IgM[15]. Numerous seroprevalence studies suggested that anti-HEV IgG is uncommon in western countries and sporadic cases of acute HEV infection in developed countries were related to travel in endemic areas, in immigrants and UN peace keeping force[16,17,18].

Anti-HEV positively increased with age; subjects aged 50 years or older had a prevalence of 24.5% against 2.2% in individuals below 50 years of age[19]. Reports suggest that patients with a high risk for hepatitis B and hepatitis C could also be infected by HEV and that there is an association between HCV and HEV infection. Halfon *et al*[20] found that 7 of 16 HEV antibody-positive subjects were also HCV-antibody positive. Pisanti, Coppola & Galli[19] also found patients with anti-HCV have an increased risk of HEV infection. They reported that anti-HEV prevalence was 27% among anti-HCV-positive individuals versus 2% among anti-HCV-negative individuals. HIV positive patients acquired through intravenous drug abuse and homosexual activities also have increased risk of HEV infection[21].

The objective of this study is to investigate the seroprevalence of anti-HEV in a group of HIV-1 reactive subjects in Malaysia.

Materials and Methods

Subjects

A total of 145 confirmed HIV-1 infected subjects were recruited in this study. All subjects consented to the study which included an interview and collection of 10ml blood by venipuncture. Eight subjects were recruited from the University Hospital, 137 subjects from various Drug Rehabilitation Centres in Klang Valley and Kajang.

A set of questionnaires modified from questionnaires used by Fogarty program of Johns Hopkins University HIV study in Thailand was used to collect demographic data, history of drug use, sexual behaviour and recent illnesses including fever, jaundice, cough, watery diarrhoea, etc. There were 3 children aged 1, 3 and 10 years old and the rest were 142 adults (Table I). There were 8 females, 133 males and 4 transsexuals. The majority of the HIV-1 infected subjects were Malays 58%, followed by Chinese 27%, Indians 14% and others 1%.

Detection of anti-HEV IgG

Abbott HEV EIA (Abbott Laboratories, Abbott Park, IL 60064, USA), an *in vitro* qualitative EIA was used in the study. The polystyrene bead was coated with two recombinant antigens: SG-3 and 8-5 derived from Open Reading Frame (ORS) 2 and ORS 3 of the Burmese strain of HEV. The assay procedure recommended by the manufacturer was followed. Briefly, it involved a specimen dilution step, two incubation steps and a step for color development. The specimens with absorbance values equal to or greater than 0.005 (minimum patient reactive absorbance) but less than the Cutoff Value were considered negative. The specimens with absorbance values greater than or equal to the Cutoff Value were considered as initially reactive and the original sample was retested in duplicate. Only repeatedly reactive samples were considered as positive for anti-HEV IgG.

Detection of anti-HEV IgM

HEV IgM ELISA (Genelabs Diagnostics, Singapore), an enzyme-linked immunosorbent assay for the detection of IgM antibodies to HEV in serum was used in this

Table I
Demographic Data and Risk Factors Associated with HIV-1 Infection

Variables	n (%)
Total	145
Gender	
Male	133 (92)
Female	8 (6)
Transsexual	4 (3)
Age groups (years)	
≤ 20	3 (2)
21 - 29	38 (26)
30 - 39	74 (51)
≥ 40	30 (21)
Ethnic group	
Malays	84 (58)
Chinese	39 (27)
Indians	20 (14)
Others	2 (1)
Risk group	
IVDU + Heterosexual	86 (59)
IVDU	32 (22)
Heterosexual	20 (14)
Homosexual	1 (1)
IVDU + Homosexual	3 (2)
Others*	3 (2)

**2 vertical transmission and 1 blood transfusion*

study. The wells of the polystyrene microplate strips were coated with 3 recombinant HEV antigens derived from the structural regions of the HEV. The samples were diluted in diluent buffer, a mouse monoclonal anti-human IgM labelled with horseradish peroxidase was used as conjugate, the substrate solution consisted of hydrogen peroxide and o-Phenylenediamine Dihydrochloride. The assay procedures consisted of standard ELISA formats and specimens with absorbance values less than the Cut-Off value were considered non-reactive to anti-HEV IgM. Specimens with absorbance values greater than or equal to the Cut-Off value were considered initially reactive and were retested in duplicate before interpretation. Only repeatedly reactive samples were considered as positive for anti-HEV IgM.

Statistical analysis

Frequency distribution of each potential risk factor were presented to describe the sample population. Age was categorized as <20, 20 - 29, 30 - 39 and ≥40. Risk group was defined as most common route for exposure to HIV-1. The association between each risk factor and anti-HEV antibody presence was evaluated using the Chi-square test or Fisher's exact test. Statistical significance was defined at $p<0.05$. Due to small numbers in the "Others" ethnic category and of homosexual or "other risk group", these categories were excluded from statistical tests.

Table II
Ethnicity and Risk Factors in HIV-1 Infected Patients

Ethnic Groups	n	Risk factors					
		IVDU + Hetero-Sexual	IVDU	Hetero-Sexual	Homo-Sexual	IVDU + Homo-Sexual	Others
Malays	84 (58%)	63	19			1	1*
Chinese	39 (27%)	12	7	16		2	2**
Indians	20 (14%)	11	6	3			
Others	2 (1%)			1	1		
Total	145 (100%)	86 (59%)	32 (22%)	20 (14%)	1 (1%)	3 (2%)	3 (2%)

**Blood transfusion **Vertical transmission*

Results

Anti-HEV IgG was detected in 10.3% (15/145) of HIV-1 infected subjects. Two of the 3 children were also found to have the antibody to HEV. The infection was found in adults of all age groups. Thirteen percent (5/38) of subjects in 20 - 29 years old were found to have anti-HEV IgG; 6.7% (5/74) and 10% (3/30) of subjects in the age-groups 30 - 39 years old and ≥40 years old, respectively, were found to have anti-HEV IgG. The anti-HEV IgG rates were significantly different by age groups (p=0.009), overall. When excluding the 3 children, however, the rates did not differ by age group (p=0.530).

Among the samples tested for anti-HEV IgM, only 4.1% (6/145) of subjects were found to be repeatedly reactive and hence have anti-HEV IgM. The adults of all age groups were also equally susceptible to HEV infection as indicated by anti-HEV IgM+ (p=0.231) (Table III). The individuals with anti-HEV IgM antibodies reported no active symptoms of hepatitis (jaundice, anorexia, fatigue). None of the subjects was found to have both anti-HEV IgG and IgM antibodies in this study.

Any anti-HEV antibody (either IgG+ or IgM+) was detected in 14% (21/145) of HIV-1 infected subjects. Prevalence of any anti-HEV antibody differed by age group while including the children (p=0.07), however, did not differ among the adult ages ≥20 (p=0.154). Thirty-seven percent of the females had detectable anti-HEV antibodies, whereas, 14% of the males had detectable anti-HEV antibodies (p=0.098). This was suggestive of gender differences, although there were too few females (n=8) to confirm this.

Table III
The Prevalence of Anti-HEV IgG and IgM Antibodies in HIV-1 Infected Patients

Risk Factor	n	IgG+ [n (%)]	IgM+ [n (%)]	HEV*+ [n (%)]
Total	145	15 (10)	6 (4)	21 (14)
Gender				
Male	133	12 (9)	6 (5)	18 (14)
Female	8	3 (37)	0 (0)	3 (37)
Transsexual	4	0 (0)	0 (0)	0 (0)
Age groups (years)				
< 20	3	2 (67)	0 (0)	2 (67)
20 - 29	38	5 (13)	2 (5)	7 (18)
30 - 39	74	5 (7)	1 (1)	6 (8)
≥ 40	30	3 (10)	3 (10)	6 (20)
Ethnic group				
Malays	84	7 (8)	3 (4)	10 (12)
Chinese	39	5 (13)	3 (8)	8 (21)
Indians	20	2 (10)	0 (0)	2 (10)
Others	2	1 (50)	0 (0)	1 (50)
Risk group				
IVDU + Heterosexual	86	6 (7)	2 (2)	8 (9)
IVDU	32	3 (9)	3 (9)	6 (19)
Heterosexual	20	4 (20)	1 (5)	5 (25)
Homosexual	1	0 (0)	0 (0)	0 (0)
IVDU + Homosexual	3	0 (0)	0 (0)	0 (0)
Others**	3	2 (67)	0 (0)	2 (67)

**defined as having either IgM + or IgG +*
***Blood transfusion and vertical transmission*

For the subjects with detectable anti-HEV antibodies (either IgG+ or IgM+), there were 8 Chinese, 10 Malays, 2 Indians and 1 Others (Table III). By excluding "others", there was no statistical difference in rates of HEV infection (p=0.378) among the three major ethnic groups. The HEV infection rate among the Chinese was higher, but not statistically higher than either Indians (p=0.469) or Malays (p=0.273). The 2 females found to have anti-HEV were both Chinese, one of the women was the mother of a child with HIV infection acquired through vertical transmission. The 10 years old Malay girl who acquired HIV through blood transfusion was also reactive to anti-HEV antibodies.

The risk factor associated with HIV infection was different in different races (Table II). The majority (86/145) of the subjects reported a combination of both IVDU and heterosexual activities as the most common risk factors associated with HIV infection. Intravenous drug use was a common risk factor among the Malays (83/84) but heterosexual risk was found more common among the Chinese (28/29). Homosexual activity was not a common risk factor among the HIV-1 infected subjects in this sample, and only one transsexual reported homosexual activity as the only risk factor linked to her HIV infection. The prevalence of anti-HEV antibodies in subjects with IVDU + heterosexual risks, IVDU risk and heterosexual risk was 9%, 19% and 25%, respectively (Table III). The 3 risk groups did not differ with respect to the prevalence of anti-HEV IgG (p=0.199), anti-HEV IgM (p=0.245) or either IgG or IgM (p=0.120). The 4 transsexual prostitutes included in the study reported having such activities, but none of them had anti-HEV antibodies. The number for those reporting homosexual or other exposure to HIV-1 were too small (n=7 total) for any statistical comparisons with IVDU or heterosexual risk.

Discussion

Prior to this study, there have been no cases of HEV infection reported in Malaysia. A seroprevalence study of anti-HEV antibodies in 315 women attending the Antenatal Clinic (ANC) in University Hospital in 1996 found that none of the women had anti-HEV IgG (unpublished data). Therefore, this is the first report on prevalence of anti-HEV antibodies in a group of HIV-1 infected subjects in Malaysia.

HIV infected subjects, being immunosuppressed, are more prone to a wide range of opportunistic infections including fungi, parasites, bacteria and viruses. The detection of anti-HEV antibodies in HIV positive subjects in this study demonstrated an additional infection that could be found in individual with HIV infection. This is a mostly adult (age >20) male IVDU+ heterosexual population. The seroprevalence of anti-HEV IgG was 10.3%, however, only 4.1% of the subjects were found to have anti-HEV IgM. The clinical significance of anti-HEV IgM was difficult to assess as none of these subjects complained of symptoms of acute hepatitis. The prevalence of anti-HEV in Chinese was higher than in Malays and Indians, although not statistically significant. The high prevalence of anti-HEV among Chinese may be related to the higher HBV infection rate in Chinese suggested by Halfon *et al*[20] on risk factors associated with HEV infection. The role of co-infection of hepatitis B, C and/or E on the natural history of HIV infection and the differences in HEV infection among different ethnic groups with HIV infection in Malaysia are definitely interest areas for research.

It has been suggested that anti-HEV IgG persisted for long periods in patient. However, there are conflicting reports on the long-term status of anti-HEV antibodies in infected persons. Goldsmith *et al*[7] noted the disappearance of anti-HEV IgG within 6 to 12 months in a group of Egyptian children after infection. Dawson *et al*[17] reported, however, that the anti-HEV antibodies can persist for 1 - 4 years; and Khuroo *et al*[22] noted that the anti-HEV IgG persisted for 14 years in the villagers in Kashmir where the infection was endemic. The persistence of anti-HEV IgG is an important event as it offers immunity. As with hepatitis A immunity, the antibodies protect subsequent infection in previously infected or immunized individuals. Unlike HAV, HEV affects mainly young adults. In developing countries, sanitary and waste disposal systems are unsatisfactory and most of the children in these endemic areas are

expected to develop immunity to such infection. The findings of anti-HEV positivity increasing with age 19 among the immunocompetent subjects in developing countries contradicted the pattern of infection caused by a virus transmitted via faecal-oral route. The explanation may be due to short- lived antibody in children after the HEV infection and the suboptimal testing system used in the laboratory for studying the seroprevalence of HEV infection. The finding of anti-HEV in 2 of the 3 HIV infected children in this study may suggest that the infection rate could be higher in immunocompromised children, but the numbers were too small to make any firm conclusions. The persistence of anti-HEV antibodies and the development of acute hepatitis in HIV infected patients including children need further study.

Transmission of HEV is reported to be mainly faecal-oral, but cases of HEV infection reported in the literature indicated that the virus could also be transmitted parenterally. Halfon *et al*[20] reported that 10.8% of the haemodialysis patients have HEV antibodies which is considerably higher than blood donors (<3%) in France. The risk factors associated with HEV infection are unknown, however, the finding of association between HEV and HCV suggested a common route of infection[19]. The results of this study indicated that faecal-oral route may be an important route of transmission for HEV among the HIV positive subjects in Malaysia because the prevalence of anti-HEV IgG and IgM class was not directly related to the risk factor associated with HIV-1 infection reported by the subjects (Table III). Although 25% of patients reported having heterosexual risk without IVDU and also 19% IVDU only were found to have anti-HEV, the numbers were too small to draw a firm conclusion on the heterosexual risk and IVDU with HEV transmission. It has also been suggested that heterosexual activity is not an important mode of transmission for HEV[23], and reported outbreaks and sporadic cases of HEV infection were found to be associated with foods and water supply.

The number of homosexuals (n=4) in this study were too small, so can not confirm if HEV infection exist in homosexual in Malaysia. However, elsewhere homosexual men were found to have a higher prevalence of anti-HEV antibodies[23,24]. There are few data on vertical transmission, but in a study by Khuroo *et al.*[1], 5 of 8 babies born to mothers who had active HEV infection in the 3rd trimester gave positive results in a polymerase chain assay. In our study, the mother and her one-year-old son were both found to have anti-HEV IgG antibodies, suggesting that the antibodies could have been transmitted vertically.

The increase risk of subjects with HCV infection to HEV infection is interesting. The high prevalence of anti-HCV antibodies in HIV positive subjects in Malaysia was reported by Yoong & Cheong[25]. In this study, among the 21 subjects with anti-HEV antibodies, 14 of them were also positive for anti-HCV antibodies, suggesting the increased risk of HEV infection in subject with anti-HCV antibodies.

Conclusion

The findings of 14.5% of the HIV-1 infected subjects with anti-HEV (IgG and IgM classes) in this study indicated that HEV infection could be found in Malaysia. The infection among the group of subjects recruited in the study was most likely transmitted via faecal-oral route. Efforts must therefore be taken to reduce the transmission and contamination of foods to prevent the potential spread of HEV infection to susceptible hosts.

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References

1. Khuroo SM, Kamili S, Jameel S. Vertical transmission of hepatitis E virus. Lancet 1995; 342: 1025-56.
2. Purcell RH. 1996. Hepatitis E virus. In Fields BM, Knipe DM, Howley PM, Chanak RM *et al.* eds. Virology. Third edition. Philadelphia: Lippincott Raven publishers. p2831-42.
3. Tashikazu Uchida. Hepatitis E: Review. Gastroenterologica Japonica 1992; 27: 687-96.
4. Gouvea V, Snellings N, Cohen SJ *et al.* Hepatitis E in Nepal: similarities with Burmese and Indian variants. Virus Research 1997; 52: 87-96.
5. Tandon BN, Joshi YK, Jain SK *et al.* An epidemic of non-A, non-B hepatitis in North India. Indian J Med Res 1982; 75: 739-44.
6. Khuroo SM, Duermeyer W, Zargar SA *et al.* Acute sporadic non-A/non-B hepatitis in India. Am J Epidemiol. 1983; 118: 360-64.
7. Goldsmith R, Yarbough PO, Reyes GR *et al.* Enzyme-linked immunosorbent assay for diagnosis of acute sporadic hepatitis E in Egyptian children. Lancet 1992; 339: 328-31.
8. Hyams KC, McCartny MC, Kaur M *et al.* Acute sporadic hepatitis E in Sudanese children: Analysis based on new Western blot assay. J Infect Dis 1992; 165: 1001-15.
9. Edemariam T, Krawazynski K, Hansson BG *et al.* Outbreak of acute hepatitis E virus infection among military personnel in Northern Ethiopia. J Med Virol 1991; 34: 232-36.
10. Corwin AL, Khiem HB, Calyson ET *et al.* A waterborne outbreak of hepatitis E virus transmission in Southeast Vietnam. Am J Trop Med Hyn 1996; 54(6): 559-62.
11. Al-Kandari S, Nordenfelt E, Al-Nakib B, Radakrishnan S, Al-Nakib W. Acute non-A, non-B hepatitis in Kuwait. Scand J Infect Dis 1987; 19: 611-18.
12. John R, Abraham P, Kurien G, Chandy G, Sridharan G. Sporadic hepatitis E in southern India. Trans Royal Soc Trop Med Hygn 1977; 91: 392-96.
13. De Tan, Stanley WKI, Ji LY, Mun HN. Acute sporadic hepatitis E virus in southern China. J Hepatology 1995; 23: 239-45.
14. Ibarra HV, Ricdemann SG, Siegel FG *et al.* Hepatitis E virus in Chile. Lancet 1994; 344 : 1501.
15. Lok ASF, Kwan W-K, Moeckli R *et al.* A seroepidemiological survey of hepatitis E in Hong Kong using recombinant based enzyme immunoassays. Lancet 1992; 340: 1205-8.
16. Fortier D, Treadwell TL, Koff RS. Enterically transmitted non-A, non-B hepatitis: importation from Mexico to Massachusetts. N Engl J Med 1989; 320: 1281-82.
17. Dawson GJ, Mushahwar IK, Chau KH, Gitnick GL. Detection of long-lasting antibody to hepatitis E virus in a US traveller to Pakistan. Lancet 1992; 340: 426.
18. Drabick JJ, Gambel JM, Gouvea VS *et al.* A cluster of acute hepatitis E infection in United Nations Bangladeshi peacekeepers in Haiti. Am J Trop Med Hyn 1997; 57(4) : 449-54.
19. Pisanti F, Coppola A, Galli C Association between hepatitis C and hepatitis E viruses in Southern Italy. Lancet 1994; 344: 746-47.
20. Halfon PH, Quzan D, Chanas M *et al.* High prevalence of hepatitis E virus antibody in haemodialysis patients. Lancet 1994; 344: 746.
21. Montella F, Rezza G, Di Sora F, Pezzotti P, Recchia O. Association between hepatitis E virus and HIV infection in homosexual men. Lancet 1994; 344: 1433.
22. Khuroo MS, Kamili S, Dar MY, Moeckii R, Jameel S. Hepatitis E and long term antibody status. Lancet 1993; 341: 1355.
23. Nandwani R. Hepatitis E virus and HIV infection in homosexual men. Lancet 1994; 345: 126-67.
24. Thomas DL, Yarbough PO, Vlahov D *et al.* Seroreactivity to hepatitis E virus in areas where the disease is not endemic. J Clin Microbiol 1997; 35(5): 1244-47.
25. Yoong KY, Cheong I. A study of Malaysian drug addicts with human immunodeficiency virus infection. Int. J STD & AIDS 1997; 8(2): 118-23.