Original Article

Seroprevalence of anti-HEV and HEV RNA among volunteer blood donors and patients with Hepatitis B and C in Iran

Hossein Keyvani[1], Mahmood Shamsi Shahrabadi[1], Saeed Najafifard[1], Bashir Hajibeigi[2], Farahnaz Fallahian[3], *Seyed-Moayed Alavian[3], [1]Department of virology, Iran University of Medical Sciences
[2]Iranian Blood Transfusion Organization Research Center, Tehran, Iran, [3]Baqiyatallah Research Center for Gastroenterology and Liver Disease, Baqiyatallah University of Medical Sciences

*Correspondence to:
Department of Internal Medicine, Gastroenterology and Hepatology Baqiyatallah Research Center for Gastroenterology and Liver Disease, Vanaq Square, Mola Sadra St. Tehran, Iran
E mail address: Alavian@thc.ir, Tell: +982188945186, Fax: +982188945188, PO BOX: 14155-3651

Abstract

Aim: To assess seroprevalence of antibodies to hepatitis E virus (HEV) in healthy blood donors and hepatitis B, C patients. **Methods:** 450 subjects consisted of 200 blood donors in Tehran blood transfusion center, 100 subjects with hepatitis C and 150 subjects with hepatitis B infection enrolled in this study. The A549 cell line was grown in mixed medium. Cells were infected with hepatitis E virus that was purified from stool sample of a patient confirmed for hepatitis E infection by reverse transcription-polymerase chain reaction (RT-PCR) method. Supernatant of infected cells was used as positive control in our RT- PCR assay. **Results:** In a total of 450 subjects, 33 (7.3%) had positive anti-HEV by enzyme-linked immunosorbent assay (ELISA). Anti-HEV was seen in (9/200) 4.5%, (7/100) 7%, and (17/150) 11.3% of healthy blood donors, hepatitis C, and hepatitis B subjects, respectively. Difference between two groups was statistically significance (P = 0.028). Difference between frequency of anti-HEV in hepatitis B in relation to healthy blood donors was significant (P = 0.014). **Conclusions:** HEV infection is more common in subjects with hepatitis B. **Keywords:** Hepatitis E virus, Seroprevalence, Transmission, Iran

Introduction

HEV infection is the major etiologic agent for acute hepatitis and acute liver failure in endemic regions. It causes severe liver disease among pregnant females and patients with chronic liver disease. The available evidence suggests that HEV may also be transmitted parenterally as well as vertically particularly in endemic areas (1) . Travel to HEV-endemic areas, veterinarians working with swine and transmission through blood have been reported. HEV infection can occur either in large epidemic in endemic regions or in sporadic forms in developed countries (2). Until 1997, hepatitis E was thought to occur only in developing countries including Africa, central Asian republics of the former Soviet Union, Afghanistan, Bangladesh, Borneo, Burma, China, India, Mexico, Mongolia, Nepal, Pakistan, Thailand, Vietnam, and some parts of the Middle East (3, 4). In Pakistan, HEV remains highly endemic, mainly affecting the adult population (5). In these countries, the disease is a significant public health concern and is both endemic and epidemic, with human outbreaks generally associated with fecal contamination of drinking water. Since 1997, HEV has been documented in humans and swine in many countries previously considered nonendemic. The hepatitis E is a neglected problem in our region (6). In a study in Iran (7), eight-hundred soldiers were selected by way of simple random sampling in army in Tehran of Iran in 2006, anti-HEV (IgG) was positive in 9 (1.1%) of soldiers, anti-HEV (IgM) was negative in all of them. For better understanding the epidemiology of HEV infection, we assessed the seroprevalence of antibodies to hepatitis E virus in healthy blood donors versus hepatitis B, or hepatitis C patients. Unfortunately the hepatitis E is a neglected problem in our region (6)

Materials and Methods

Asymptomatic and chronic hepatitis B patients and hepatitis C patients were referred according to constitutional and biochemical evidences of hepatitis. Patients with concurrent hepatitis B and hepatitis C infection were excluded. Voluntary blood donors included in this study that was tested negative for hepatitis B and hepatitis C.

A total of 450 samples consisted of 200 consecutive voluntary blood donors, and 100 hepatitis C patients and 150 hepatitis B patients were included in this study. All samples were tested for hepatitis B surface antigen (HBsAg) (DiaSorin-Madrid-Spain). Samples repeatedly reactive or inde-

terminate for HBsAg were further analyzed with a second independent HBsAg EIA, and if further reactive, tested by a neutralization assay. All reactive samples were verified in a recognized confirmatory test, and quantitative HB-VDNA (Assay HBV DNA was extracted from sera using a commercial DNGTM-plus DNA extraction kit (CinnaGen, Tehran, Iran).). Anti-HCV was done by Elisa test (Dia-Sorin-Madrid-Spain). Positive test were evaluated by Reverse Transcriptase (RT) PCR (Amplicor 2 Roche). The positive cases with RT PCR were considered HCV infected. Samples repeatedly reactive or indeterminate for anti-HCV were confirmed with an additional independent anti-HCV EIA and confirmed by HCV RIBA 3.0 (Genelabs Diagnostics-Singapore).

All samples were subjected to antibody testing against HEV by means of ELISA (DRG, Diagnostics GmbH, Germany). The kit used had not cross-reactivity with several viral antigens and antibodies.

Cell culture: The A549 cell line was grown in mixed medium as described previously by Huang et al (8). Cells were infected with Hepatitis E virus that was purified from stool sample of a patient confirmed for hepatitis E infection by RT-PCR method. Supernatant of infected cells was used as positive control in our PT- PCR assays. Standard RT-PCR was performed as described by Meng et al (9).

Of 100 patients with hepatitis C 62 were male and 38 were female; of 150 subjects with hepatitis B infection 90 were male and 60 were female; in 200 healthy blood donors 110 were male and 90 were female. When we divided subjects according to the age groups of 20-34, 35-49, and ≥ 50 years: 73 subjects of healthy blood donors and 97 of total hepatitis B, C patients were in age group 20-34 years. 95 subjects of healthy blood donors and 98 of total hepatitis B, C patients were in age group 35-49 years, and 32 subjects of healthy blood donors and 55 of total hepatitis B, C patients were in age group ≥ 50 years.

The study protocol approved by the ethics committee of the Baqyiatallah University of Medical Sciences and for checking their sample for hepatitis E virus written informed consent was obtained.

Results

In a total of 450 subjects, with range of age 20-61 years: 33 (7.3%) had positive anti HEV. Positive anti-HEV in healthy blood donors, hepatitis C, and hepatitis B subjects was 4.5% (9/200), 7% (7/100), and 11.3% (17/150), respectively. Frequency of anti-HEV in healthy blood donors was 4.5%, versus 9.6% in total of hepatitis C, and hepatitis B subjects. Difference of frequency of anti-HEV between two groups was statistically significance (P= 0.028). Frequency of anti-HEV in healthy and liver disease group had no difference between male and female sex. Frequency of Anti-HEV in healthy blood donors, hepatitis B, and hepatitis C subjects is shown in table 1. Comparison of frequency of Anti-HEV in healthy blood donors, hepatitis B, and hepatitis C subjects according to sex are shown in table 2. Difference between frequency of anti-HEV in hepatitis B in relation to healthy blood donors was significant (P= 0.014). When the subjects divided into three age groups: 20-34 years, 35-49 years, and ≥50 years: difference between frequency of anti-HEV between healthy (5.3%) and viral hepatitis B, hepatitis C group (14.3%) was significant in age group 35-49 years (P= 0.03). Also the difference between frequency of anti-HEV between healthy (5.3%) and hepatitis B (16.9%) was significant in age group 35-49 years (P= 0.019). Comparison of frequency of Anti-HEV in healthy blood donors, hepatitis B, and hepatitis C subjects according to age is depicted in table 3.

From 33 ELISA positive sera, HEV genome was detected in one serum by RT-PCR test. He was a soldier from Khuzestan Province (South and West of Iran) and the HBV and HCV markers were negative.

Discussion

A few studies have performed to estimate the incidence of human cases of acute, clinically apparent hepatitis E in Iran. To investigate seroprevalence of HEV infection, we selected voluntary blood donors and patients with hepatitis B or C. Here reported routes of transmission of HEV through drinking water, transfusion, and parenteral would be reviewed. Iran is located in an area that hepatitis E is endemic in its neighboring countries, mostly due to lack of waste pipe lines and access to sanitary water supplies.

In an outbreak of acute viral hepatitis was reported from a military unit at Mardan, in north Pakistan; about 10% of the exposed personnel developed jaundice. The maximum number of cases occurred in whose main water supply was near a polluted area, where a leaking pipe of water supply passing through a drain (10). This hepatitis epidemic stopped when the pipeline was repaired and the contamination of the water was prevented (11). In the absence of an effective vaccine, public health measures such as clean water supply, improved sanitation and public education are the major tools to prevent HEV epidemics in developing nations (1).

In countries with habit of consuming pork, this animal reported as a reservoir of HEV infection. In Iran consuming pork is banded and we had no report of HEV in animals. The mean positivity rates of anti-HEV antibody for pigs and cattle were 78.8% and 6.3% in serum samples from pigs, and cattle, respectively from various regions of China (12). Balinese people in Indonesia are mostly Hindu and have a habit of consuming pork. Serum samples were obtained from the 99 farm pigs in Bali and tested for anti-HEV and HEV RNA. The sera from 71 pigs (72%) were positive for anti-HEV and a 2-month-old pig had detectable HEV RNA. The results indicate that a presumably indigenous HEV strain is circulating in Bali, Indonesia and that HEV infection may occur via zoonosis (13).

There are reports of parenteral HEV exposure, transmission through transfusion or dialysis of hepatitis E both in hyperendemic and nonendemic areas. A potential risk of post-transfusion hepatitis E should be considered even in nonendemic countries (14). Of 200 voluntary blood donors screened for HEV RNA, three were found to be positive (1.5%). Overall seroprevalence of IgG anti-HEV was 18.6% (15). Recent hepatitis E infection was documented in 10% of thalassemic patients in India (16). In Egypt the prevalence of anti-HEV IgG was 45.2% (43/95) in blood donors and 39.6% (38/96) in hemodialysis patients. Anti-HEV IgG was found in 69.2% (18/26) and 28.6% (20/70) in hemodialysis patients positive and negative for HCV, respectively. This study showing evidence of hepatitis C virus infection as in hemodialysis patients suggesting either shared parenteral risk or increased sensitivity to HEV coinfection; that is to say a possibility of combined route of transmission for HEV (17).

In a study in Tabriz of Iran, the prevalence of anti-HEV IgG was 7.8% in serum samples of 399 voluntary male blood donors in 2004. Risk factors for infection included age and a low educational level (18). In a study (19) , the seroprevalence of hepatitis in 324 patients on hemodialysis in Tabriz of Iran was 7.4 %. The prevalence rate of HBV and HCV infection were 4.6% and 20.4%, respectively. No significant association was found between anti-HEV positivity and duration of hemodialysis, positivity for hepatitis B or C virus infection, and history of transfusion.

In a study among 190 adult patients with chronic hepatitis B virus (HBV) and 174 with chronic hepatitis C virus (HCV) infection, anti-HEV IgG antibodies were positive in 26/190 (13.7%) of chronic HBV and 94/174 (54%) of chronic HCV patients. In the 178 individuals without known liver disease as control group, anti-HEV positivity was 15.7% (28/178). The presence of HEV infection was significantly higher in chronic HCV patients (20).

In our study, anti-HEV is more frequent in hepatitis B and hepatitis C infected subjects versus healthy blood donors, but difference between anti-HEV frequency in hepatitis B and healthy blood donors is statistically significant. In this study we did not regard risk factors of hepatitis E as tripe to endemic areas, or consuming uncooked meat of pigs. Also, history of blood transfusion, injection drug use by sharing devices, educational levels, and socioeconomic status of subjects were not investigated. It needs to design studies with questionnaires regarding routes of HEV transmission in cases suspected to viral hepatitis infections. Because diagnostic testing is limited due to the lack of commercially available tests, illness due to HEV infection may be undiagnosed. Serodiagnosis of HEV is now available and should be used routinely for diagnosis of suspicious cases.

In conclusion, unexplained hepatitis in those lacking sanitary water supplies, traveling to endemic areas, receiving transfusion or on hemodialysis, subjects that breed animals, and in those use pork meat should be tested for hepatitis E antibodies. Regarding HEV infection in fulminant hepatic failure in pregnant women is emphasized. We recommend regarding HEV infection in co-infection with other hepatitis, especially hepatitis B infection. It needs further studies to define all routes of HEV exposure and epidemiological characteristics of hepatitis E infection and to identify additional risk factors involved in the pathogenesis of this infection, duration of immunity, and natural course of hepatitis E infection.

References

1. Acharya SK, Panda SK. Hepatitis E virus: epidemiology, diagnosis, pathology and prevention. Trop Gastroenterol. 2006 Apr-Jun;27(2):63-8.
2. Peron JM, Mansuy JM, Izopet J, Vinel JP. [Hepatitis E virus: an emerging disease]. Sante. 2006 Oct-Dec;16(4):239-43.
3. Centers for Disease Control and Prevention. Hepatitis E among U.S. travelers. . Morb Mortal Wkly Rep. 1993;42:1-4.
4. Meng XJ, Dea S, Engle RE, Friendship R, Lyoo YS, Sirinarumitr T, et al. Prevalence of antibodies to the hepatitis E virus in pigs from countries where hepatitis E is common or is rare in the human population. J Med Virol. 1999 Nov;59(3):297-302.
5. Malik IA, Tariq WUZ. The prevalence and pattern of viral hepatitis in Pakistan. . J Coll Physicians Surg Pak. 1995;5:2-3.
6. Alavian SM. Hepatitis E Virus Infection: A Neglected Problem in Our Region. Hep Mon. 2007;7(3):119-21.
7. Ghorbani GA, Alavian SM, Esfahani AA, Assari S. Seroepidemiology of Hepatitis E Virus in Iranian Soldiers. Hep Mon. 2007;7(3):123-6.
8. Huang R, Li D, Wei S, Li Q, Yuan X, Geng L, et al. Cell culture of sporadic hepatitis E virus in China. Clin Diagn Lab Immunol. 1999 Sep;6(5):729-33.

9. Meng J, Dubreuil P, Pillot J. A new PCR-based seroneutralization assay in cell culture for diagnosis of hepatitis E. J Clin Microbiol. 1997 Jun;35(6):1373-7.
10. Bradley DW. Enterically-transmitted non-A, non-B hepatitis. Br Med Bull. 1990 Apr;46(2):442-61.
11. Quraishi MS, Ahmad M, ul Rashid H, Mushtaq S, Ahmed SA. Hepatitis non-A, non-B--report of a water-borne outbreak. . J Pak Med Assoc 1988;38:203-4.
12. Wang YC, Zhang HY, Xia NS, Peng G, Lan HY, Zhuang H, et al. Prevalence, isolation, and partial sequence analysis of hepatitis E virus from domestic animals in China. J Med Virol. 2002 Aug;67(4):516-21.
13. Wibawa ID, Muljono DH, Mulyanto, Suryadarma IG, Tsuda F, Takahashi M, et al. Prevalence of antibodies to hepatitis E virus among apparently healthy humans and pigs in Bali, Indonesia: Identification of a pig infected with a genotype 4 hepatitis E virus. J Med Virol. 2004 May;73(1):38-44.
14. Matsubayashi K, Nagaoka Y, Sakata H, Sato S, Fukai K, Kato T, et al. Transfusion-transmitted hepatitis E caused by apparently indigenous hepatitis E virus strain in Hokkaido, Japan. Transfusion. 2004 Jun;44(6):934-40.
15. Arankalle VA, Chobe LP. Hepatitis E virus: can it be transmitted parenterally? J Viral Hepat. 1999 Mar;6(2):161-4.
16. Irshad M, Peter S. Spectrum of viral hepatitis in thalassemic children receiving multiple blood transfusions. Indian J Gastroenterol. 2002 Sep-Oct;21(5):183-4.
17. Abdel Hady SI, El-Din MS, El-Din ME. A high hepatitis E virus (HEV) seroprevalence among unpaid blood donors and haemodialysis patients in Egypt. J Egypt Public Health Assoc. 1998;73(3-4):165-79.
18. Taremi M, Gachkar L, MahmoudArabi S, Kheradpezhouh M, Khoshbaten M. Prevalence of antibodies to hepatitis E virus among male blood donors in Tabriz, Islamic Republic of Iran. East Mediterr Health J. 2007 Jan-Feb;13(1):98-102.
19. Taremi M, Khoshbaten M, Gachkar L, EhsaniArdakani M, Zali M. Hepatitis E virus infection in hemodialysis patients: a seroepidemiological survey in Iran. BMC Infect Dis. 2005;5(1):36.
20. Bayram A, Eksi F, Mehli M, Sozen E. Prevalence of hepatitis E virus antibodies in patients with chronic hepatitis B and chronic hepatitis C. Intervirology. 2007;50(4):281-6.

Table 1: Frequency of Anti-HEV in blood donors, hepatitis B, and hepatitis C subjects

	Anti-HEV			
	Blood donors	**Hepatitis B**	**Hepatitis C**	**Total**
Test positive (%)	9 (4.5%)	17(11.3%)	7(7%)	33(7.3%)
Test positive (%)	191(95.5%)	133(88.7%)	93(93%)	417(92.7%)
Total (%)	200(100%)	150(100%)	100(100%)	450(100%)

Table 2: Comparison of frequency of Anti-HEV in healthy blood donors, hepatitis B, and hepatitis C subjects according to sex

	Anti-HEV			
Sex	**Blood donors**	**Hepatitis B**	**Hepatitis C**	**Total**
Male Positive number (%)	5 (4.5%)	11(12.2%)	4(6.5%)	20(7.6%)
Total number (%)	110(100%)	90(100%)	62(100%)	262(100%)
Female Positive number (%)	4(4.4%)	6(10%)	3(7.9%)	13(6.9%)
Total number (%)	90(100%)	60(100%)	38(100%)	188(100%)

Table 3: Comparison of frequency of Anti-HEV in blood donors, hepatitis B, and hepatitis C subjects according to age

	Anti HEV			
	Age	**Blood donors**	**Hepatitis B and C hepatitis**	**Total**
20-34	Positive number (%)	2 (2.7%)	4(4.1%)	6(3.5%)
	Total number (%)	73(100%)	97(100%)	170(100%)
35-49	Positive number (%)	5(5.3%)	14(14.3%)	19(9.8%)
	Total number (%)	95(100%)	98(100%)	193(100%)
≥50	Positive number (%)	2(6.3%)	6(10.9%)	8(9.2%)
	Total number (%)	32(100%)	55(100%)	87(100%)