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ORIGINAL ARTICLE

Prevalence of hepatitis E virus viremia and antibodies among healthy blood donors in India

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Abstract

Background Hepatitis E virus (HEV) is transmitted primarily through contaminated water and food. Recently, HEV viremia in blood donors and transfusion-related transmission of HEV have been reported, leading to calls to screen donated blood for this virus. However, these data are from regions where genotype 3 HEV is predominant. In India, where human infections are caused only by genotype 1 HEV, the frequency of subclinical HEV viremia is unknown.

Methods Minipools of sera prepared from three donor units each from our institution's blood bank in Lucknow, India, were tested for HEV RNA using a sensitive amplification-based assay. A randomly selected subset was also tested for IgG anti-HEV antibodies using a commercial (Wantai) immunoassay.

Results Sera from 1799 donors (median [range] age 30 [18–63] years; 1746 [97.0%] men) were collected (June–July 2016, 900; November–December 2016, 899). Of these, 17 (0.95%), 16 (0.90%), and 3 (0.17%) tested positive for HBsAg, anti-HCV, and anti-HIV antibodies, respectively. None of the donors tested positive for HEV RNA. Of 633 randomly selected donors (age 30 [18–63] years, 613 [96.8%] male) tested for IgG anti-HEV, 383 (60.5%) tested positive. Seropositivity rate increased with age, being 70/136 (52%), 177/299 (59%), 100/154 (65%), 30/34 (88%), and 6/10 (60%) in the 18–24, 25–34, 35–44, 45–54, and 55 years or older age groups, respectively.

Conclusions In healthy blood donors from northern India, HEV viremia is infrequent though anti-HEV antibody prevalence is high. This suggests that asymptomatic HEV viremia may be less frequent in areas with genotype 1 predominance than those with genotype 3 predominance.

Keywords Hepatitis E virus · Seroprevalence · Viral hepatitis · Viremia

Introduction

Hepatitis E virus (HEV) is the most common among the hepatotropic viruses as the cause of acute viral hepatitis in India. Human HEV has four major distinct genotypes, named 1 to 4 [1], with occasional reports of disease caused by genotype 7 [2]. Though all the four major HEV genotypes have primarily fecal-oral transmission, genotype 1 and 2 viruses differ from genotype 3 and 4 HEV in the source of infection (human vs. animal source), modes of transmission (water-borne vs. food-borne), clinical features (more marked liver injury presenting more often as acute icteric hepatitis or acute liver failure vs. milder illness), lack of propensity to cause chronic infection among immunosuppressed persons (none or extremely infrequent vs. high), and severity of liver injury when the infection occurs in pregnant women (increased vs. unchanged) [1, 3].

Genotypes 1 and 2 HEV have a human-to-human transmission, primarily through consumption of contaminated drinking water supplies [4, 5]. The traditional water-borne fecal-oral transmission cannot explain the locally-acquired cases of hepatitis E in developed countries, where sanitary and hygienic conditions are excellent. In such countries, zoonotic transmission through ingestion of undercooked meat has been found to be the main route of acquisition of HEV infection [6, 7]. In these low-endemicity areas, transfusion-mediated HEV transmission has recently been

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described. In a study in the UK, HEV viremia was detected at a rate of approximately one per 2500 donated blood units, and evidence of HEV infection, albeit often asymptomatic, was found among a proportion of those who had received blood components prepared from these viremic units [8]. HEV viremia, mainly with genotype 3 HEV, has also been reported in healthy blood donors in other developed countries [9, 10]. These studies have been used to support the need for screening of blood and blood components for HEV by nucleic acid testing [11], and such screening has been introduced in some countries [12].

In India, burden of HEV infection is very high as indicated by frequent occurrence of outbreaks and sporadic cases of acute hepatitis E. With nearly 9 million blood units collected and used in the country every year, the issue of transfusion-transmitted HEV infection is of great public health relevance here. Also, a large proportion of donated blood units are used to prepare multiple blood components, which amplifies the potential risk of blood-borne HEV transmission—by exposing several individuals to each contaminated blood unit. The issue has led to experts providing conflicting opinions on the need for HEV RNA testing in Indian blood banks [13, 14]. These differing opinions are related primarily to lack of data on the subject from India, except for a few small studies done several years ago [15–17]. Hence, there is a clear need to study the prevalence of HEV viremia in Indian healthy population, in particular blood donors.

In the current study, we studied the prevalence of HEV viremia and anti-HEV IgG antibody in serum specimens from a group of healthy blood donors in our institution, located in northern part of India.

Methods

Blood specimens were collected from blood donors at our institution during two time periods, i.e. during June–July 2016 (the monsoon season, when hepatitis E is more frequent) and during November–December 2016 (winter months, when the disease is much less frequent). During the monsoon season of 2016, the number of cases of hepatitis E in our city was particularly large, as compared to other years.

Healthy blood donors, either voluntary or replacement, were screened, using the standard operating procedure followed in our blood bank, for selection or deferral of blood donors. In brief, a questionnaire was administered followed by physical examination by a physician. Persons with low body weight (< 45 kg), low hemoglobin (< 12.5 g/dL), uncontrolled major systemic illnesses, and history of jaundice in previous 2 years were excluded. From those deemed eligible to donate, a blood specimen was collected and aliquots of freshly separated serum were immediately frozen at − 80 °C till analysis.

Detection of HEV RNA and measurement of its titer

Thawed serum specimens were used to prepare minipools by mixing 47 μl of serum each from three donors. From each minipool (~ 140 μl), RNA was extracted using QIAamp viral RNA mini kit (Qiagen, Valencia, CA, USA) in 30 μl of water, and 10 μl of this was used in a real-time, one-step, quantitative reverse transcription and polymerase chain reaction (RT-PCR) assay. For any specimen that tested positive, the individual specimens (140 μl each) constituting the minipool were individually tested for HEV RNA. The RT-PCR assay has been described previously [18]. It used primers based on open reading frame 1 region of the HEV genome, which are known to amplify genotype 1 to 4 HEV RNA, and had a lower limit of HEV RNA detection of 100 IU/mL.

In each assay run, two different concentrations of a stool suspension known to contain HEV which had been calibrated against a WHO HEV RNA standard was processed similar to the serum specimens as positive controls, and to permit quantitation of HEV RNA, if present.

For specimens that test positive for HEV RNA, amplification of a part of the HEV genome and sequencing to determine the viral genotype was planned.

Anti-HEV antibody testing

A subset of specimens were also tested for the presence of IgG anti-HEV antibodies using a commercial immunoassay (Wantai HEV IgG assay; Beijing Wantai, China).

The study was approved by our institution's Ethics Committee, which waived off the requirement for individual consent. Categorical and numerical data were expressed as proportions, and mean and standard deviation, respectively, and compared between groups using chi-squared test and Student's *t* test, respectively.

Results

The study included 1799 blood donors. They were aged between 18 and 63 years (mean 31.2 years, standard deviation 8.2 years, median 30 years) and were mostly men ($n = 1749$; 97%). Of these, 900 specimens were collected during June-July 2016, and 899 were collected during November-December 2016. HBsAg, anti-HCV, and anti-HIV antibodies tested positive in 17 (0.95%), 16 (0.90%), and 3 (0.17%) of these donors, respectively.

All the minipools tested negative for HEV RNA, except two that tested weak positive. Tests on all the six individual serum specimens that constituted these two minipools tested negative for HEV RNA. Thus, on final interpretation, all the donor sera were considered as negative for HEV RNA. Since none of the donors tested positive for HEV viremia, estimation of the circulating viral

load and determination of HEV genotype, or comparison of viremia rate between the two seasons were not possible.

IgG anti-HEV was tested in 633 donors (35.2% of the total cohort). The age and gender distribution of this subset of donors was comparable to that for those who were not tested (male 613/633 vs. 1120/1166, $p = 0.72$; age [mean ± standard deviation] 31.3 ± 8.2 years vs. 31.1 ± 8.3 years, $p = 0.48$). Of the 633 sera tested, 383 (60.5%) tested positive for HEV IgG antibody. The anti-HEV positivity rate increased with increasing age (Fig. 1; $p < 0.001$, Chi-squared test for linear trend). The anti-HEV positivity rates were similar among men and women (376/613 vs. 7/20; $p = 0.05$); however, the number of women studied was quite small.

Discussion

Our data revealed that HEV RNA was not detectable in any of the 1799 blood donors from northern India included in this study and that nearly 60% of these donors were positive for IgG anti-HEV antibody, and the antibody positivity rate increased with age.

HEV infection shows two distinct epidemiologic patterns worldwide. In geographical areas where sanitation and water quality are poor, HEV infection is caused almost exclusively by genotype 1 and 2 viruses from human source, which are transmitted primarily through contamination of water and possibly food. These infections occur predominantly in young, immunocompetent adults and manifest usually as acute viral hepatitis, with occasional cases developing acute liver failure, and many infected persons having asymptomatic infection. In developed countries with good sanitation and hygiene, HEV infection is primarily caused by genotype 3 and 4 viruses, which circulate naturally in several animal species (primarily pigs, but also deer, wild boars, goat, sheep, etc.), through ingestion of undercooked meat or other forms of animal-human contact [1, 6].

The interest in transmission of HEV infection through blood transfusion has been based on reports in recent years from several developed countries, particularly those in Europe, where HEV viremia has been detected in a small proportion of otherwise healthy persons. The positivity rate of HEV RNA in healthy persons has varied widely between countries (Table 1) [9, 10]. Nearly all these subjects with viremia have had infection with genotype 3 HEV, which is the prevalent type in these countries [8, 19, 20]. This has led to calls in several resource-rich countries to test transfused blood and blood components, in particular those meant for use in immunosuppressed recipients, for HEV RNA, and introduction of such screening in some countries [12]. However, such testing is quite costly, and the cost-effectiveness of this measure remains doubtful, even in high-income countries.

Data on HEV viremia in healthy blood donors from developing countries where genotype 1 and 2 HEV infection are prevalent, including India, are extremely limited. Several years ago, in a study from western India, HEV viremia was reported in 3 of 200 blood donors [15]. Soon thereafter, the same group also reported HEV seroconversion in 2 of 37 HEV-seronegative transfusion recipients [17]. In another study from Kashmir, India, an even higher rate of viremia was reported among blood donors in that region (4/107; 4%) and of HEV infection among recipients of blood transfusions (3 of 22 seronegative recipients) [16]. These data were generated when the methods for HEV RNA testing were not as advanced and reliable. Also, the high rates of HEV viremia among healthy persons reported in these studies were not compatible with anti-HEV antibody rates of 30% to 40% in the Indian population reported around the same time [21]. More recent Indian data on this subject are not available.

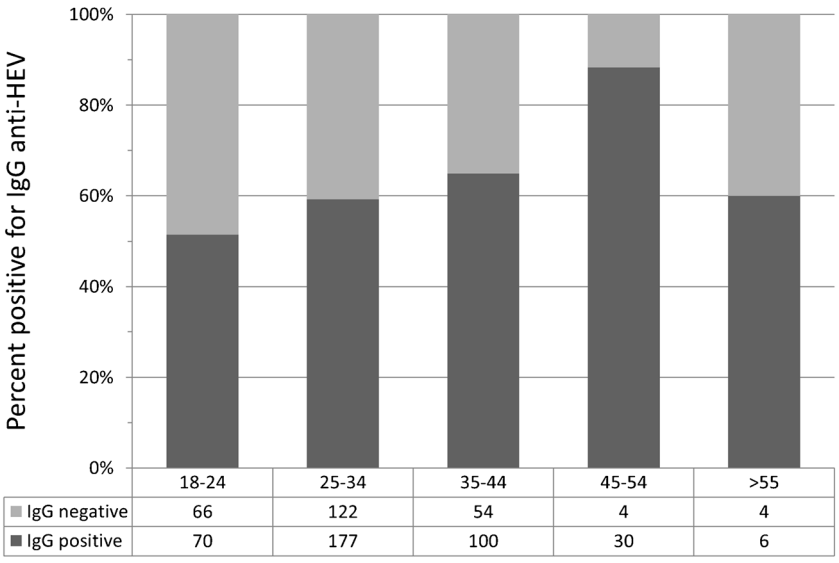

Fig. 1 IgG anti-hepatitis E virus (HEV) seropositivity rates in healthy northern Indian blood donors according to age

Table 1 Hepatitis E virus (HEV) ribonucleic acid (RNA) positivity rates reported in blood donors in different countries (adapted from reference [10])

Country	HEV RNA positivity rate
England	1:2848–1:7000
Scotland	1:14,520
Germany	1:1240–1:4525
Australia	0:3237
The Netherlands	1:658–1:2436
France	1:2218
Spain	1:3333
USA	0–1:9500
India (current study)	0:1799

A recent report from Nepal reported detection of HEV genotype 1 RNA in 1.5% of 581 donated blood units [22]. This prevalence of asymptomatic viremia is much higher than the rates reported from developed countries, suggesting that HEV infection is quite common among otherwise healthy blood donors in South Asia. However, in this study, the prevalence rate of IgG anti-HEV antibody was only 8.3%, indicating that HEV infection was not very prevalent in this population. Furthermore, the prevalence of IgM anti-HEV antibody was quite high (3.6%). These findings together suggest that an outbreak of HEV infection may have existed during the period of specimen collection in that study. In any case, the HEV RNA detection rate of 1.5% observed in that study was not consistent with the IgG anti-HEV antibody prevalence rate, given that anti-HEV antibody persists for several years after exposure.

Our study has some advantages over the previous Indian data. First, it had a much larger sample size than in the previous studies. Second, we included blood units collected during two different periods of time, including one during the monsoon season when HEV transmission is likely to be high. Third, we used a technique for HEV RNA detection which is widely used by several groups working on HEV infection and has been shown to be highly sensitive.

How does one reconcile our results of absence of HEV viremia despite high HEV endemicity in India, with the presence of HEV viremia in blood donors in Europe and North America? The HEV genotypes prevalent in these parts of the world are quite different. Thus, whereas genotype 3 HEV predominates in the developed regions, genotype 1 is the only HEV genotype reported from humans in India [23]. Barring a case report, genotype 1 has not been shown to cause chronic infection [24]. At least three studies have been reported among organ transplant recipients from India; in these studies, HEV viremia was not detected in any of nearly 300 subjects receiving immunosuppressive drugs [25–27]. Thus, the HEV genotypes differ markedly in their propensity to cause disease and in viral persistence. Hence, it is not surprising if these also differ in the likelihood of viremia among healthy persons.

As a secondary objective, we also assessed the prevalence of anti-HEV antibodies in our subjects. Previous studies from India have shown anti-HEV antibody prevalence rates of ~ 30% [21]. The higher antibody prevalence rate observed could be related to the use of Wantai assay, which has been shown to be more sensitive [28]. The high anti-HEV antibody rate detected in our study may actually support our finding of lack of detectable HEV viremia, with a high population seroprevalence of anti-HEV, which may reflect a high level of protection against HEV infection in the Indian population. Several pieces of evidence, in particular, the data from follow up of recipients of a recombinant hepatitis E vaccine trial [29], show that anti-HEV antibodies offer protection against HEV infection, though the exact titer which is protective remains unclear.

Though our data may need verification in further studies, these suggest that pre-transfusion HEV RNA screening may not be applicable in India, despite a high endemicity of disease. Such testing would have a very low yield, while being quite costly and technically highly demanding.

In conclusion, our study suggests that HEV viremia is infrequent in healthy blood donors in India, and hence provides evidence against the need to introduce HEV RNA testing in Indian blood banks.

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Compliance with ethical standards

Conflict of interest HK, AG, AS, VY, SS, RC, and RA declare that they have no conflict of interest.

Informed consent Written informed consent was taken from all the subjects.

Ethics statement The authors declare that the study was performed in a manner to conform with the Helsinki Declaration of 1975, as revised in 2000 and 2008 concerning human and animal rights, and the authors followed the policy concerning informed consent as shown on Springer.com.

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