Original Article

Hepatitis E seroprevalence among blood donors: A pilot study from Western India

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Abstract

Background: Hepatitis E virus (HEV) is emerging as a potential threat to blood safety after several cases of transmission by transfusion or transplantation have been described. Currently, blood donors in India are not screened for HEV. The studies conducted on HEV in recent times in India have focused on epidemiology and future perspectives, but there is no published study on blood donors. To address possible issues surrounding blood safety and risk of HEV transmission within the Indian blood supply, HEV seroprevalence study was conducted in blood donors at our center. **Materials and Methods:** A total of 460 male voluntary blood donors were selected for the study and after taking their written consent. Serum anti-HEV IgM was detected by Dia.Pro HEV kit (Diagnostic Bioprobes Srl, Milano, Italy). **Results:** The study population was composed of 460 male voluntary blood donors and their age ranged from 18 to 60 years with a mean age of 30.48 years. Out of 460 donors, 22 (4.78%) donors were tested positive for IgM anti-HEV and the mean value alanine aminotransferase (ALT) was 26.06 IU/L, the highest being 93.5 IU/L. Normal reference value of ALT in our center was 40 IU/L. Out of 22 anti-HEV positive donors, 19 (86.36%) had ALT values above 40 IU/L. **Conclusion:** HEV seroprevalence of 4.78% in our center. Though reports of HEV transmission through blood has been reported from various parts of the world, before making it as a mandatory screening test among blood donors in India, further studies with confirmatory assay of HEV need to be done.

Key words:

Blood donors, hepatitis E virus, seroprevalence

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Introduction

Hepatitis E virus (HEV) was discovered in 1983 and cloned in 1991. The virus is transmitted predominantly by enteral route and causes water-borne epidemics of hepatitis in developing countries particularly in South-East Asia and the Indian subcontinent.[1-3] Therefore, human to human transmission appears to be exceptional, mother-to-child and blood transfusion transmission have been described.[4,5] HEV isolates are classified into five major genotypes, which belong to the same serotype. Genotypes differ with respect to host species and epidemiological distribution. Genotypes 1 and 2 infect only humans and are endemic in many parts of Asia, Africa and South America. Genotypes 3 and 4 infect humans, pigs and other animal species. Genotype 3 causes sporadic cases of an acute hepatitis in North and South America, Europe and Asia whereas genotype 4 is essentially restricted to Asia. Genotype 5 infects avian species.[5,6]

HEV is emerging as a potential threat to blood safety after several cases of transmission by transfusion or transplantation have been described.[7-9] Poor sanitation in developing countries leads to higher anti-HEV seroprevalence among the general population and thereby giving an opportunity for blood donors to infect recipients. HEV was thought to result only in a self-limiting acute infection. However, reports of persistent HEV infection have been described in immunosuppressed groups with reports of progression of liver inflammation and fibrosis with HEV-related cirrhosis.[10,11] In pregnant women, HEV infection severity is increased with mortality rates up to 20%[12] and in patients with underlying liver disease, it may reach up to 60%.[13] Currently, blood donors in India are not screened for HEV. The studies conducted on HEV in recent times in India have focused on epidemiology and future perspectives and only one study with relatively smaller sample size is reported from Pune has shown the HEV ribonucleic acid (RNA) prevalence of 1.5%.[14-16] To address possible issues surrounding blood safety and risk of HEV transmission within the Indian blood supply, HEV seroprevalence study was conducted in blood donors at our center.

Materials and Methods

A total of 460 male voluntary blood donors were selected randomly for the study from August 2012 to

December 2012, after they were found medically fit for donation as per the regulatory requirements in India and after taking their written consent. Serum anti-HEV IgM was detected by DiaPro HEV ELISA kit (Diagnostic BioprobesSrl, Milano, Italy) along with mandatory screening tests in India (anti-human immunodeficiency virus [HIV-1] and HIV-2, anti-hepatitis C virus (HCV), hepatitis B surface antigen (HBsAg), syphilis and malarial parasite. The ELISA kit has diagnostic sensitivity and specificity of 98%. This assay uses two HEV-specific synthetic antigens encoding for conservative and immunodominant determinants derived from open reading frame (ORF) two and ORF three of all the four subtypes. The tests were performed and the results were analyzed according to the manufacturer's instructions. Initially reactive samples were retested and those with repeatedly reactive results were considered to be positive. All donor samples were also tested for alanine aminotransferase (ALT) levels. The statistical analysis of the data was performed using *t*-test and Fisher's exact test.

Results

The study population which composed of 460 male voluntary blood donors and their age ranged from 18 to 60 years with a mean age of 30.48 years. The donors were stratified on the basis of age into three groups: 266 (57.8%) from 18 to 30 years, 136 (29.5%) from 31 to 40 years and 58 (12.6%) from 41 to 60 years.

Out of 460 donors, 22 (4.78%) were tested positive for IgM anti-HEV. The distribution of IgM anti-HEV positive and negative donors in various age groups are shown in Table 1 ($P = 0.1718$).

The mean value ALT was 26.06 IU/L, the highest being 93.5 IU/L. Normal reference value of ALT in our center was 40 IU/L. Out of 22 anti-HEV positive donors, 19 (86.36%) had ALT values above 40 IU/L [Figure 1].

Out of 460 blood donors tested, four were found positive for HBsAg, one positive for anti-HCV and one positive for Syphilis but all of those positive for other infectious diseases were negative for anti-HEV IgM antibody.

Discussion

A total of 460 blood samples from voluntary male blood donors were screened for anti-HEV in our center and the seroprevalence was found to be 4.78%. In our study, older donors tended to have higher HEV seroprevalence rates, although the differences were not statistically significant [Table 1] ($P = 0.1718$). The studies from USA and Denmark have found older age to be a risk factor for anti-HEV positivity.[17,18] Comparison of HEV seroprevalence among blood donors in various countries are shown in Table 2.

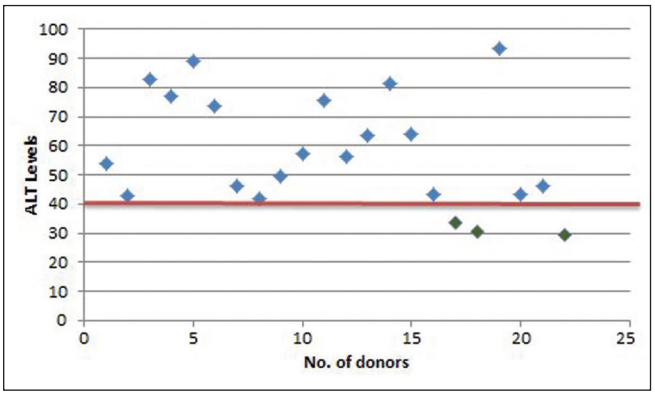

Figure 1: Distribution of alanine aminotransferase levels in donors positive for IgM anti-hepatitis E virus

The seroprevalence in our study is comparable with that of Switzerland.[23] The study conducted among 200 blood donors in Pune, India has shown HEV RNA prevalence of 1.5%.[16] The higher seroprevalence (4.78%) observed in our study may be due to the larger sample size ($n = 460$) and HEV RNA is more specific than HEV IgM ELISA. Neighboring countries such as China and Korea have higher seroprevalence of 19.7% and 23.1% respectively. As HEV infection in developed countries is mainly a zoonotic disease transmitted primarily through contaminated meat and in developing countries through contaminated water, which may reflect the differences in seroprevalence in different countries. Most of the subjects under study belonged to areas of proper sanitation facilities which may be responsible for lower seroprevalence. A wide variation of seroprevalence among blood donors in various countries may be due to different marker selected of HEV. Another possible reason for the lower seroprevalence we observed is that the distribution of risk factors such as profession, hobbies, diet, and social status may differ. A wide variation of seroprevalence among blood donors in various countries may be due to different marker selected. We were unable to assess these factors in detail in our study population. Comparisons between studies are difficult due to differences in the demographics of the population studied and in the HEV antibody detection assays used. The various commercially available tests show important differences in sensitivity. Further, sensitivity and specificity of a test depends upon the prevalence, as well as on the viral genotype present in the study population. Thus, in the absence of standardized

Table 1: Age-wise distribution of IgM anti-HEV in blood donors

Age (years)	IgM anti-HEV negative	IgM anti-HEV positive	Seroprevalence in the age group %
18-30	257	9	3.38
31-40	129	7	5.14
41-60	52	6	10.34
Total	438	22	4.78

(*P:* 0.1718); HEV: Hepatitis E virus; IgM: Immunoglobulin M

Table 2: Comparison of HEV seroprevalence among blood donors in different countries

Country of study	Seroprevalence %	Year	Subjects studied
Netherlands[19]	1.1	1993	1275
Italy[20]	1.0	1994	948
Spain[17]	2.8	1998	863
India[16]	1.5	2000	200
USA[18]	18.3	2002	400
Denmark[21]	20.6	2008	461
England[22]	16	2008	500
France[23]	16.6	2008	529
Switzerland[24]	4.9	2010	550
China[25]	19.7	2012	14028
Korea[26]	23.1	2012	147
Present study	4.78	—	460

HEV: Hepatitis E virus

Table 3: ALT levels in IgM anti-HEV positive and negative donors

IgM anti-HEV	ALT <40	ALT ≥40	Total
Positive	3	19	22
Negative	434	4	438
Total	437	23	460

HEV: Hepatitis E virus; IgM: Immunoglobulin M; ALT: Alanine aminotransferase

commercially available confirmatory assays such as Western blot, differences in seroprevalence rates between different populations must be interpreted with caution.

ALT levels of donors positive and negative for IgM anti-HEV are shown in Table 3.

Our study showed a significant correlation ($P < 0.0005$) between raised ALT levels and IgM anti-HEV positivity. Although this correlation is statistically significant, screening for HEV based on ALT levels is unsatisfactory because the elevation in ALT level occurs after the peak of HEV RNA is subsided and even HEV positive donor may be asymptomatic with normal ALT levels.[27] IgM anti-HEV is more sensitive than IgG anti-HEV and less specific than HEV RNA, making it as a mandatory screening test in blood donors will lead to higher discard rates of blood units.

Conclusion

In summary, we have found anti-HEV IgM seroprevalence of 4.78% among male voluntary blood donors in our center, but the positive results were not confirmed with HEV RNA. Though reports of HEV transmission through blood has been reported from various parts of the world, before making it as a mandatory screening test among blood donors in India, further studies with confirmatory assay of HEV need to be done.

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