



## SEROPREVALENCE OF HEPATITIS E VIRUS AMONG VOLUNTARY BLOOD DONORS IN CHENNAI –A CROSS SECTIONAL STUDY

### Immunohaematology

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### ABSTRACT

HEV is a transfusion transmissible infection. Samples from 89 whole blood donors were tested for anti-HEV immunoglobulin IgG and IgM by enzyme linked immunosorbent assay (ELISA). Enzyme alanine aminotransferase (ALT) testing was also performed as a surrogate marker. A total of 10 of 89 blood donors tested positive for anti-HEV IgG (11.2% seroprevalence). None of the 89 donors were positive for IgM anti-HEV. Enzyme Alanine Amino Transferase (ALT) assay on all samples were within normal range (6-30 IU/ml). Most of the IgG HEV positive donors were from lower socioeconomic status, they reside in areas with poor infrastructure facilities wherein other water and food borne infectious diseases are also common. Since HEV infection is more severe among high risk populations like pregnant mothers, immunocompromised individuals and dialysis patients. At least for these restricted population, blood components if needed have to be screened for HEV.

### KEYWORDS

HEV- Hepatitis E virus, ELISA-Enzyme linked immunosorbent assay, ALT-alanine aminotransferase, IgM-Immunoglobulin-M, IgG-Immunoglobulin-G.

### INTRODUCTION

Blood transfusion is an important part of modern medicine and considered as a lifesaving procedure. The transfusion of blood and blood products is considerably safer than ever before but it will take long way to attain safe blood transfusion.<sup>1</sup> Viral infections assume a great importance in transfusion associated mortality and morbidity in patients. Important transfusion transmitted viruses are HIV, HBV, HCV, HTLV, Parvo virus B-19 and cytomegalovirus.<sup>2</sup> In spite of the use strict donor selection criteria, screening for serological markers for virus infection, testing of blood products by polymerase chain reaction (PCR) and the use of inactivation procedures, the risk of viral transmission cannot be totally eradicated as reported by S. Madrow et al.<sup>3</sup>

Hepatitis E virus (HEV) is a major international public health problem and it is estimated that 2.3 billion people are infected worldwide. HEV is the important cause of acute viral hepatitis in the world, especially in developing countries. In hepatitis E endemic regions, the presence of HEV viremia among healthy blood donors and subsequent transmission of this infection to transfusion recipients has been documented. I K Mushaahar et al quoted that, an anti-HEV IgG prevalence rate of 7.8% to 45% has been reported in volunteer blood donors in endemic countries, in contrast to 1–4% in industrialized countries.<sup>4</sup>

Khuroo *et al.*, had shown that increased markers for acute HEV (anti-IgM and HEV RNA) are present in multitransfused patients as compared to controls. It was also proved that patients who were positive for HEV markers has received more transfusions, had increased incidence of icteric disease and higher ALT levels.<sup>5</sup>

Antibodies to HEV IgM & IgG increase after infection in the asymptomatic phase. Anti-HEV IgM titers increase during symptomatic stage and decrease to baseline within 3-6 months. After symptomatic disease. Anti-IgG titers may be detectable up to 15 years as stated by Mushahwar et al<sup>18</sup>

Hepatitis E virus (HEV) has long been considered an enterically transmitted virus causing self-limiting acute viral hepatitis. Currently screening of blood and blood products for HEV is not done and there are also no recommendations. But there is increased risk of parenterally transmitted HEV risk to transfusion safety.

The aim of the study is to find out the seroprevalence of Hepatitis E virus among voluntary blood donors in Chennai and the objectives are

- To detect IgM and IgG anti-HEV antibodies among healthy blood donors.
- ALT analysis- surrogate marker.
- IgM anti-HEV antibody positive samples will be confirmed by PCR.

### MATERIALS AND METHODS

This Cross sectional study was conducted at the Department of Transfusion Medicine, The Tamilnadu Dr. MGR Medical University, Guindy, Chennai. Ethical clearance of the study was obtained from the Institutional Ethical Committee of The Tamilnadu Dr. M.G.R Medical University, Chennai- 32. Samples were collected randomly from a total of 89 healthy voluntary blood donors from 10 camps, conducted at different areas in Chennai over a period from June 2013 – August 2014 after obtaining informed consent from the donors.

### Sample Collection

10 ml of blood from each donor was collected in two sterile capped tubes as 5 ml each. One tube was centrifuged; serum was then separated and stored at -20°C for further use for ELISA assay. Fresh serum from the other 5 ml sample was used for ALT assay.

Detection of IgG and IgM antibodies for Hepatitis- E by the enzyme-linked immunosorbent assay (ELISA) test., HEV RNA detection by RT-PCR in the serum of IgM HEV positive samples and estimation of Alanine aminotransferase level as a surrogate marker. Serum samples that were frozen earlier were thawed and were tested for IgG and IgM antibodies for Hepatitis- E by the Enzyme-Linked Immunosorbent Assay (ELISA) test. The Hep E-specific antibodies were studied by the commercial DIA.PRO Hepatitis- E IgM and IgG ELISA Kit.

ALANINE AMINOTRANSFERASE (ALT) Enzyme estimation was done in the Semi-Automated analyser by International Federation of Clinical chemistry (IFCC) Kinetic Method.

### Statistical Analysis

Data entry was done in EXCEL and analysis was done using SPSS software. Qualitative data were given in frequencies and percentage. Quantitative data were given in mean and standard deviation. Comparison of values were performed by chi-square test.

### RESULTS

In our study on serum samples from 89 voluntary blood donors, 36 (40.4%) belonged to 18- 19 years of age, 10(11.2%) were between 20-

22 years of age, 22 (24.7%) were between 23-30 years and 21 of them were greater than 30 years of age.

Out of 89 voluntary blood donors 83 (93.25%) were males and 6 (6.75%) were females. On the basis of occupation, 18(20.2 %) were professionals, 23 (25.8%) were doing of clerical work, 42 (47.2%) were students, and 3 (3.4 %) were coolies. The remaining 3.4 % were unemployed.

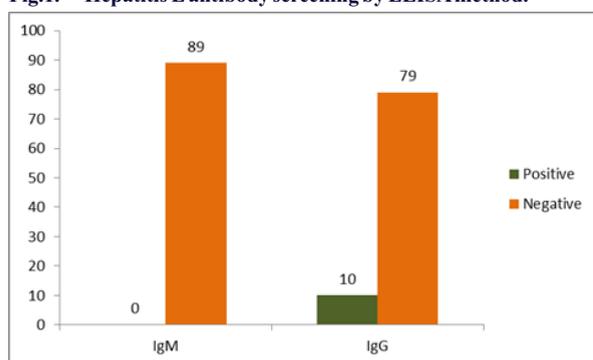
Of the total 89 donors, 29(32.58%) were first time donors and 60(67.41%) were regular donors. Majority of donors belonged to higher and middle classes (27.0% and 65.2% respectively) and the remaining 7.9% belonged to of lower class.

All 89 donors in our study were negative for mandatory screening tests for routine transfusion transmitted infections as per DGHS guidelines. Out of 89 donor samples screened for HEV IgG -antibody by ELISA, 10 showed positivity with prevalence rate of 11.2%. (Table-1, Fig-1).

**Table 1: Anti-HEV Antibody screening by ELISA**

Anti-HEV Antibody	Positive	Negative
IgM	0	89
IgG	10	79

**Fig.1. Hepatitis E antibody screening by ELISA method.**



Out of 10 donors who were positive for HEV IgG, 3 (8.33%) were 18-19 years of age, the remaining 7 (33.33%) were more than 30 years of age (p =0.002) Among the total of 83 male donors, 10(12.04%) were positive for HEV IgG. All 6 female donors in our study were negative for HEV IgG. (P=0.478). Out of 10 HEV IgG positive donors, 2 (11.1%) were professionals, 3 (13%) were doing clerical job, 3(7.1%) were students and 2 (66.7%) were coolies. (P=0.034). Out of 10 donors positive for HEV IgG, 6.89% were First time donors and 13.33% were regular donors. (P=0.303).

Among HEV IgG positive donor, 8.3% belonged to higher class, 8.6% were from middle class and 42.9% were from lower class. In low socioeconomic status, there is a statistical significance for IgG HEV positivity (P=0.022). (Table-2, Fig-2)

**Table 2: IgG seropositive as per socioeconomic status**

Socioeconomic status	IgG seropositive donors	IgG seropositive donors	Total donors
High	2	22	24
Middle	5	53	58
Low	3	4	7
Total	10	79	89

**Chi square value=7.618**

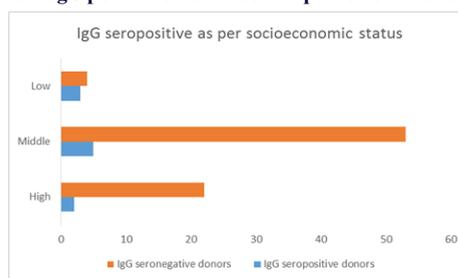
**P value=0.022**

In our study all the donors included were voluntary healthy blood donors. In the total of 89 study group screening for the mandatory tests were negative for Transfusion transmitted infections.(HIV, HBV, HCV, Syphilis and Malaria). Unlike our study Labrique AB et al in Bangladesh<sup>17</sup> reported there is associated infection of Hepatitis-B-35.2%, Hepatitis-C-1.5% and Hepatitis-A-95%. Gaetano Scotto et al<sup>13</sup> in his study at Italy reported that there is confection with HBV and HCV infections along in HEV IgG positive donors.

**Table 3: comparison of our study with other studies worldwide**

	Present study	Arankalle Et a <sup>6</sup>	Furong Ren et al <sup>16</sup>	Jutavijittum Et al <sup>7</sup>	Fukuda s et al <sup>11</sup>	David Juhl et al <sup>12</sup>	T.Pittaras Et al <sup>8</sup>
Place of the study	Chennai	Pune	China	Thailand	Japan	German	Greece
Seropositivity	11.2%	18.6%	27.4%	8.7%	3.7%	6.8%	9.43%
Age	18-19 Yrs.	8.33%		6.9%		3.3%	
	>30 yrs.	33.33%		10.6%			

**Fig.2. HEV IgG positive blood donors as per socioeconomic status.**



Among HEV IgG positive donors, none of them showed elevated ALT enzyme levels. All donors were negative for HEV IgM-antibodies done by ELISA. Enzyme Alanine Amino Transferase (ALT) assay on all samples were within normal range (6-30 IU/ml). Since none of the donors among the 89 study group showed IgM positivity PCR was not done.

**DISCUSSION**

The present study was undertaken to detect the seroprevalance of Hepatitis E Virus infection among voluntary blood donor population. In our study, the seroprevalance of HEV among voluntary blood donors is 11.2%. Similar studies done by Arankalle et al at Pune,<sup>6</sup> Jutavijittum et al at Thailand,<sup>7</sup> and T.Pittaras et al at Greece<sup>8</sup> revealed HEV seroprevalance of 18.6%, 8.7%, and 9.43%, respectively.

In the present study, seroprevalance among voluntary blood donors belonging to different occupation showed significant correlation. Similarly studies done by Furong Ren et al<sup>1</sup> at China showed high HEV Seroprevalance rate among donors who worked in contact with pork.

In our study, out of 89 donors 10 were IgG HEV antibody positive, none were IgM positive, which is similar to the studies done by Arankalle V.A et al in Pune<sup>6</sup>, India and Maria Luisa Mateos et al in their study at Spain.<sup>10</sup> But in a study done by S.Fukuda et al in Japan,<sup>11</sup> and David Juhl et al in Germany reported revealed positivity for IgM HEV antibody.<sup>12</sup> These donors are asymptomatic, potential transmissible donors.

In our study, about 8.33% (2 out of 24) IgG HEV positive donors were from higher socio economic group, while 8.62% (5 out of 58) and 42.8% (3 out of 7) were from middle and lower socio economic groups. This is in concordance with the study done by Jutavijittum et al in Thailand<sup>7</sup> who reported that the seropositivity increases in lower socio economic group when compared to higher socioeconomic group. Gaetano et al in Italy<sup>13</sup> also reported that there was a decline in seropositivity with rising socioeconomic status. Naveen et al<sup>14</sup> reported that infection with HEV is endemic in the developing countries and in areas of low socioeconomic conditions.

In our study, estimation of Alanine aminotransferase (ALT) on all IgG HEV positive donors showed normal levels (6-30IU/L). This is similar to the study done by Maria Luisa Mateos et al<sup>10</sup> in Spain and H.Takeda et al in Japan. These studies also showed no correlation between IgG HEV positivity and increase in ALT enzyme levels. On the other hand there is an increase in ALT enzyme level (4-5 times of normal) among IgM HEV positive donors as reported by Gaetano Scotto et al in Japan.<sup>13</sup> Yuhko Gotanda et al in Japan<sup>15</sup> in his Study on 6700 from healthy donors with elevated ALT level were tested for antibodies to HEV. Positivity for HEV IgG was 7.1% and 1.7% for HEV IgM. Among the eight samples positive for IgM five samples were positive for HEV RNA by RT-PCR. These results shows subclinical infection of the donors.

Sex	Male	12.04%		30.7%	9.0%		62.3%	10.65%
	Female	0		24.2%	7.9%		37.6%	4.08%
Occupation	Professional	11.1%						
	Clerical	13.04%						
	Coolie	66.66%		Pork meat producer				
	Student	7.1%						
Socioeconomic status	Higher	8.33%						
	Middle	8.62%						
	Lower	42.85%			Increased			
No of donation	First time donor	6.89%					2.6%	
	Regular donor	13.33%					7.3%	
IgM positivity	0	0	1.02%			Positive		
TTI	0							
ALT	N	Increased	Increased			Increased 2.9%		N

## CONCLUSION

In our study on the seroprevalence of Hepatitis E Virus infection among voluntary blood donors, none of them were either IgM positive or showing elevated enzyme levels suggestive of asymptomatic carrier state. However, there were a significant proportion of voluntary blood donors positive for IgG HEV with seroprevalence rate of 11.2%.

Most of the IgG HEV positive donors were from low socioeconomic status, they reside in areas with poor infrastructure facilities wherein other water and food borne infectious diseases are also common. Donors in age group of > 30 years showed higher percentage of IgG HEV Positivity.

Even though studies reveal that there is a possibility of HEV transmission by blood transfusion from asymptomatic donors, none of the donors in our study showed IgM HEV positivity. However, to arrive at a definite conclusion, the study has to be conducted on larger number of donors. Since HEV infection is more severe among high risk populations like pregnant mothers, immunocompromised individuals and dialysis patients. At least for these restricted population, blood components if needed have to be screened for HEV.

## REFERENCES

1. Universal access to safe blood transfusion. WHO manual.2008
2. Nabajyoti Choudhury et al. Transfusion transmitted infections: How many more? Asian Journal of Transfusion science 2010; 4:71-72
3. S.Madrow et al. Prevalence of nucleic acid sequences specific for human parvoviruses, hepatitis A and hepatitis E viruses in coagulation factor concentrates. Vox Sanguinis 2011;100:351-358
4. I.K. Mushahwar. HEV: molecular virology, clinical features, diagnosis, transmission, epidemiology and prevention. J Med Virol.2008; 80: 646-658.
5. Khuroo MS, Rustgi VK, Dawson GJ, Mushahwar IK, Yatoo GN, Kamili S, Khan BA. Spectrum of hepatitis E virus infection in India. J Med Virol. 1994; 43:281-286.
6. Arankle VA, Chobe LP. Retrospective analysis of blood transfusion recipients: Evidence for post-transfusion hepatitis E. Vox Sang. 2000; 79:72-4.
7. Jutavijittum et al. Seroprevalence of antibody to Hepatitis E Virus in Voluntary Blood Donors in Northern Thailand. Trop. Med 2000 June; 42 (2): 135-139.
8. T. Pittaras et al. Seroprevalence of hepatitis E in blood donors in Greece. Vox Sanguinis 2014; (106):387-387.
9. K.K. Prahalathan et al. Transfusion Transmissible infections among Blood Donors at a South Indian City. Int. Conf. AIDS, 2006, Aug. 13-18, 16. Abstract No. TuPe0299.
10. Maria Luisa Mateos et al. Hepatitis E Virus: Relevance in Blood Donors and Risk Groups. Vox Sang 1999; 76:78-80.
11. Fukuda et al. Prevalence of Antibodies to Hepatitis E Virus among Japanese Blood Donors: Identification of Three Blood Donors Infected With a Genotype 3 Hepatitis E Virus. J. Med. Virol.2004; 73(4):554-61.
12. David Juhl et al. Seroprevalence and incidence of hepatitis E virus infection in German blood donors. Transfusion 2014; 54:49-56.
13. Gaetano Scotto et al. Prevalence of Antibodies to Hepatitis E virus in Immigrants: A Seroepidemiological Survey in the District of Foggia. Journal of Medical Virology 2013; 85:261-265.
14. Naveen Kumar et. al. Hepatitis E-Is it a risk to transfusion safety? Asian journal of transfusion science. 2013; 7: 1-3.
15. Yuhko Gotanda et al. Ongoing Subclinical Infection of Hepatitis E Virus Among Blood Donors With an Elevated Alanine Aminotransferase Level in Japan. Journal of medical Virology 79:734-742 (2007).
16. Furong Ren et al. Hepatitis E virus seroprevalence and molecular study among blood donors in China. Transfusion 2014; 54:910-917.
17. AB Labrique et al. Epidemiology and Risk Factors of Incident Hepatitis E Virus infection in Bangladesh. Am J Epidemiol 2010; 172(8):952-961.
18. I.K. Mushahwar. HEV: molecular virology, clinical features, diagnosis, transmission, epidemiology and prevention. J Med Virol.2008; 80: 646-658.