

Full Length Research Paper

# High prevalence of anti-hepatitis E virus among Egyptian blood donors

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**This study evaluated the seroprevalence of hepatitis E virus among blood donors attending blood transfusion Center of Suez Canal University Hospital from March to September, 2010. Four hundred eighty eight (488) subjects which consisted of 137 Anti- Hepatitis C virus positive donors, 35 Hepatitis B surface antigens positive donors and 316 blood donors who were negative Hepatitis B surface antigen, Anti- Hepatitis C virus and HIV were included in this study. Anti-hepatitis E virus (IgG and IgM) was detected in 17.7, 28.57, and 26.28% of blood donors negative for Hepatitis B surface antigen (HBsAg) and Anti- Hepatitis C virus, Hepatitis B surface antigen (HBsAg) positive and Anti- Hepatitis C virus positive donors, respectively. No significant ( $P > 0.05$ ) association was found between anti- Hepatitis E virus positivity and Hepatitis B surface antigen (HBsAg) positivity and anti-Hepatitis C virus positivity subjects. The overall prevalence of anti- Hepatitis E virus antibodies (IgG and IgM) was 20.9% (102/488). Seroprevalence increased significantly with age; from 8.3% in subjects below 20 years of age, 16.94% in 20-34 years of age, 34.5% in 35-49 years of age and a slight decline of 33.3% over those of 50 years of age. All anti-HEV antibodies samples were negative for Hepatitis E virus RNA by reverse transcriptase polymerase chain reaction (RT-PCR) method. Even though, seroprevalence of hepatitis E virus antibody among blood donors in our study in Ismailia, Egypt is high, transfusion-associated with hepatitis E infection still needs further investigation.**

**Key words:** Hepatitis E virus, Blood donors, seroprevalence.

## INTRODUCTION

Hepatitis E virus (HEV) is the etiological agent of acute hepatitis. It is a non-enveloped, positive-sense, single stranded RNA virus. Originally classified within the family of caliciviruses, HEV is now classified as the sole member of the genus *Hepevirus* in the family *Hepeviridae* (Emerson et al., 2005, Tam et al. 1991).

Even if hepatitis E is a self-limiting viral infection followed by recovery, occasionally, HEV infection leads, in 1 to 2% of cases, to lethal fulminant hepatitis, and this level reaches 20% in pregnant women infected during their

third trimester in areas of endemicity (Singh et al., 2003).

Hepatitis E is endemic in many developing countries of Asia and Africa where sanitation is sub optimal (Dalton et al., 2008) and is also endemic in many industrialized countries including the United States, European countries, and Japan (Emerson and Purcell, 2004; Purcell and Emerson, 2001; Okamoto et al., 2003).

Antibody to HEV, which is indicative of past infection, has been detected in 5 to 60% of the general population in developing countries where the disease is endemic

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(Purcell, 1996). In Egypt, recent studies reported very high levels of anti-HEV prevalence among healthy adults and pregnant females in rural areas; 67.7 and 84.3%, respectively (Stoszek et al., 2006).

The virus is excreted in feces and primarily transmitted via the fecal-oral route through contaminated water or food. In developing countries with poor sanitation conditions, rare outbreaks of acute hepatitis E in more explosive epidemic form are generally associated with fecal contamination of drinking water (Arankalle et al., 2001; Purcell and Emerson, 2001). In addition most of the HEV outbreaks have been observed during the rainy seasons or after floods (Uchida, 1992).

Alternatively, the detection of serum antibodies to HEV and its viral genome in various animal species; like, swine, rodents, chickens, dogs, cows, sheep and goats (Arankalle et al., 2001; Favorov et al., 1998; Tien et al., 1997) amplified the idea of a possibility of zoonotic transmission of the virus.

Although transmission of HEV is generally via the faecal-oral route, person-to-person transmission, and transmission via the parenteral route or blood transfusion have been suggested (Dawson et al., 1997; Schlauder et al., 1993).

Blood borne transmission of HEV had been investigated as indirect evidence implicating HEV as a potential transfusion risk by many investigators worldwide. It has been reported that a substantial proportion of blood donors (1.5%) were positive for HEV RNA and viraemic blood donors are potentially able to cause transfusion-associated hepatitis E in areas of high endemicity (Arankalle and Chobe, 1999; Arankalle and Chobe, 2000).

This work was carried out to determine the seroprevalence of HEV and attempt to gain insight into the possible blood-borne transmission of HEV among blood donor who attended blood transfusion center of Suez Canal University Hospital during the study period.

## MATERIALS AND METHODS

### Serum samples

Between March 1, 2010, and September 15, 2010, serum samples were collected from consecutive, voluntary, apparently health blood donors attending blood transfusion Center of Suez Canal University Hospital, Ismailia, Egypt. Demographic data were collected using a questionnaire. After routine blood screening for Hepatitis B surface Antigen (HBsAg), anti-HCV and human immunodeficiency virus (anti-HIV) markers and alanine transaminase (ALT) level, 488 serum samples consisted of 137 anti-HCV positive samples, 35 HBsAg positive samples, and 316 samples who were negative HBsAg, anti-HCV and anti-HIV were recruited and the serum were stored in duplicate at -20°C for anti-HEV antibodies detection and HEV RNA extraction.

### Detecting anti-HEV antibodies

Anti-HEV IgG and IgM were detected using Third generation Enzyme Immuno Assay (EIA) according to the manufacturer's instructions (DIA.PRO, Milano, Italy). The Cut-off was calculated by

addition of 0.350 with mean optical density value of the Negative Control (NC) and samples were considered as positive when ratio of the test result of sample (od450nm) and the cut-off value was above 1 (or  $\leq 1$ ), according to the manufacturer's instruction. All positive samples were retested in duplicate with the same EIA assay to confirm the initial results.

### Purification of viral RNA primer used

The RNA was extracted from 140  $\mu$ m serum samples using the QIAamp® Viral RNA Mini kit (Germany) according to the manufacturer's instructions. The viral RNA was eluted from the spin column with 45  $\mu$ L of the elution buffer and stored at -20°C. Two sets of primers for reverse transcriptase polymerase chain reaction (RT-PCR) and reverse transcriptase-nested polymerase chain reaction (RTnPCR) to amplify different region of the capsid gene were used (Huang et al., 2002).

### RT-PCR and nested PCR

RT-PCR was performed by using a RevertAid TMFirst Stand cDNA Synthesis RT-PCR kit (Fermentas, Canada). The RT step was carried out in a 20  $\mu$ L reaction mixture volume containing 1  $\mu$ L (concentration) Primer S1HE-R (Metabion GmbH, Germany), 4  $\mu$ L 5xbuffer, 1  $\mu$ L (units/uL) RNase inhibitor, 2  $\mu$ L reverse transcriptase (units/uL); 2  $\mu$ L dNTPs mix (Fermentas, Canada). The mixture was incubated at 37°C for 1 h, heated for 5 min at 70°C, and placed immediately on ice. The first round of PCR was carried out in a total reaction mixture volume of 25  $\mu$ L including 2.5  $\mu$ L 5 x buffer, 0.5  $\mu$ L dNTPs mix (Fermentas, Canada), 0.5  $\mu$ L Primer S1HE-R, 0.5  $\mu$ L Primer S1HE-F, (Metabion, GmbH, Germany), 0.25  $\mu$ L BioReady rTaq polymerase (Hangzhou, China), 18.5  $\mu$ L RNase-free water and 2.5  $\mu$ L of cDNA. 35 cycles of PCR (94°C for 5 min, 94°C for 30 s, 50°C for 30 s and 72°C for 1min) were carried out.

For the second round of polymerase chain reaction (PCR), 2.5  $\mu$ L of the first PCR product was amplified as described above (The first round of PCR), except that the primers used were Primer S2HE-R and Primer S2HE-F (Metabion GmbH, Germany). The expected size of the PCR product amplified with the nest of primers was 348 bp (Huang et al., 2002).

### Statistical analysis

Data were entered and analysis was performed using the statistical package for the social sciences (SPSS V-16) for windows (Version 16, SPSS). Descriptive summaries were the study findings presented, and were explained in words and tables. Chi-square test ( $\chi^2$ ) was used to compare and assess difference between the proportions between groups. In all cases, P-value less than 0.05 was taken and considered as statistically significant.

The study was endorsed by the ethical committee of the faculty of Medicine, Suez Canal University. Participation was fully voluntary and consent was obtained from all participants. Any information obtained during the study was kept confidential.

## RESULTS

A total of 488 samples which consisted of 137 anti-HCV positive blood donors, 35 HBsAg positive donors and 316 blood donors who were negative HBsAg, anti-HCV, and HIV were included in this study. The mean and SD of age in the study group was  $29.90 \pm 7.44$  years. Overall seroprevalence of HEV was 20.90% (102/488). Seroprevalence

**Table 1.** Seroprevalence of HEV Ab in different age groups of blood donors.

Age (year)	HEV Ab		Total	P Value
	Positive	Negative		
< 20	1 (8.3%)	11 (91.7%)	12	0.0031*SA
20- 34	61(16.94%)	299(83.06%)	360	
35-49	39 (34.5%)	74(65.5%)	113	
≥50	1(33.3%)	2 (66.7%)	3	
Total	102 (20.90%)	386 (79.1%)	488(100%)	

HEV Ab, Hepatitis E virus Antibody; \*Chi-square test ( $\chi^2$ ); SA, significant association ( $p < 0.05$ ) (Between  $\leq 35$  year old and  $> 35$  years old).

**Table 2.** Frequency of anti-HEV in blood donor positive for HCV Ab and HBsAg and Blood donors negative for HCV Ab, HBsAg and HIV.

Parameter	Anti- HEV		Total	P value
	Test positive (%)	Test negative (%)		
HCV Ab	36 (26.28%)	101 (73.72%)	137	0.068 *NS
HBsAg	10 (28.57%)	25 (71.43%)	35	0.247 *NS
Blood donors negative for HCV Ab ,HBsAg and HIV	56 (17.7%)	260 (82.3%)	316	
Total	102 (20.9%)	386 (79.1%)	488 (100%)	

HBsAg, Hepatitis B surface Antigen; HCV Ab, Hepatitis C virus Antibody; HIV, Human immunodeficiency Virus; \* Chi-square test ( $\chi^2$ ); NS, no significant association ( $p > 0.05$ ) of anti-HEV positivity between of HBsAg or anti -HCV and Blood donors negative for HCV Ab, HBsAg and HIV .

**Table 3.** Frequency of Anti- HEV among blood donors negative for HCV Ab, HBs Ag and HIV and its relation with ALT levels.

Anti- HEV	ALT≤40IU/L	>40IU/L	TOTAL	P value
Test positive (%)	43 (19.6%)	13 (13.4%)	56 (17.7%)	0.183*NS
Test Negative (%)	176 (80.4%)	84 (86.6%)	260 (82.3%)	
Total	219	97	316 (100%)	

ALT, Alanine aminotransferase, \* Chi-square test ( $\chi^2$ ); NP, No significant association ( $p > 0.05$ ) of HEV seropositivity between specimens with ALT level  $\leq 40$  and  $> 40$ .

in male and female was 20.96% (100/477) and 18.82 % (2/11), respectively.

Age-specific prevalence of anti-HEV increased significantly with age (Table 1), which was 8.33% in subjects under 20 years, 16.94% in individuals 20 to 34 years, 34.51% in individuals 35 to 49 years and 33.33% over 50 years old. There was significant difference ( $p < 0.05$ ) of seropositivity between age groups of  $\leq 35$  year old and  $> 35$  years old.

The highest seroprevalence of HEV was detected in specimens positive for HBsAg (28.57%), followed by specimens positive for anti- HCV (26.28%) while blood donors negative for HBsAg, Anti HCV and HIV had the lowest seroprevalence (17.7%) (Table 2). There was no statistical significant difference ( $p > 0.05$ ) in anti-HEV positivity between HBsAg or anti HCV and blood donors negative for HCV Ab, HBsAg and HIV.

The frequency of Anti- HEV among blood donors with elevated ALT ( $> 40$  IU/L) and normal ALT level ( $< 40$  IU/L) who was negative for HCV Ab, HBs Ag and HIV was 19.6 and 13.4% respectively (Table 3). There was no meaningful difference ( $p > 0.05$ ) in seropositivity between the ALT level  $\leq 40$  IU/L and  $> 40$  IU/L.

From 102 ELISA sera, 92 samples were tested for RNA by RT-PCR and all were negative, the other 10 samples were not tested because of inadequate sample volume.

## DISCUSSION

Hepatitis E is one of the important hygienic infectious problems of the world with a high incidence in developing countries, mainly in Asia and in Africa (Dalton et al., 2008). A research conducted in rural area of Egypt reported 67.7 and 84% of anti-HEV prevalence among healthy

adults and pregnant women respectively (Stoszek et al., 2006). The authors concluded that both zoonotic and anthroponetic transmission of virulent HEV occurs widely in these rural villages and that the rate of positive antibodies increased with age.

In this study, the overall prevalence of anti-HEV antibodies among our blood donors was 20.9%, which is more than figures obtained from blood donors in Germany (5.94%) (Vollmer et al., 2012), Spain (2.8%) (Mateos, et al., 1999), Ghana (4.6% anti-HEV IgG and 5.9% anti-HEV IgM) (Meldal et al., 2013) and in Saudi Arabia (16.4%), (Abdelaal et al., 1998) general population in Pakistan (17.5%) (Hamid et al., 2002) and healthy blood donors in Riyadh (8.37%) (Arif, 1996), but it is lower than what was reported in previous Egypt study which was 45.2% (43/95) in blood donors and 39.6% (38/96) in hemodialysis patients (AbdelHady et al., 1998). This can be explained by varying epidemiologic condition in different geographical area and difference in diagnostic techniques between studies. This study was not able to assess the sex association of anti-HEV due to low flow of female donors to the center.

In the current study, seroprevalence increased significantly with age; from 8.3% in subjects below 20 years of age, 16.94% in 20 to 34 years of age, 34.5% in 35 to 49 years of age and a slight decline of 33.3% over those 50 years of age. The slight decrease of anti-HEV in age group over 50 may be the small study subjects in this age group. Similar finding of sero activity associated with increasing age was also reported in other studies among persons living in HEV endemic (Arankalle et al., 1995; Cheng, 2012) and non-endemic regions (Bernal et al, 1996).

In contrast to the above study, in the present work, all of 92 anti-HEV study subjects were negative for polymerase chain reaction PCR test, which point to the absence of viremia in the blood donors. A study conducted in Iran also showed that out of the 33 ELISA positive sera, only one was for RT-PCR, which is nearly similar to other finding (Keyvani et al., 2009). Another study conducted in Ghanaian blood donors also showed that all anti HEV IgG and anti-HEV IgM positive sera were HEV-RNA negative by RT-qPCR (Meldal et al., 2013).

The present study also searched the seroprevalence of HEV antibodies among HBsAg and HCV antibodies. Out of 137 individual positive for Anti-HCV, 36 (26.28%) were positive for anti-HEV and out of 36 individuals positive for HBsAg, 10/36 27.78% individuals were positive for anti-HEV antibodies. In 316 individuals negative for anti-HCV, HBsAg and anti-HIV as control group, anti-HEV positivity was 56/316 (17.7%). The prevalence of anti-HEV in HBsAg and anti-HCV positive individuals was slightly higher than from individual without anti-HCV, HBsAg and anti-HIV. This study observed no statistical association between anti-HEV and anti-HCV or HBsAg positivity. One study conducted in Egypt has shown a striking association between HCV and HEV, pointing to similar or overlapping routes of transmission (AbdelHady

et al., 1998).

From 97 individual without anti-HCV, anti-HIV and HBsAg, only 13 individual were positive for anti-HEV with elevated ALT (>40). Out of this positive individual 9 were with ALT level range of 41-60 IU/L, 3 were with 61-80 IU/L and only one was with ALT level >120IU/L. This study shows that, the increase of ALT level has no correlation with anti-HEV prevalence, so the cause for elevated ALT level might be other factors.

In conclusion, Seroprevalence of HEV-antibody among blood donors in our study in Ismailia, Egypt is high, but we cannot recommend screening of all blood donors for HEV until more data becomes available and until more is known about the parenteral route of transmission. A careful surveillance in the general population is required and further appropriate investigations are needed to identify the exact mode of transmission and risk groups in Egypt.

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