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ORIGINAL ARTICLE

Status of anti-HEV IgG and IgM antibodies among the hemodialysis patients in southwest region of Iran

Roya Pirmoradi[1], Manoochehr Makvandi[1*], Azadeh Haghi Navand[1], Shokouh Shayanpour[2], Heshmatolah Shahbazian[3], Kimia Makvandi[4], Saeed Eynali Varnosfaderani[4], Sepideh Nasimzadeh[1]

[1]Department of Virology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[2]Department of Nephrology, Imam Khomeini Hospital, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[3]Department of Nephrology, Golestan Hospital, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[4]Deputy of Health, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

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ABSTRACT

Background and Objectives: Hepatitis E Virus (HEV) account for acute hepatitis, fulminant liver failure and chronic hepatitis worldwide. Several high risk groups including hemodialysis (HD) patients are at risk of HEV infection. Based on consequences of HEV infection it is important to determine the serological and molecular epidemiology of HEV in HD patients. The aim of this study was to evaluate the frequency of HEV antibodies and HEV RNA in HD patients.
Materials and Methods: The sera of 84 HD patients were collected and tested for anti- HEV IgG and anti IgM antibodies using enzyme-linked immunosorbent assay (ELISA) at Golestan hospital in Ahvaz city during October 2014 and November 2014. HEV RNA was tested in HD patients using RT PCR. The prevalence of anti- HEV IgG was evaluated in the age group (52/84) > 50 and (31/84) < 50 years.
Results: Out of 84 patients, 52 (61.9%) were males and 32 (38.1%) females. The mean age of participants was 52 ± 1.57 years. 43/84 (51.19%) cases including 26/52 (50%) males and 17/32 (53.1%) females were positive for anti-HEV IgG (p=0.95). Among the 43 cases positive anti-HEV IgG 8 cases including 5 (9.61%) males and 3 (9.37%) females tested positive for anti-HEV IgM (p=0.729) while the HEV RNA was negative in HD patients. The distribution of anti- HEV IgG was 62.75% and 33.33% among the age group >50 and <50 respectively (p=0.015).
Conclusion: This study showed high prevalence of anti-HEV IgG antibodies (51.19%) were observed among the HD patients while the HEV RNA tested negative in HD patients. The rate of HEV IgG is significantly higher with increased age. Further investigation require to identify the factors account for high seroprevalence of HEV in Ahvaz HD units.

Keywords: Hepatitis E virus; Hemodialysis patients; Enzyme-linked immunosorbent assay

*Corresponding author: Manoochehr Makvandi, Ph.D, Department of Virology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. Tel: +98-9166181683 Fax: +98-6133738123 Email: manoochehrmakvandi299@gmail.com

INTRODUCTION

HEV account for acute and fulminant hepatic failure (1). The subclinical course of HEV infection reported, in blood donors (2). Chronic hepatitis E has been observed in immunocompromised patients (3, 4). The occult HEV infection was reported in patients with cirrhosis of liver (5). The association of HEV infection has been reported in patients with neurologic disorders, acute pancreatitis, autoimmune symptoms and hematologic abnormalities (6, 7).

Approximately 20 million HEV infections take place annually, with more than 3 million symptomatic cases and about 60,000 fatalities. (8). Hepatitis E virus (HEV) is a positive-sense, single-stranded RNA virus, and is classified in the genus *Orthohepevirus*, the family *Hepeviridae* (9).

HEV is a small, non-enveloped virus with a 7.2 kb, positive-sense RNA genome (8, 9). Its genomic RNA contains 4 ORFs. ORF1 encodes non-structural proteins including methyltransferase, protease, helicase, polymerase and need for HEV replication. ORF2 encodes the capsid protein, which is the major structural protein of the HEV virion. ORF3 encodes a small protein with a molecular weight of 13 kDa. ORF4 is overlapped with ORF1 and cap-independent (10). HEV have been classified into eight genotypes with several subtypes, among them genotypes HEV-1 to HEV-4 have been detected in human while HEV-5 and HEV-6 have been isolated in wild boars (11, 12). HEV-7 have been infected humans and cause chronic hepatitis E (13). HEV-8 has been identified in Bactrian camels (14).

HEV can be transmitted via fecal-oral, subclinical blood donors, blood transfusion and plasma donations (15, 16). Several investigators have been reported the subclinical donors with HEV infection have transmitted HEV infection to recipients blood such as HD patients (17-19).

The serological HEV IgG, IgM and nucleic acid tests have been developed for diagnosis of HEV infection. Anti-HEV IgM antibodies and HEV RNA can be identified during the acute phase of sickness (20), whereas detection of anti-HEV IgG antibodies alone, indicates the past infection can last more than 10 years (21). The seroprevalence of HEV found in HD patients in Iran (22, 23). The circulation of HEV genotype 1 reported in blood donors in Shiraz, Iran (24).

HD patients on dialysis have anemia and their kidneys cannot make enough erythropoietin to help their body make red blood cells. So HD patients frequently receive blood transfusions which expose them to HEV infection (17-19). Therefore, this study was conducted to determine the rate of HEVIgM and HEV IgG and HEV RNA in HD patients in Golestan hospital, Ahvaz city, Iran.

MATERIALS AND METHODS

This was a cross sectional study and conducted on 84 sera samples of HD patients, including 32 females and 52 males who referred to Golestan Hospital, Ahvaz, Iran. Written consent was taken from each participant. The Ethics Committee of the Ahvaz Jundishapur University of Medical Sciences has approved this study.

The sera were collected from 84 patients undergoing hemodialysis at Golestan hospital in Ahvaz city during October 2014 and November 2014. The duration of dialysis was recorded >3 and <3 years. All the sera samples were stored at -80°C until further analyses.

ELISA tests. All the sera samples were tested for anti-HEV IgM and anti-HEV IgG by ELISA test kit (Diapro, Milano, Italy) according to the manufacturer's instruction. The specificity of the HEV IgM and HEV IgG DIA.PRO ELISA kits > 95% and > 99.5%, respectively, and the sensitivity of these kits was 100% (24).

RNA extraction and amplification. RNA was extracted from positive HEV IgM and HEV IgG sera samples using pars to Kit (Pars Toos, Iran) according to manufacturer's instructions. cDNA was synthesized (Yektatajhis, Iran) according to manufacturer's instructions.

Then, cDNA template was amplified using outer primers [forward primer (HE361): GCRGTGGTTTCTGGGGTGAC; reverse primer (HE364): CTGGGMYTGGTCD CGCCAAG] and inner primers [forward primer (HE366): GGGYTGATTCTCAGCCCTTCGC; reverse primer (HE363): GMYTGGTCDCGCCAAGHGGA] by nested RT-PCR. The 139 bp PCR product indicate positive (25). The second nested RT-PCR was carried out to amplify 731 nucleotides and 348 nucleotides of the ORF2 region, the outer primers [forward primer (3156N):

AATTATGCYCAGTAYCGRGTTG; reverse primer (3157N): CCCTTRTCYTGCTGMGCATTCTC] and inner primers [forward primer (3158N): GTWATGCTYTGCATWCATGGCT; reverse primer (3159N): AGCCGACGAAATCAAT TCTGTC] (26). The First round PCR was achieved with 25 µl volume, containing 8 µl template, 2.5 µl 10× reaction buffer, 0.75 µl $MgCl_2$ (50 mM), 0.5 µl forward/reverse primer, 1 µL dNTP (10 mM), 0.2 Cinna Gen Taq DNA Polymerase (5 u/µl), and 12.55 µl D/W. The reaction mixture was subjected to thermal cycler (TC-512, Techne, UK) using the following program: 1 cycle with initial denaturation at 94°C for 5 minutes, followed by 35 cycles, 94°C for 1 minute, 60°C for 1 minute, 72°C for 2 minutes, and final extension at 72°C for 10 minutes. The first round of PCR product was used as the template for the second round. The amount of PCR components was the same as the first round except for the DNA template, which 7 µl was used in the second round. The thermal cycler was programmed as follows: initial denaturation at 94°C for 5 minutes, 30 cycles consisting of 94°C for 1 minute, 44°C for 1 minute, 72°C for 1.5 minutes, and final extension at 72°C for 10 minutes. All reactions were performed along with negative and positive controls. The 348 bp PCR product indicate positive.

Statistical analysis. SPSS 17 (SPSS Inc., Chicago, IL, USA) was used to perform all statistical analyses. Data were displayed as frequencies, percentage and mean ± standard deviation (SD) following analysis by descriptive statistics. Quantitative data were compared between HEV seropositive and HEV seronegative gender by Chi-square test P value < .05 was considered statistically significant.

RESULTS

Out of 84 patients, 52 (61.9%) were males and 32 (38.1%) females. The mean age of participants was 52 ± 1.57 years. 43/84 (51.19%) cases including 26/52 (50%) males and 17/32 (53.1%) females were positive for anti HEV IgG (p=0.95). Among the 43 cases positive anti-HEV IgG, 8 cases including 5 (9.61%) males and 3 (9.37%) females tested positive for anti-HEV IgM (p=0.729) while the HEV RNA was negative in HD patients. The distribution of anti- HEV IgG was 32/51 (62.75%) and 11/33 (33.33%) among the age group >50 and <50 respectively (p=0.015).

Table 1. Frequency of HEV IgG among the HD patients

Category	Test N	HEV IgG Positive	P value	HEV IgM Positive	P value
Sex					
Males	52	26 (50%)	0.95	5	0.729
Females	32	17 (53.1%)		3	
Age group (years)					
>50	51	32 (62.75%)	0.015	5	0.07
<50	33	11 (33.33%)			
Duration of dialysis (years)					
<3	66	37 (56.06%)	0. 148		
>3	18	6 (33.33%)			

Table 1 shows the distribution of anti-HEG IgG: among the males and females was 50% and 53.1% respectively (p=0.95), among the age-group > 50 (62.75%) and > 50 years (33.33%) was significant (p=0.015), and the duration of hemodialysis <3 (56.06%) and >3 years (33.33%) was not significant (p=0.148). The distribuation of HEVIgM among gender (0.729) and age groups (p=0.07) was not significant.

The results of HEV RNA negative in HD patients positive for anti-HEV IgG and anti-HEV IgM. Table 1 shows the distribution of anti-HEV IgG among the gender, age groups and duration of dialysis.

DISCUSSION

Hemodialysis patients have debilitated immune systems, which increase their risk for infection, and they require frequent hospitalizations and surgery where they might acquire an infection. The seroprevalence of anti-HEV IgG varies among patients undergoing hemodialysis (HD).

In our survey 43/84 (51.19%) cases including 26/52 (50%) males and 17/32 (53.1%) females tested positive for anti-HEV IgG (p=0.95). Among the 43 cases positive for anti-HEV IgG, 8 cases including 5 (9.61%) males and 3 (9.37%) females tested positive for anti-HEV IgM (p=0.729) while the HEV RNA negative in all HD patients. The distribution of anti- HEV IgG among the age groups was >50 and <50 years 62.75% and 33.33% respectively (p = 0.015).

Marıa Belen Pisano et al. in Cordoba, Argentina

(2018) have conducted a cohort study and reported 8/82 (9.7%) of HD patients showed positive for HEV IgG, and found that 5/8 of positive anti-HEV IgG cases tested positive for anti-HEV IgM while the HEV RNA was not detected among the HD patients (27).

Harrison et al. (2013) in the southwest England have reported among the 28/76 (36.8%) HD patients positive for anti-HEV IgG, 2 cases were also positive for HEV IgM, while HEV RNA was not detectable among the HD patients (28). In our analysis high prevalence of 33/84 (39.28%) of anti-HEV IgG were observed in HD patients which is in accordance with Harrison et al. findings (28). Ismail et al. (2019) in the Lebanon have reported 37/171 HD patients including 22/96 (22.92%) males and 15/75 (20%) females showed positivity for anti HEV IgG (29). Mrzljak et al. (2020) in Croatia have described the HEV IgG antibodies was detected in 110 (27.9%) HD patients. They have reported the status of seroprevalence of HEV IgG antibodies were ranging from 5.2 to 43.4% in the different dialysis centers ($p = 0.001$). The HEV IgM antibodies were found in 0.04% of IgG positive HD patients while all the HD patients tested negative for HEV RNA (30). Scotto et al. (2015) have described 14/ 231 (6%) of the HD were positive for HEV IgG and among the positive cases 2 of them were also positive for HEV IgM and 3 patients tested positive for HEV RNA (31).

Several factors may influence the spread of HEV infection in HD patients. These are as follow, heparin is extracted from porcine small intestine and HEV RNA has been detected in concentration of heparin derived from these animals, so the contaminated heparin with HEV infection may transmit HEV to HD patients (32). Patients with chronic kidney disease have weakened erythropoiesis and thus HD patients need to receive blood transfusion (33). Therefore, the contaminated blood transfusion with HEV infection could transmit the virus to recipients.

The detection of HEV RNA has been detected among blood donors in Shiraz city, Iran (24). The HEV RNA detected in the blood donors in Germany (34) France (35), Netherland (36), England (37) and Spain (38). Presently, blood transfusions are regularly tested for HEV RNA in the UK, Ireland, and Netherlands (39).

Remarkably, the other risk factor frequently reported to be associated with HEV infection, such as consumption of drinking of tap water (40, 41). The HEV RNA has not been detected in the drinking water in our region but needs further study. Foodborne transmission is a major pathway for HEV infection. The HEV RNA has been detected in shellfish (42, 43). Also, high seroprevalence of HEV infection has been reported in seafood processing workers in China (44). Our region Ahvaz city is close to Persian Golf and most of people are consuming sea food but so far no investigation has been carried out to detect HEV RNA in variant fishe or Shrimp in this region.

A limitation of this study is lack of molecular epidemiology studies of HEV (of all genotypes and subtypes), in different regions of Iran. The complete sequencing of HEV genomes need to evaluate the phylogenetic and evolutionary relationships of the HEV strains in different region of Iran.

CONCLUSION

In conclusion, this study showed the high prevalence of anti-HEV IgG antibodies (51.19%) tested positive among the HD patients while the HEV RNA test was negative in these patients. The rate of positive HEV IgG are significantly higher with increased age. Further investigation is required to identify the factors account for high seroprevalence of HEV in Ahvaz HD units.

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