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Original Article

Evaluation of anti-hepatitis E virus antibody among hemodialysis patients in Gorgan, north of Iran

Naziri H[1], Tahamtan A[1], Moradi A[2], Tabarraei A[2*]

1. Department of Virology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
2. Department of Microbiology, School of Medicine, Golestan University of Medical Sciences, Gorgan, Iran

Abstract

Background and Aims: Hepatitis E virus is a public health concern and about a third of people in the world live in endemic areas. Although HEV is believed to be transmitted by fecal-oral routes between humans in low sanitary conditions, but evidence of transmission have been reported in hemodialysis (HD) patients.

Materials and Methods: The aim of present study was to determine the prevalence of anti-HEV antibody among HD patients in Gorgan, north of Iran. In this cross-sectional study, totally 300 HD patients from May to December 2014 were tested for detection of anti-HEV IgG and IgM by commercial HEV enzyme-linked immunosorbent assay (ELISA) kit. Demographic variables were collected in pre-designed questionnaires.

Results: Out of 300 individuals, 148 (49.3%) were male and 152 (50.7%) were female. The overall anti-HEV IgG seroprevalence rate was 4%. Result showed significant association between anti-HEV IgG and duration of dialysis ($p = 0.03$), but there were no significant differences between the subjects grouped according to gender, Ethnicity, dialysis per week and age. No anti-HEV IgM were detected in patients.

Conclusions: Since our finding showed statically significant relationship between duration of hemodialysis and anti-HEV. Therefore, hemodialysis maybe be considered as a risk factor in HEV transmission. However further studies are needed to confirm our data.

Keywords: HEV, Hemodialysis, Seroprevalence, Iran.

Introduction

Hepatitis E Virus (HEV) is a non-enveloped single stranded RNA virus that is approximately 7.2 kb in size. It was determined in an unknown outbreak hepatitis in Russian soldiers in Afghanistan in 1983 (1) . HEV is a public health concern and about one-third of people in the world lives in endemic areas at risk for infection, as a result of limited access to clean water and improved sanitation facilities (2) . Elderly people are more likely to experience more severe HEV infection that it may be due to the host factors (3) . HEV has at least 4 genotypes, genotypes 1 and 2 have been found only in human but in addition to human sources, HEV strains of genotypes 3 and 4 have been detected in several animals. Unlike several genotypes, only one serotype of HEV has been identified. It is believed that HEV has been transmitted by fecal-oral routes between humans in low sanitary conditions and improper disposal of sewage nevertheless other mode of transmission like parenteral routes, transfusion and hemodialysis has been reported (4) . HEV causes asymptomatic infection to acute hepatitis in healthy individuals and non-pregnant women with spontaneous recovery in almost all cases. More severe disease and high mortality rate (20-25%) has been observed among pregnant women (5) . hormonal shifts

[*] **Corresponding author:** Alijan Tabarai PhD, Department of Microbiology, School of Medicine, Golestan University of Medical Sciences, Falsafi Educational Campus, Shaskalate, 5th km Gorgan - Tehran Rd., Gorgan, Iran. E-mail: alijant@yahoo.com , Tel: +9817132440225

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(estrogen and progesterone) and consequently immunological changes during pregnancy may effect on the severity of HEV infection (6) . HEV RNA has been detected in 1.5% of blood donors in high endemic areas suggesting that transfusion-transmitted HEV is possible in these regions (7) . Although some studies reported an unexpectedly high prevalence of anti-HEV antibody in their HD patients and considered nosocomial transmission via infected blood and contaminated instruments as a route of transmission but it remains to be investigated (8) . In contrast other studies found low rate of anti-HEV-positivity in their HD patients (9, 10) . As far as we know, there are few studies with conflicting results about presence of detectable HEV RNA in serum and serorprevalence rate of HEV in HD patients in Iran as an endemic area. In our study we assessed the presence of HEV RNA among HEV-seropositive HD patients in Gorgan, north of Iran. Gorgan is a big city with population of about 350000 people that include two main ethnic: Fars and Turkmen.

Methods

Patients. This cross-sectional study was approved by the Ethical Committee of Golestan University of Medical Science. It was performed on 300 HD patients attending in 5th Azar hospital from May to December 2014. Demographic variables such as age, sex, ethnicity, duration of dialysis, history of blood transfusion were collected in pre-designed questionnaires.

Laboratory evaluation. Five ml blood samples were collected from all patients after obtaining an informed consent. Serum stored in -80 ˚C until anti-HEV IgG and IgM determination. Anti-HEV IgG and IgM were detected by commercial HEV enzyme-linked immunosorbent assay (ELISA) kit (HEV IgG/IgM; DIA.PRO Srl, Milan, Italy) according to manufacturer's instruction. The DIA.PRO assay uses HEV-specific synthetic antigens encoding for conservative and immunodominant determinants derived from Mexican and Burma virus strains. When the optical density (OD) value was more than cut-off value, the samples were considered positive.

All positive anti-HEV IgG and IgM were tested for HEV-RNA by Reverse Transcriptase- Polymerase Chain Reaction (RT-PCR). Blood samples were obtained in sterile EDTA-containing tubes and plasma was separated and stored at -70 ˚C. RNA was extracted from plasma according to the manufacturer protocol (Qiagen, Hilden, Germany). cDNA synthesis was performed at 50˚C for 25 minutes by one step kit as indicated by manufacturer (Invitrogen). The HEV open reading frame 3 (ORF3) genes was amplified using specific HEV primers following standard method. In brief, The HEVORF3 gene was amplified using the following reaction mixture: 4 µL of cDNA, 4 µL of 10X PCR buffer, 2µL of 5 mM dNTP, 0.5 µL of 5 U Taq DNA polymerase , 6 µL of 100 mM MgCl2, 0.5 µL of each PCR primer (10 pM) and 33 µL of DEPC-treated water. The following primer pairs were used: forward primer 5'-GGTGGTTTCTGGGGTGAC-3' and reverse primer 5'-AGGGGTTGGTTGGATGAA-3' (11). Annealing was done at 62.5˚C, extension at 72˚C and denaturation at 94˚C. Each step of a cycle was carried out for 1 minute. The PCR consisted of 45cycles and final extension step at 72˚C for 5 minutes.

Statistical analysis. Statistical analyses were performed by SPSS (version17) statistical software (Scientific Package for Social Sciences, Chicago, IL). using ANOVA test was used to compare the proportions between groups. The level of P-value less than 0.05 were considered significant. The study was approved by the responsible ethics committee of Golestan university of medical sciences.

Results

Totally 300 HD patients were tested for detection of anti-HEV IgG and IgM. The mean age was 54 ± 8 (range from 18 to 70). Out of 300 individuals, 148 (49.3%) were male and 152 (50.7%) were female. The overall anti-HEV was found in 12 of 300 patients, the anti-

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Table1: Hepatitis E seroprevalence among hemodialysis patients in north of Iran, Gorgan.

Variables	HEV seropositive patients N=12 100%	HEV seronegative patients N=288 100%	Prevalence in total HD patients	P-Value
Gender				
Male	7(58.3 %)	141(49%)	2.3%	>0.05
Female	5(41.7%)	147(51%)	1.7%	
Ethnicity				
Fars	9(75%)	239(83%)	3%	>0.05
Turkmen	3(25%)	49(17%)	1%	
Dialysis per week				
2	4(33.3%)	124(43%)	1.3%	>0.05
3	8(66.7%)	164(57%)	2.7%	
Age (year)				
<30	0(0%)	24(8.4%)	0%	>0.05
31-40	1(8.3%)	21(7.3%)	0.3%	
41-50	3(25%)	42(14.6%)	1%	
51-60	5 (41.7%)	101(35%)	1.7%	
61-70	2(16.7%)	49(17%)	0.7%	
>70	1(8.3%)	51(17.7%)	0.3%	
Duration of dialysis(Month)	56.7±3.4	19.2±1.3		<0.05

HEV prevalence in our population was 4%. None of seropositive patients exhibited any clinical symptoms similar to acute or chronic hepatitis at the time of sampling. Table 1 represents the data and results in HEV seropositive and seronegative patients. There were no a statistically significant between the subjects grouped according to gender (1.7% in females and 2.3% in males), ethnicity and age (p >0.05). No anti-HEV IgM were detected in patients. The median duration of hemodialysis was 56.7±3.4 months for the IgG positive patients and19.2±1.3 months for the IgG negative patients. Our results showed a significant association between dialysis time in patients with the positive serology against HEV (p < 0.05) (Fig 1). Also no significant correlation has been found between Dialysis per week and HEV-IgG positivity (p >0.05). The HEV RT-PCR assay was evaluated for the detection of HEV-RNA in the IgG positive patients. The HEV-RNA was detected in none of these 12 patients.

Discussion

HEV is the major cause of acute viral hepatitis in the developing world, especially in Asia (12) . Industrial countries have a lower prevalence of anti-HEV between general populations (0.4% - 3.9%) (13) . It is believed that the seroprevalence of HEV is underestimated as a result of low sensitivity of diagnostic kit used for detection and asymptomatic infection in most cases (3) .

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Comprehensive study about seroprevalence of HEV in Iran does not exist especially in HD patient's group as an endemic region. In present study, we determined anti-HEV antibody among 300 HD individuals in Gorgan, north of Iran. Hepatitis E usually diagnosed by serological marker in patients, nevertheless the RNA of HEV can be detected by RT-PCR in blood or stool. Anti-HEV IgM antibody is elevated in early infection and then IgG antibody is raised for 6 month to 14 years after decreasing in IgM. (14)

On the basis of our observation, the percentage of HD patients with positive HEV IgG was 4%, which is extremely lower than the measurement documented in the HD population of Hamedan (19.2%). It may be result from the differences between source of water (26% on non-piping water in Hamedan), while all our study patients were using tap water or more hemodialysis time .(15) The main route of transmission is fecal-oral and blood was not as an important route for transmission because the virus can not cause a chronic carrier state. (16) But studies over the past decade showed the possibility of intravenous passage from person to person like hepatitis B and C virus.(17) Since it has been reported outbreak on HD patients in few studies (18), Consequently, there is a risk of transmission through blood in HD patients.

Various prevalence of HEV in HD patients have been reported all over the world from 2.6% in Italy to 7.3% in Brazil.(19) Compared with other studies on general population, the acquired value is dramatically lower than surrounding country, e.g., general population in Pakistan (17.5%) (20) and the Iraqi-Kurdish refugees (14.8%) (21) but more than Ankara, Turkey (3.8%). (22) In other regions of Iran the prevalence of anti-HEV was higher than our findings, for example: 9.3% in Nahavand (23), 8.1% in Isfahan (24), 7.3% in sari (25), 7.9–15% in Tehran (26). In the present study, we have investigated the fewest seroprevalence of HEV among HD patients to date in Iran, although Pourahmad et al (27)found 7% seroprevalence of HEV in small population of HD patients, which is close to our findings. We detected significant association between anti-HEV and duration of dialysis in our patients, a finding consistent with previous studies among HD patients in Iran (14, 17), it is probably due to HEV transmission through contaminated instrument during hemodialysis.

The result from our study is approximately lower than that reported in other reports from several part of Iran, this might be due to differences in sample size, geographical region, situation of public health services, type of kit is used and the population under study. All samples in our report were collected from urban area, therefore they had access to better public health services, such as equipped hospital, tap water and proper sewage disposal system that appear to decrease risk of infection. These issue remain to be investigated in future studies.

We did not observe increasing in seroprevalence associated with age and there was no remarkable relationship among age and higher HEV prevalence. This result contradict with other study in Isfahan and Bushehr, central and sourth of Iran respectively (24, 28) In one study by Farshadpour et al (29) the highest prevalence of anti-HEV was so far reported in general papulation (almost 46%), this should be considered as an alarm for health authorities for raising HEV in south-west of Iran. Although there are few studies in seroprevalence of HEV in city of Gorgan, Our study on HD patients showed a moderate rate of HEV and emphasizes the need for more studies on general population in order to determine the prevalence of HEV in province of Golestan. In this study, none of the variable were not associated with increased risk of HEV-antibody positive similar to some previous studies in Iran. (15, 30)

Conclusion

In summary, our findings showed notable lower level compared to the prevalence of HEV in the general population in Iran. Since there is no study on general population in Gorgan, we recommend more studies on general population in order to determine the prevalence of HEV in province of Golestan. In conclusion, our data indicate significant

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relationship between duration of hemodialysis and increasing in seroprevalence of HEV among HD patients in this part of Iran, maybe contaminated tolls should be considered as a risk factor for HEV transmission. Further studies are needed to confirm our data. There are no conflicts of interest to disclose. The authors only are responsible for the content and writing of the paper. The research leading to these results has received funding from the Research Deputy at Golestan University of Medical Sciences.

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