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Original Article

Hepatitis E Virus Seroprevalence and Viremia in Hemodialysis and HIV Infected Patients in Iran

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Abstract

Background and Aims: Hepatitis E virus (HEV) infection is usually a self-limited viral disease that causes acute hepatitis and may progress to chronic hepatitis in immunosuppressed individuals. It seems that hemodialysis patients and HIV infected people are more exposed to HEV infection. The aim of this study was to evaluate the extent of HEV infection in hemodialysis and HIV infected patients in Iran using serological molecular methods.

Materials and Methods: Serum and plasma samples were collected from 149 patients undergoing hemodialysis and also 102 proved HIV infected patients. Theses sera were used for detection of HEV total antibodies with Enzyme immunoassay and HEV RNA by Real Time PCR. Demographic and clinical data were obtained and analyzed by SPSS version 16.

Results: HEV antibody for hemodialysis patients and HIV infected individuals were (4%) and (33.3%) respectively. No viremia was observed in both HIV and hemodialysis serum samples. There was no association between demographic and clinical data and HEV antibody positive people.

Conclusions: This study showed some different results in comparison with other studies in Iran. These conflicting results showed differences between HEV infection in hemodialysis and HIV-infected patients in Iran.

Keywords: Hepatitis E, HIV, Hemodialysis, serology, viremia.

Introduction

Hepatitis E virus (HEV) that firstly was recognized in the early 1980s, is a small, non-enveloped, single-stranded positive RNA virus. HEV belongs to the Hepevirus genus, Hepeviridae family and it has one serotype with four genotypes[1].Genotypes 1 and 2 of HEV are distributed in Asia, Africa and Central America, restricted to human and transmitted by fecal-oral route. Genotypes 3 and 4 are circulating in both human and animals and it is believed that they are zoonotic in developed countries[1]. Iran is located in Asia, where considered as one of the endemic regions for HEV[2]. Recent general population studies in Iran, showed that a range of 2% to 14% of population had anti HEV antibody[3]. Although the fecal-oral route is the main route of HEV transmission, the recent studies have demonstrated transfusion of HEV infection via blood products [4-6]. Previous seroprevalence studies among blood donors in Iran showed variable rates of anti- HEV antibodies of 4.5-16.7% [7-11]. As patients undergoing chronic hemodialysis have impaired immunity and a history of blood transfusion, they are at risk of nosocomially and parenterally transmition agents [12, 13]. The studies among hemodialysis patients in Iran have shown prevalence range of 6-28.3% HEV antibody [3, 14]. Since 2008, several studies reported chronic HEV infections in subjects of immunosuppressed individuals such as HIV infected patients [15]. A few

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surveys reported in Iran showed a prevalence of 10-16% anti HEV Ab in HIV infected patients [16, 17]. Because of diversity results in HEV prevalence among HIV infected and hemodialysis patients in Iran, more studies in this field is required. The aim of this study was to evaluate the presence of HEV in HIV infected and hemodialysis patients using serological and molecular methods in northern Iran.

Methods

Patient Recruitment. Serum and plasma samples collected from 149 patients undergoing hemodialysis and 102 proved HIV infected patients were used for detection of HEV total antibodies and HEV RNA. Golestan University of Medical Sciences' Ethics Committee approved our study (IR.goums.REC.1394.25). Written consent, Demographic and clinical data such as age, gender, high risk behavior, CD4+count (for HIV positive patients), number of dialysis per week, ethnicity (for patients undergoing hemodialysis) were obtained.

Serological assessment. Detection of total HEV antibody was done using Enzyme Immuno Assay (Dia.Pro-Italy) with 100% sensitivity and 99.5% specificity according to manufacturer's protocol. The optical densities that were greater than the cut off value were considered positive.

RNA Extraction. Total RNA was extracted from the serum/plasma using commercially available kit (High Pure Extraction Kit; Roche Diagnostics GmbH, Mannheim, Germany) according to manufacturer's instructions. The RNA concentration was quantified by UV spectrophotometer at 260 nm and the purity and integrity was determined using the A260/A280 ratio. GAPDH PCR was used as an internal control to assess the quality of RNA.

Plasmid preparation. Genomic region at positions of 5256 to 5334 of HEV NCBI reference sequence (Accession number: NC_001434.13) was constructed (Macrogen, Korea) and cloned into the T/A plasmid by a commercially available kit (RBC T/A Cloning Kit, Taiwan), according to the manufacturer's protocol. The construct transformed into Escherichia coli strain DH5 alpha. Colony PCR was done to show the accuracy of cloning and transforming process. Plasmid was extracted with kit (Qiagen, Germanyss). The recombinant plasmid stock was quantified using the PicoDrop (PicoDrop, England) according to the manufacturer's instructions and converted into genome copy numbers in this way: copy number= [(concentration of linearized plasmid)/(molar mass)] ×(6.023 × 1023) [18].

RNA reverse transcription. Total RNA was reverse transcribed using Oligo dT primers and M-MuLV Reverse Transcriptase (Thermo, USA) according to the manufacturer's protocol.

2.6. Real Time PCR

Presence of HEV RNA for all serum/plasma were determined by Real Time PCR with TaqMan probe and primers targeting the overlapping region ORF2/3 (nt 5261– 5330 of HEV reference gene): (F, 5/-GGTGGTTTCTGGGGTGAC-3/; R, 5/ AGGGGTTGGTTGGATGAA-3/; 5/-FAM-TGATTCTCAGCCCTTCGC-BHQ-3/)[19].

The reaction was carried out in 25 µl volumes comprising 20µl of master mix (Ampliqon, Germany) with 20 pmol concentration of primers and probe and 5 µl of cDNA according to instruction provided by the manufacturer. The assay was performed on an ABI 7300 Real Time PCR instrument in a 96-well format under the following conditions: 15 min at 95 ◦C for initial denaturation and Taq polymerase activation then followed by 35 cycles of amplification with denaturation at 95 ◦C for 15 s, annealing at 60 ◦C for 20 s and extension at 72 ◦C for 30 s. Distilled water and HEV RNA / HEV antibody negative sample were used as negative controls. The lower limit of detection in our Real Time PCR determined 1.4×104 copy/ml.

Statistical analysis. Data were analyzed using Statistical Package for Social Sciences (SPSS) version 16.0s. P-value of <0.05 was considered significant. A chi-square test was performed to evaluate the distribution of qualitative data in association with HEV infection. For analysis of quantitative data t-test was used after the

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normal distribution of data was assessed with the Kolmogorov-Smirnov.

Results

Anti-HEV Ab was detected in 4% (6/149) of patients undergoing hemodialysis and 33.3% (34/102) in HIV infected patients. The median age was 55.09±15.92 years (range: 15-90 years) in patients undergoing hemodialysis and 35.34±19.8 years (range: 17-54 years) in patients infected with HIV. Patients undergoing hemodialysis were composed of 73 (49%) men and 76 (51%) women. Ethnicity distribution pattern of hemodialysis patients were 117 (78.55%) Fars, and 32 (21.5%) people from other ethnicities such as Ghazagh, Sistaniani, Torkeman and Tork. The reason of kidney failure in 54 cases (36.2%) of these patients were the diabetic, 43 cases (28.9%) due to hypertension, 16 cases (10.7%) due to both diabetes and hypertension, and 36 patients (24.2%) were due to other reasons. In these patients, 64 patients (43%) were dialysed two times a week and 84 cases (56.4%) were dialysed three times a week (one unknown). HIV infected patients, were comprised of 70 (68.6%) men and 32 (31.4%) women. The CD4 count in all of HIV infected patients was less than 250 cells/mm3 with a mean of 126 cells/mm3 (range: 40-250 cells/mm3). There was not any association between anti-HEV-positive status and age, sex, ethnicity, number of dialysis per week, high risk behavior and CD4 cell count (Table1). HEV RNA was not detected in any of patients either in HIV infected and hemodialysis patients.

Table1: Clinical and demographic features of HEV infection in hemodialysis and HIV infected patients

Patient characteristic		Hemodialysis patients			HIV infectd Patients		
		HEV Ab+	HEV Ab-	p-value	HEV Ab+	HEV Ab-	p-value
Mean of age		65.83	54.64	0.092	36.61	34.72	>0.05
Gender							
	Male	4(66.7%)	69(48.3%)	>0.05	24(70.6%)	46(67.6%)	>0.05
	Female	2(33.3%)	74(51.7%)		10(29.4%)	22(32.4%)	
Ethnicity							
	Fars	4(66.7%)	112(78.9%)	>0.05	-	-	-
	Others	2(33.3%)	31(21.1%)		-	-	-
Number of dialysis per week							
	Tow	3(50%)	61(42.7%)	>0.05	-	-	-
	Three	3(50%)	81(56.6%)		-	-	-
CD4 count (cells/mm3)		-	-	-	115	130	>0.05
HEV RNA		0	0	-	0	0	-

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Discussion

Studies have shown different results of HEV seroprevalence in hemodialysis and HIV infected patients. This study found low HEV antibody rate (4%) in hemodialysis patients and high HEV seroprevalence rate (33.3%) in HIV infected individuals. No viremia was observed in both HIV and hemodialysis serum/plasma samples. We did not see any association between assesed parametres and HEV antibody positive results.

Recent studies have considered hemodialysis patients as a high-risk group of HEV infection [12] via transmission through blood transfusion[4-6]. The result of this study showed lower prevalence of HEV antibody in hemodialysis patients (4%) in comparison with distribution of HEV antibody among blood donors in Iran (4.5% to 16.7%) [7, 8, 10, 20]. Overall HEV infection seroprevalence studies among hemodialysis patients in Iran were shown range of 6 to 28.3% [3, 14]. HEV infection in this study (4%) is lower than all of previous studies reported from Iran and other foreign countries by Ben-Ayed et al (10.2%)[12], Harrison et al (36.8%) [21] and Mitsui et al (9.4%) [22]. It is somewhat consistent with the results of Zekavat et al (6%) [23], Scotto et al (6%) [24], Ayoola et al (4.8%) [25] and Sylvan et al (6%) [13]. Most of case-control studies in Iran and in the rest of the world were shown a higher prevalence of HEV among hemodialysis patients compared to healthy people [5, 12, 14, 21, 23-25]. This research was not a case-control study but HEV seroprevalence in our previous studies among women of childbearing age and pregnant women in this region of Iran with 1600 subjects were shown a prevalence of 6.3% and 7.3% respectively[26, 27]. This can partially reflect the spread of HEV among general population. With this approach, anti HEV antibody prevalence among hemodialysis patients (4%) was lower than healthy people and it is in contrary to other case-control studies [5, 12, 14, 21, 23-25]. Therefore HEV infection is not a serious risk among hemodialysis patients in this region. This differences could be due to the low prevalence of HEV, level of hygiene in hemodialysis centers and suppling safe water and good sewage in this area.

Chronic HEV infection was reported in patients with incomplete immune system such as HIV-positive people [15]. The high HEV seroprevalence result in HIV infected people of this study (33.3%) is in contrast with previously published rate of infection in Iran (10% and 16.4%) [16, 17] as well as many foreign studies such as Jacob et al (71%) [28] and Scotto et al (7.6%)[29]. While consistency of this result was confirmed with studies of Nouhin et al (41.7%) [30] and Feldt et al (45.3%) [31]. The result of this study showed a high rate of HEV antibody in comparision with HEV seroprevalence studies in the general population of Iran that was between 2% and 14% [3]. A case control report in Iran was shown no HEV seropositivity differences between HIV infected patients and general population [17]. To clarify the situation of HEV prevalence among HIV infected patients in Iran, further case-control studies in large sample size are required.

We did not detect any HEV RNA in both HIV infected and hemodialysis cases. In a period of time, Immune respon doesn't increase while viremia occures [1]. So detection of HEV infected patients will misss in some cases if we use serological tests only [19]. HIV infected patients with low CD4 cells count had delayed antibody response which causse negative HEV antibody tests in contrast of HEV positivity [15]. So we completed our serological study with molecular tests. We did not detect any HEV RNA. This can happen for several reasons. Firstly, absent of HEV RNA in samples indicating that none of our specimens were in the limited phase of the HEV RNA existence in serum. Most probably, the acute and persistent HEV infection didn't present in the studied samples but for an exact description IgM/IgG evaluation or a cohort study with follow-up of the patients must be carried out. Secondly, our study was cross-sectional and consecutive serum samples were not available. In a cohort study, we can investigate the chronic situation of infection in HIV infected patients and the route of transmission in

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hemodialysis patients. Thirdly, the limit of detection of our Real Time PCR test was 1.4×104 copies/ml. In immunocompetent participants, HEV RNA concentration varies from 126->107 copy /ml in an acute phase [15]. Thus, one of the reasons for negative molecular results, can be the presence of HEV RNA less than 104 copies/ml in the serum samples of this study. Fourthly, the quality of kits, were used for RNA extraction and molecular test may have influence for increasing precise of RNA detection. In general, distinct results may be explained by differences in geographical regions, sanitation system, nutrition, type of study and tests with various sensitivity and specificity.

In conclusion, the results of this research were different with the other studies in this field in Iran. These conflicting results show further studies are needed to clear the situation of HEV in hemodialysis and HIV-infected patients in Iran.

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