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BRIEF REPORT

Hepatitis E Virus Infection in Hemodialysis Patients: A Seroepidemiological Survey in Jahrom, Southern Iran

Morteza Pourahmad*, Abdol Reza Sotoodeh, Hamid Nasiri

Faculty of Medicine, Jahrom University of Medical Sciences, Jahrom, Iran

Background and Aims: **Hepatitis E virus (HEV) is mainly the causative agent of waterborne epidemics, but some authors have found that patients on chronic hemodialysis have an increased risk of exposure to HEV. We conducted this study to reveal HEV seroprevalence in hemodialysis patients as a specific group in Iran, and to evaluate age, duration of hemodialysis, and the levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in them.**

Methods: **The presence of immunoglobulin G antibodies to HEV(anti-HEV IgG) was measured by enzyme-linked immunosorbent assay (ELISA) in the patients' sera. Both ALT and AST serum levels were measured. The duration of hemodialysis and the age and sex of the participants were obtained from the medical records of patients, and the data were made into quantitative variables, which were expressed as mean ± standard deviation (SD).**

Results: **43 patients (29 males and 14 females) enrolled in this study. 3 of these patients (7% of the sample) were HEV antibody positive (2 males and 1 female). The mean levels of AST and ALT in all of the studied patients were 22.3 ± 23.3 IU/L and 21.3 ± 27.6 IU/L, respectively. An association between HEV positivity and duration of hemodialysis was revealed by our results, but there was no significant association between HEV antibody positivity and patient age. All 3 patients who were positive for anti-HEV antibody in our study also had elevated liver enzymes.**

Conclusions: **The finding that HEV infection was associated with elevated liver enzymes in patients who were on chronic hemodialysis may indicate that hemodialysis is a route for HEV transmission, and more controlled studies are needed to explore this association in Iran.**

Keywords: **Hepatitis E, Viral Hepatitis, Hemodialysis**

Introduction

Hepatitis E virus (HEV), a small non-enveloped RNA virus, is a causative agent of acute hepatitis that is infrequent in industrialized countries but is one of the most important infectious problems in developing countries, including Iran [1-3]. Today we know that HEV is a zoonotic virus and is found in both wild and domestic animals [1]. This virus is the causative agent of both waterborne epidemics and sporadic cases of viral hepatitis in regions with inadequate sanitation. On the other hand, vertical transmission of HEV from infected mothers to their children has been observed [4]. In addition, dental treatment and blood transfusion have been found to be associated with HEV transmission [5-7]. Some authors have observed a high prevalence of antibody to HEV (anti-HEV Ab) in their hemodialysis patients and have thus hypothesized that the fecal-oral route may not be the only route of transmission of HEV [8].

Moreover, this has led to the conclusion that patients on chronic hemodialysis have an increased risk of exposure to nosocomially transmitted agents, such as HEV. Iran, with few suspected outbreaks of HEV, is thought to have a high probability of hepatitis E occurrence [9]. Iran is categorized as an endemic country for hepatitis E, and seroprevalence of this type of hepatitis increases significantly with age, from 3.3% in subjects under 30 years of age to 37.5% in individuals over 50 [10]. In Iran it is

**** Correspondence:***
Morteza Pourahmad, M.D., Vali Asr St., Peymanieh Hospital, Jahrom, Iran.

Tel: *+98 917 791 6203*
Fax: *+98 791 223 0021*

E-mail: *Mortezapourahmad@yahoo.com*

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emphasized that HEV infection should be considered in any cases of suspicious viral hepatitis that do not show evidence of hepatitis A virus (HAV) or hepatitis B virus (HBV) infection [11].

Unfortunately, few studies have been conducted in Iran to explain the statistical characteristics of this type of infection in specific groups of people, such as patients who are on hemodialysis [12]. Thus, we conducted this study to reveal HEV seroprevalence in hemodialysis patients as a specific group in Iran and to evaluate age, duration of hemodialysis, and the levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in patients with positive and negative HEV Ab.

Materials and Methods

This cross-sectional descriptive study was carried out in November 2007 and included all 43 patients on maintenance hemodialysis at a dialysis center in Jahrom, southern Iran. These patients were receiving routine, 3- to 4-hour dialysis treatments 3 times a week. Five milliliters of venous blood were obtained from each selected patient. The blood samples were centrifuged immediately, and after serum separation, were stored at -20°C. After collecting all of the samples, the presence of anti-HEV immunoglobulin G (IgG) antibody in the patients' sera (as evidence for HEV infection) was measured by enzyme-linked immunosorbent assay (ELISA; DIA-PRO, Italy); this test was a quantitative assay. Serum levels of ALT and AST were also measured in patients. The age, sex, and duration of hemodialysis for each patient were obtained from the patient's medical records. Afterwards, SPSS software ver. 11.5 was used for the statistical analysis of the data, and descriptive statistics were reported. Quantitative variables were expressed as mean±standard deviation (SD), and comparisons were performed using two-sample t test. Statistical significance was set at $P<0.05$.

Results

Forty-three patients (29 males and 14 females) participated in this study. The mean age of the patients was 59.3 ± 14.4 years. Three patients (7% of the enrolled patients) were HEV antibody positive (2 males and 1 female). These three patients did not have any clinical signs or symptoms for hepatitis. The mean levels of AST and ALT in all of the studied patients were 22.3 ± 23.3 IU/L and 21.3±27.6 IU/L, respectively. Table 1 shows the ages and durations of hemodialysis for patients with positive and negative HEV antibody, as well as their levels of ALT and AST.

Table 1. Age and duration of hemodialysis and the levels of ALT and AST in patients with positive and negative HEV Ab.

Variable	HEV antibody	Mean	SD	P value
Age (years)	Positive	68	10.14	0.28
	Negative	58.67	14.58	
Dialysis duration (Months)	Positive	76	42.14	< 0.0001
	Negative	22.72	22.03	
AST (IU/L)	Positive	103.66	18.23	0.014
	Negative	16.10	4.68	
ALT (IU/L)	Positive	119.33	8.32	< 0.0001
	Negative	13.92	4.76	

Furthermore, to avoid possible bias (in the associations between seropositivity and duration of dialysis, age, and sex) we used logistic regression. A forward stepwise model was used, and only the duration of dialysis ($\chi^2 = 7.149$, $P = 0.007$) was significantly related to the positivity and/or negativity of HEV Ab.

Discussion

HEV infection is mainly transmitted via the enteric route and is widespread in many developing countries, especially in Asia and Africa [1, 13]. The prevalence of HEV antibody varies across countries (*e.g.*, 26% and 11%, in Egypt and Taiwan, respectively) [14, 15]. Such differences, are also seen between various cities in Iran; for instance, the rates for Isfahan, Khozestan, Tabriz, and Nahavand (various cities in Iran) are 3.8%, 11.5%, 7.8%, and 9.3%, respectively [10, 16-18].

These differences may be due to the various routes of virus transmission and levels of sanitation in different regions and countries.

Although some authors believe that the prevalence of HEV antibody in patients with multiple transfusion and intravenous drug users is not different from the general population [19, 20], some reports have suggested parenteral transmission of HEV [21, 22]. Therefore, hemodialysis may also be a means for transmission of this virus. On the other hand, anti-HEV prevalence data among chronic hemodialysis patients are few, and analyses utilizing them have found conflicting results [23].

In our study 7% of the studied patients were anti-HEV IgG positive. A similar proportion (7.4%) was

reported in another similar study in Iran [24]. These proportions are near the ratios for anti-HEV IgG in the general population in Iran (based on data from blood donors), so these results may indicate that hemodialysis is not a route for HEV transmission. However, one of the other results in our study was the association between HEV positivity and duration of hemodialysis. Specifically, the duration of hemodialysis in patients with positive HEV antibody was significantly longer (76 months) than in patients with negative HEV antibody (22.72 months), and the logistic regression analysis also showed that duration of hemodialysis was the only variable significantly related with HEV seropositivity. These results indicate the possibility that HEV infection may increase with a prolonged duration of hemodialysis. It is possible that these findings are a result of our small sample size, but this association was also found in another study, which was conducted in the north of Iran [25]. However, this association was not revealed in another study in Iran [23]. On the other hand, it is important to note that we used ELISA to check for anti-HEV IgG in the patients in our study, and the positive patients may have been infected before being dialyzed. Checking for the anti-HEV IgM in addition to anti-HEV IgG could help to clarify this issue, although anti-HEV IgM is a momentary antibody.

In a study conducted in Greece, 4.8% of the patients who were on chronic hemodialysis were positive for HEV antibody, whereas in blood donors this ratio was 0.26% [26]. Unfortunately, we did not find this ratio in blood donors in Jahrom. However, these results suggest that risk for infection with HEV may be increased by hemodialysis, but more controlled studies are necessary to better evaluate this association.

In our study, the analyses revealed that the presence of positive HEV antibody was not associated with age. In similar studies of hemodialysis patients, no association between the age and anti-HEV positivity was found [23, 26]. In contrast, in some studies conducted on the general population and normal blood donors, an association between age and anti-HEV positivity was observed [10, 15, 18, 27]. This may be due to the limited age range of the patients in our study and similar studies, all of which were conducted only on patients with renal failure.

All three patients who were positive for anti-HEV antibody in our study had elevated liver enzymes. It should be noted that the presence of IgG anti-HEV may represent the convalescent phase of an acute illness or previous exposure [19]. In two other studies, which were conducted in Japan on voluntary blood donors with elevated ALT levels, anti-HEV IgG was positive in 7.1% and 3.7% of participants, respectively. These studies suggest that these patients had ongoing sub-clinical infection of HEV and were potentially able to cause transfusion-associated hepatitis E [21, 22]. In a similar study, which was conducted in the Nile Delta, viral hepatitis markers were tested for in 47 subjects, all of whom had ALT levels that were at least twice the normal level, and anti-HEV IgG was detected in 40 of these patients [28]. Still, the proportion of HEV viremia and its duration have not been found to be directly related to serum ALT or HEV antibody levels [29], but sub-clinical infection of HEV may be the cause of abnormal ALT [30].

Conclusions

We think that a subclinical infection of HEV may have caused the elevated ALT and AST levels in our patients and that this infection may have been due to hemodialysis.

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