Hepatitis E Virus Antibodies in Hemodialysis Patients: Epidemiological Survey in Thessalia (central Greece)

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Introduction

Hepatitis E, previously known as enterically transmitted non-A non-B hepatitis, is a self-limited infection with clinical and morphological features of acute viral hepatitis. The disease, first documented during an epidemic of viral hepatitis in 1955-56 in India (1), was not recognized as a distinct clinical entity until 1980, when sera from affected patients were shown to lack serological markers of acute hepatitis due to A and B viruses (2).

Seroepidemiological studies based on immunoassays for antibodies against recombinant virus antigens showed 1-3% prevalence of anti-HEV in blood donors and in pregnant women in developed countries. Furthermore, a high percentage of the serologically positive or the diseased individuals reported no recent travel to HEV endemic areas. These data indicate that contamination with HEV does not preclude a previous travel to an endemic region and that HEV may be endemic in western countries, albeit at a low prevalence (3-5).

A high prevalence of anti-HEV antibody in patients undergoing chronic HD (10.9%), reported in an early study, led to the hypothesis that the oral–fecal may not be the only route of transmission of HEV in this setting (6).

The main purpose of the study presented here was to determine the prevalence of IgG anti-HEV in HD patients in the Thessalia region of central Greece. A second aim of the study was to re-evaluate the risk of exposure to HEV associated with chronic HD and its relation to contamination with other blood transmitted viruses such as hepatitis B (HBV), C (HBC) and human T-lymphotropic virus (HTLV).

Patients and Methods

All patients (n=351; 234 males, 117 females; mean age 60.4 ± 14 years) with end stage renal disease, who were on chronic HD treatment in Thessalia, were included in the study. Thessalia is a semi-rural region located in central Greece and at the time of the study there were five HD units in function (four in public hospitals and one in a private clinic).

The clinical profile of the HD patients included in the study is presented on Table 1. HD was performed with standardized treatment techniques, for 3 – 4,5 hours three times a week in all patients.

Serum was stored at -20°C and tested by enzyme immunoassays (EIAs). Thawing-freezing of serum specimens was strictly avoided. For detection of anti-HEV IgG a commercially available anti-HEV EIA (Abbott Diagnostika, Wiesbaden-Delkenheim, Germany) was used (7,8). The optical density for sample (S) over Cut-Off (CO) ratio (S/CO) was calculated. Samples with a S/CO value >1 were considered reactive and testing was then repeated in duplicate.

Antibodies to hepatitis C virus (anti-HCV) were assayed by a third generation EIA (Murex, Temple Hill, UK). Commercially available EIAs (Abbott Diagnostika, Wiesbaden-Delkenheim, Germany) were used to detect hepatitis B virus (HBV) surface antigen (HBsAg) and antibodies to the HBV core antigen (anti-HBc), to human immunodeficiency virus types 1 and 2 (anti-HIV 1/2) or to HTLV types I and II (anti-HTLV I/II)as previously described (5,9,10).

Serum aspartate and alanine aminotransferase (AST and ALT) levels were determined by spectrophotometry (reference levels: AST < 46 and ALT < 40 IU/l). In the study presented here a value of ALT 1.5 times higher than the upper limit was arbitrarily considered to be significant and designated the group of patients with elevated aminotransferase levels (11,12).

Data for continuous variables are given as a mean value and standard deviation (x ± SD) or as median with 5 and 95 percentiles in brackets. Categorical variables are presented as percentages with 95% confidence intervals (95% CI) in brackets. The probability of error for comparison of the mean values was calculated using the Student's t-test for unpaired data or the Mann-Whitney U-test where applicable. For data frequencies comparison analysis was performed by the χ^2 test and the Fisher's exact probability test. P-values < 0.05 were considered as statistically significant.

Results

IgG anti-HEV reactivity was detected by this EIA in the serum of 17 among the 351 HD patients studied (Table 2). The prevalence of IgG anti-HEV was 4.8% (95% CI 2.6 – 7.1). The S/CO value of anti-HEV-positive patients was 3.34 ± 1.65 and in most of the cases (n=12) a high reactivity (S/CO value >2) was observed. In five patients S/CO was between 1 and 2. The prevalence of IgG anti-HEV was varying in the different HD units and it was lowest in the HD unit of the General Hospital of Trikala (1/55, 1.8%)

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and highest (6/55, 9.8%) in the unit of the General Hospital of Karditsa.

Table 1: Clinical profiles and IgG anti-HEV status of the patients (n=351) with end stage renal disease (ESRD) treated with hemodialysis (HD) at the renal units of central Greece (region Thessalia) in 2001.

	Hemodialysis patients		
Primary disease	**Total n (%)**	**anti-HEV– (n)**	**anti-HEV+ (n)**
Diabetic nephropathy	57 (16.2)	55	2
Vascular renal disease	34 (9.7)	34	
Chronic glomerulonephritis	52 (14.8)	51	1
Polycystic kidney disease	25 (7.1)	22	3
Obsrtuctive uropathy	33 (9.4)	29	4
Chronic interstitial nephritis	5 (1.4)	5	0
Systemic disease	11 (3.1)	10	1
	12 (3.4)	11	1
Other causes			
Unknown etiology[1]	122 (34.8)	117	5
Sum n (%)	351 (100)	334 (95.2)	17 (4.8)
Mean age (years)	60±14	60±14	61±18
Sex (m/f)	234/117	226/108	8/9
Mean time on HD (months)	49±48	49±47	51±59

[1]The high rate of patients with unknown primary disease is probably due to an underestimation of vascular kidney disease as a cause of ESRD.

We found 169 (48.1%; 95% C.I. 43 – 53) with serological signs of a previous hepatitis B virus infection (anti-HBc positive). 20/251 patients (5.7%; C.I. 3.3 – 8.1) had signs of a persistent hepatitis B virus infection (HBsAg positive). There were 82 patients with anti-HCV antibody and the prevalence of anti-HCV positivity was 23.4% (95% C.I. 19 – 28).

No significant association was found between anti-HEV positivity and age, duration of HD treatment, elevated aminotransferase levels, history of transfusion, hepatitis C virus infection (anti-HCV) or previous hepatitis B virus infection (anti-HBc) (Tables 1 and 2). Furthermore, no association could be identified between the primary causes of end stage renal disease ant the anti-HEV status (p=0.29; Table 1).

Table 2: Clinical and laboratory characteristics of hemodialysis (HD) patients treated in the renal units of central Greece (region Thessalia) including the serological HEV status. Comparative descriptive data and statistical analysis of anti-HEV positive and anti-HEV negative patients are presented.

	Hemodialysis patients[1]		
		Anti-HEV status	
	Total	positive	negative
Patients (n)	351	17	334
Patients > 63 (median) years	174 (49.6)	10 (58.8)	164 (49.1)
Male patients	234 (66.7)	8 (47.1)	226 (67.7)
On HD > 33.5 (median) months	176 (50.1)	7 (40.2)	169 (50.6)
Immigrants	5 (1.4)	0	5
Transfused patients	129 (36.7)	6 (35.3)	123 (36.8)
Raised aminotransferases (ALT)[2]	19 (5.4)	1 (5.9)	18 (5.4)
History of raised ALT	76 (21.7)	5 (29.4)	71 (21.3)
HBsAg-positive patients	20 (5.7)	2 (11.8)	18 (5.4)
Anti-HCV-positive patients	82 (23.4)	3 (17.6)	79 (23.6)
Anti-HIV 1/2-positive patients	0	0	0
Anti-HTLV I/II positive patients	0	0	0

[1] On the left side of the table values represent number of patients (n) and percentages (%), except from rows in them it is otherwise denoted.

[2] Aminotransferase levels were considered as raised in patients with values of ALT 1.5 times higher than the upper reference limit (40IU/l).

Serum aminotransferases did not differ between the anti-HEV positive (AST 22 ± 12 and ALT 22 ± 15 U/l) and the anti-HEV negative group (AST 23 ± 22 and ALT 26 ± 25 U/l). Five anti-HEV positive patients had a history of aminotransferasemia and one had elevated values during the study (AST: 50 U/l and ALT: 65 U/l).

Five of the 351 patients (1.4%) were immigrants from Albania and none of the immigrants was anti-HEV-positive. The IgG anti-HEV positive patients did not report any travel abroad especially in HEV endemic regions or any contact with individuals coming from these regions before the entry into the study.

Discussion

We found a prevalence of IgG anti-HEV of 4.8% among chronic HD patients, who were attending at the five HD units of Thessaly, located in central Greece. This prevalence was significantly higher than that of 0.26% (95% CI

0.08 – 0.41) found in our own previous study among 3016 healthy blood donors in Greece (5).

In this study as in most of the seroepidemiological surveys previously reported EIA was used for the detection of IgG anti-HEV (anti-HEV EIA). This serological assay (anti-HEV EIA) has been developed in the last decade based on recombinant virus antigens (7,8). Along with IgM anti-HEV determination, the detection of IgG anti-HEV by EIA has been established for the diagnosis of acute hepatitis E, particularly in endemic regions. Currently anti-HEV EIA is also used in raising frequency for serological surveillance studies in selected epidemiological settings (13,14).

The present study is the third epidemiological survey of IgG anti-HEV prevalence among HD patients in Greece (Table 3). The results of the first study, which we conducted in the western region of the country (Epirus and Agrinion), showed among HD patients from Epirus an anti-HEV prevalence of 1.34% and among HD patients from Agrinion a prevalence as high as to 9.7%, which was significantly higher compared to that among healthy blood donors from the same region (0.53%) (5). In a survey of five renal units in Athens an anti-HEV prevalence of 6.4% was reported among HD patients. The authors did not attribute this high prevalence to an increased viral exposure during HD treatment but to the effect of sex and age (15).

Hepatitis E is endemic in many tropical countries whereas only sporadic cases have been recognized in industrialized countries. Previous studies from developed countries regarding HEV in HD are still few and have reported a prevalence of anti-HEV positivity among HD patients which varies between 1.3 – 10.9% (4-6,15-19) with the exceptional prevalence of zero and 17.3% in two reports (20,21) both based however, on a small number of HD patients.

In the different HD units of Thessalia region a high variation of the prevalence of IgG anti-HEV was observed. Anti-HEV prevalence was lowest (1.8%) in the HD unit of the General Hospital of Trikala and highest (9.8%) in the unit of the General Hospital of Karditsa. These centers are located both in a semi-rural region at the west part of Thessalia. Although no general population survey has been done in these regions, this finding along with the previously observed high prevalence in the HD unit of Agrinion (5) support our hypothesis that there may be an undefined intra-unit or other local factors, which promote the transmission of HEV and which have to be identified.

Prospective studies from HD units in areas of high HEV prevalence are needed to determine whether HD treatment is a risk factor for hepatitis E transmission. Patients on HD have a considerable high risk of exposure to blood transmitted viruses. From the descriptive data of the anti-HEV positive HD patients in comparison with the anti-HEV negative patients, in our study, it is already clear that no risk factor for IgG anti-HEV reactivity could be identified (Tables 1 and 2). Especially no association between anti-HEV and blood transmitted viruses (HBV, HCV) was observed. According to the above findings the possibility of blood transmission of HEV during HD treatment seems not to be high but cannot be totally excluded.

On the other hand, we did not either find any past history of travel to endemic areas of the world among the anti-HEV positive HD patients. The high prevalence of anti-HEV IgG in some renal units observed in the present and in previous studies may be the result of a local HEV infection (5,6). Traveling is generally uncommon in chronic HD patients.

In conclusion, our cross-sectional investigation of HEV infection in the cohort of HD patients of the Thessalia region in central Greece showed that the prevalence of IgG anti-HEV was higher compared to healthy blood donors. No association of anti-HEV reactivity to blood borne infections (HBV, HCV) could be determined. The high prevalence of anti-HEV we found in one HD unit was probably related to a local infection. However, still undefined intra-unit factors may be associated with HEV transmission. Long-term prospective studies with large number of HD patients are needed in order to accurately address whether anti-HEV IgG seropositivity is an acquired phenomenon during the HD process.

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