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Prevalence of Hepatitis E Virus Infection in Hemodialysis Patients

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ABSTRACT

Background: There are many studies about the epidemiology of hepatitis E virus (HEV) in the general population, but the data about HEV infection among patients with end-stage renal disease (ESRD) give conflicting results. Although the parenteral route may be involved in the transmission of HEV infection, several investigators have suggested that fecal-oral transmission is the primary transmission mode. We aimed to identify the seroprevalence of HEV in hemodialysis (HD) patients compared to the seroprevalence of HBV and HCV infection, and examine the role of parenteral transmission. **Methodology:** Eighty-four patients from TBRI's HD unit who had been receiving dialysis for more than six months were involved in the study. All the patients were subjected to detailed medical history, full clinical examination, and routine investigations, including virology screening and abdominal ultrasound. HEV seropositivity was investigated by ELISA for HEV-Ab (IgG), while active viremia was assessed by RT-PCR for HEV RNA. **Results:** Out of the 84 patients, anti-HEV IgG antibodies were detected in five cases (6.0%). A minor (P=0.001) and a moderate (P=0.01) increase in liver echogenicity by ultrasound were significantly correlated with HEV seropositivity. Blood transfusion and HEV seropositivity did not significantly correlate (P=0.6). Neither HBsAg nor HCV-Ab was related to anti-HEV antibody seropositivity. HEV RT-PCR was only positive in one case.

Conclusion: Compared to HCV (34.5%), the prevalence of HEV seropositivity was low (6%) in our patients. Parenteral transmission of HEV was less likely. HEV routine screening may help lower the related morbidity and mortality in HD patients.

Keywords: HEV-IgG, Non-A Hepatitis, Hemodialysis, HEV prevalence.

INTRODUCTION

Enteric non-A Hepatitis is commonly caused by HEV. It was believed until recently that HEV exists only in developing countries, where it was associated with outbreaks through contaminated water supplies[(1)]. However, recent data have defined the virus as a worldwide infection, probably related to either parenteral/vertical transmission or zoonotic fecal-oral infection. In addition, it has recently been discovered that a changing rate of blood and/or solid organ donors was positive for HEV-RNA [(2, 3)].

In most cases HEV infection presents with an acute self-limited disease; however, people who already have chronic liver disease are more likely to get acute on top of chronic liver failure. [(4)]. The same applies to people with impaired immune systems (like HIV-positive cases, organ transplant recipients, and cases with hematological malignancies) [(5,6)].

These groups of cases have a greater incidence of chronic infection progression; the incidence may reach up to 50% in solid-organ transplant recipients [(7)]. When an infection becomes chronic, the liver may rapidly get fibrosis and decompensates, which can sometimes result in mortality[(8)].

Patients with End-Stage Renal Disease (ESRD) have been acknowledged as a severe challenge in global public health. All over the world, hemodialysis (HD) is still the main therapy method used to treat ESRD patients. It is well-recognized that HD cases are more likely to contract viral infections because they share dialysis machines, receive blood transfusions frequently, require repeated hospital stays, and have poor cellular immunity, making them especially susceptible to blood-borne viruses[(9)]. Conflicting data were published in the last two decades about the real prevalence of HEV in HD cases. Some investigators remarked on the great level of anti-HEV antibodies in those patients and considered other transmission modes by nosocomial in addition to the fecal-oral route [(10-12)]. Other authors reported low rates of anti-HEV-positivity in their HD patients [(13, 14)]. This study aimed to identify the seroprevalence of HEV in HD patients compared to the seroprevalence of HBV and HCV infection and examine the role of parenteral transmission.

PATIENTS AND METHODS

A- Patients

The current work was performed at the Hemodialysis Unit in the Nephrology Department and Biochemistry & Mol. Biology Dep., Theodor Bilharz Research Institute. Eighty-four patients with ESRD on maintenance hemodialysis for more than six months were listed in the work.

The patient's ages ranged between 19 and 76 years, 33 (39.3%) were females and 51(60.7%) were males. All were subjected to full medical history and clinical assessment for signs of liver cell failure. Abdominal Ultrasonography (U/S) was performed for all patients.

The causes of ESRD were as follows: hypertension (HTN) in 44 (52.4%), diabetes mellitus (T2DM) in 16 (19.0%), congenital in 4 (4.8%), nephrocalcinosis in 4 (4.8%), obstructive uropathy in 4 (4.8%), Non-steroidal anti-inflammatory drug (NSAID) in 4 (4.8%), chronic glomerulonephritis (GN) in 2 (2.4%), and other causes which include (amyloidosis, Systemic lupus erythematosus (SLE), and tubulointerstitial renal disease) in 6 (7.1%) cases.

The age (mean ± SD) of patients with HEV positive and negative serology was 53.4±12.5 and 55.9±14.4 respectively, with no significant difference (**Table 1**).

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Table 1: Demographic and laboratory results of 84 cases of hemodialysis

		Frequency or Min – Max	Percent or Mean ± SD
Age (years)		17.0 - 83.0	55.7±14.2
Sex	Female	33	39.3%
	Male	51	60.7%
Cause of ESRD	HTN	44	52.4%
	T2DM	16	19.0%
	Congenital	4	4.8%
	Nephrocalcinosis	4	4.8%
	Obstructive Uropathy	4	4.8%
	NSAID	4	4.8%
	Chronic GN	2	2.4%
	Others	6	7.1%
HD Duration/ yrs		1.0 - 25.0	7.5±4.1
Blood Transfusion	No	20	23.8%
	Yes	64	76.2%
Liver Ultrasound	Normal	23	27.4%
	Mild	26	30.9%
	Mod	24	28.6%
	Cirrhotic	11	13.1%
Hepatomegaly	No	55	65.5%
	Yes	29	34.5%
Splenomegaly	No	51	60.7%
	Yes	33	39.3%
Ascites	No	82	97.6%
	Mild	1	1.2%
	Moderate	1	1.2%
Hb (g/dl)		6.8 - 15.1	10.0±1.7
TLC(x103/µl)		3.1 - 13.1	6.6±2.3
Platelets (x106/µl)		77.0 - 440.0	216.3±76.3
AST (U/l)		12.0 - 61.0	21.7±9.7
ALT (U/l)		11.0 - 157.0	19.4±9.6
S. Albumin (g/l)		3.1 - 4.9	4.1±0.3
HBV s-Ag	Negative	79	94.0%
	Positive	5	6.0%
HCV-Ab	Negative	55	65.5%
	Positive	29	34.5%
HEV-IgG	Negative	79	94.0%
	Positive	5	6.0%
HEV-RNA	Negative	83	98.8%
	Positive	1	1.2%

Age, HD Duration, Hb, TLC, Platelets, AST, ALT, and S. Albumin were represented as Mean ± SD.
While Sex, Cause of ESRD, Blood Transfusion, Liver Ultrasound, Hepatomegaly, Splenomegaly, Ascites, HBVs-Ag, HCV-Ab, HEV-IgG, and HEV-RNA were represented as F (%) frequency and percent.

B- Laboratory Investigations:

(1) Routine Investigations

- A complete blood count was done utilizing an automated cell counter, (Medonic, Boule Diagnostics, Sweden).
- Blood Chemistry involving alanine aminotransferase (ALT) aspartate aminotransferase (AST), and serum albumin was carried out on Olympus AU480 Chemistry Analyzer, Beckman Coulter, USA.

(2) Serological Investigations

- Viral markers involving (HCV IgG) and (HBsAg) were carried out on ADVIA Centaur CP automated chemiluminescence Immunoassay System, Siemens, Germany.
- Antibodiesdetection to (HEV IgG) using commercially available ELISA test kit (Lot No 20200905), Precheck, Bio. Inc., South Korea. The manufacturer's instructions were strictly followed.

(3) Molecular Investigation:

Primer design of HEV gene

The primers utilized in this work **(Table 2)** were designed based on the HEV genotype 1 (Gene ID: NC_001434.1) using the online program primer-3 tool. The designed oligonucleotide primers were assessed utilizing an online primer program called IDT-sequence analyzer (http://www.idtdna.com) for calculation of primer melting temperature (Tm) and GC-content and examined for hairpin loop, validity self-complementary, and primer dimer creation.

Table 2: Primer design of HEV gene

Primer	Sequence	Amplified gene product size
HEV-F	5′TTCCAACCTCATGCTCCACT 3′	(~ 488 bp)
HEV-R	5′ACGAATCGCAGTCCTACAGT 3′	

Construction of the plasmid DNA standard

Hepatitis E virus synthetic gene at nucleotide positions from 908 to 1608, open reading frame 1 (ORF1) of a genotype 1 human HEV strain was generated after regaining the HEV genome sequence (Gene ID: NC_001434.1) from the National Centre for Biotechnology Information (NCBI) server (www.ncbi.nlm.nih.gov) and the European Bioinformatics Institute (EMBL-EBI). The synthesized gene was inserted in the pUC57 cloning vector (Cat.No.GTE006, Bio Basic, Canada) (**Fig. 1).**

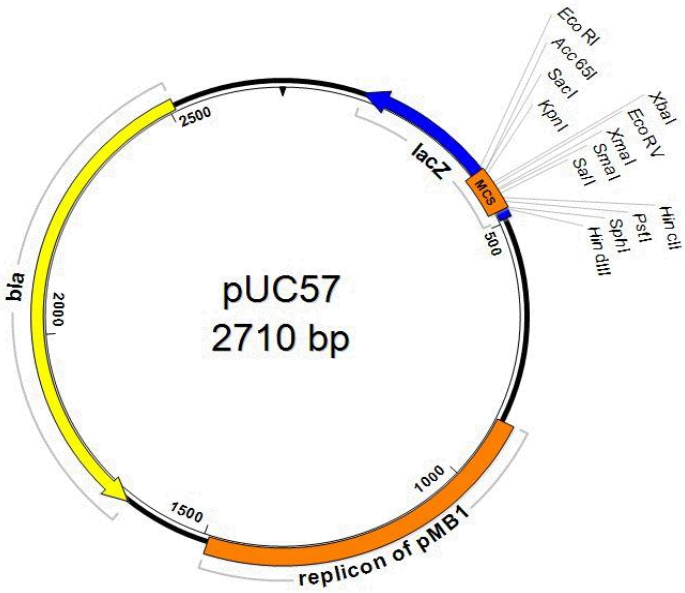

Figure 1: The pUC57cloning vector map with m13 sequencing primer.

The Hepatitis E virus recombinant plasmid was transfected into E-coli TOP10 bacteria (Invitrogen, Foster City, USA) by heat shock at 42oC for 90 seconds followed by incubation on ice for 5 minutes. A volume of 500 µl Luria-Bertani (LB) medium free of antibiotics was then added and incubated at 37°C for 1 h. Then, about 150 µltransformants of the HEV gene were diffused on LB-agar plates inclosing ampicillin (50µg/ml) (Cat No: AB0028, Bio Basic Inc, Canada), and incubated at 37°Covernight. Thereafter, one separate colony was sub-cultured in LB medium containing ampicillin followed by plasmid DNA mini-preparation usingGeneJET plasmid miniprep kit (Cat. No. K0502, Thermo Fisher Scientific, USA). The extracted DNA was quantified at 260 nm utilizing Nanodrop® 2000c Spectrophotometer (Thermo Fisher Scientific Inc, USA), aliquoted, and kept at -80°C till usage.

Sample processing and RNA extraction

Hepatitis E virus RNA was separated from 400 µl serum samples utilizing the Abbott mSample preparation system (MD1355, Promega Corporation, Madison, USA) in accordance with the instructions of manufacturer, aliquoted, kept at -80°C and then dissolved once.

HEV-RNA Detection

Hepatitis E virus RNA was reverse transcribed into cDNA utilizing the RevertAid First Strand cDNA Synthesis kit (Thermo Fisher Scientific, USA). Until utilization, the produced cDNA was stored at -20°C. All patients in this study were examined to detect any presence of HEV-RNA using PCR assay. PCR duplication was performed in 25 µl volume [5 ng viral cDNA, 1x PCR buffer (20 mM Tris-HCl, 50 mM KCl, 2 mM MgCl2), 0.2 mM dNTPs, 0.4 mM MgCl2, 1 µM HEV specific primer pairs, 1unit Taq polymerase (Promega Co, Madison, USA)] was prepared for the PCR reaction. Cycling parameters were denaturation at 95 °C for 3 min, 40 cycles of 30 sec at 95 °C denaturation, 30 sec at 55 °C annealing, and 30 sec at 72 °C extension, followed by a final extension of 5 min at 72 °C using the T100 PCR Thermal cycler (Bio-Rad, Singapore). A plasmid containing HEV DNA served as positive control and Nuclease–free distilled H2O was utilized as a negative control in each run. All PCR runs were carried out at certified management conditions, while every step of the PCR workflow was performed in isolated laboratory rooms.

Amplicons (488 bp) were visualized on 1.5% agarose gel stained with ethidium bromide.

Ethical Approval:

All participants gave informed written consent following the 1975 Helsinki Declaration's ethical guidelines. The IRB approval number is (FWA#000010609/PT. 602) on 31 May 2021. No fund was needed.

The study was approved by the Ethical Committee of Theodor Bilharz Research Institute, which was fully aligned with the code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans. All the participants signed a written informed consent form.

Statistical Analysis

The data were analyzed utilizing the statistical package for social science 'IBM SPSS Statistics for Windows, version 26 (IBM Corp., Armonk, NY, USA) and Microsoft Excel 2016. A p-value < 0.05 indicates that the results are significant statistically. Continuous normally distributed variables were revealed as mean ± SD with a 95% confidence interval and utilizing the percentage and frequencies for categorical variables. To compare the means of normally distributed variables between categories, the Student's t-test was used. To determine how categorical variables were distributed among categories, Fisher's exact test or the $\chi 2$ test was used. Effect variations were assessed by stratification, and statistical interaction was evaluated using logistic regression analysis, as well as multinominal logistic regression analysis, that included the primary risk variables and their product terms.

RESULTS

Of eighty-four patients with ESRD on maintenance HD, 29 patients were positive (34.5%) for HCV-Ab, five patients (6.0%) were positive for HBsAg, and five patients (6.0%), of whom one was a female, were positive for HEV Ab. The patient's clinical characteristics, demographic, and laboratory investigations are revealed in **(Table 2).**

In terms of the correlation of gender to HEV, males compromised 80.0% (4/5) of HEV-positive patients and only 59.5% (47/79) of HEV-negative cases. No significant difference was discovered between men and women regarding HEV seropositivity **(Table 3).**

Table 3: Risk assessment of positive HEV-IgG antibodies in 84 patients on hemodialysis

		Demographic and laboratory data			Risk assessment		
		Negative HEV-IgG N=79	Positive HEV-IgG N=5	P-value	OR	95%C.I	P-value
Age (years)		55.9±14.4	53.4±12.5	0.7	0.988	0.930 - 1.050	0.7
Sex	Female	32(40.5%)	1(20.0%)	0.4	2.723	0.291 - 25.502	0.4
	Male	47(59.5%)	4(80.0%)				
HD Duration/ yrs		7.5±4.2	6.8±3.8	0.7	0.965	0.778 - 1.196	0.7
Blood Transfusion	No	19(24.1%)	1(20.0%)	0.6	1.267	0.133 - 12.033	0.8
	Yes	60(75.9%)	4(80.0%)				
Liver Ultrasound	Normal	23(29.1%)	0(0.0%)	0.01*	-	-	-
	Mild	24(30.4%)	2(40.0%)	0.001**	11.500	2.711 - 48.777	0.001**
	Mod	21(26.6%)	3(60.0%)	0.01*	7.000	2.088 - 23.468	0.01*
	Cirrhotic	11(13.9%)	0(0.0%)	0.01*	-	-	-
Hepatomegaly	No	53(67.1%)	2(40.0%)	0.2	3.058	0.481 - 19.441	0.2
	Yes	26(32.9%)	3(60.0%)				
Splenomegaly	No	49(62.0%)	2(40.0%)	0.3	2.450	0.387 - 15.519	0.3
	Yes	30(38.0%)	3(60.0%)				
Ascites	No	77(97.5%)	5(100.0%)	0.8	1.400	0.233 - 8.050	0.9
	Mild	1(1.3%)	0(0.0%)	0.9	-	-	-
	Moderate	1(1.3%)	0(0.0%)	0.9	-	-	-
Hb (g/dl)		9.9±1.6	10.7±2.2	0.5	1.279	0.778 - 2.101	0.3
TLC (x103/µl)		6.5±2.3	7.9±2.6	0.3	1.244	0.878 - 1.763	0.2
Platelets (x103/µl)		218.0±78.1	191.2±34.0	0.2	0.995	0.982 - 1.008	0.4
AST (U/l)		22.0±9.9	17.2±3.7	0.1	0.908	0.758 - 1.087	0.3
ALT (U/l)		19.8±8.2	13.4±2.6	0.1	0.710	0.446 - 1.131	0.1
S. Albumin (g/l)		4.1±0.3	4.2±0.1	0.3	2.156	0.091 - 51.185	0.6
HBVs-Ag	Negative	74(93.7%)	5(100.0%)	0.6	0.937	0.885 - 0.992	0.6
	Positive	5(6.3%)	0(0.0%)				
HCV-Ab	Negative	51(64.6%)	4(80.0%)	0.5	0.455	0.049 - 4.274	0.5
	Positive	28(35.4%)	1(20.0%)				
HEV-RNA	Negative	78(98.7%)	5(100.0%)	0.8	0.940	0.890 - 0.992	0.8
	Positive	1(1.3%)	0(0.0%)				

NB: Other causes of ESRD include Amyloidosis, SLE, and Tubulointerstitial renal disease

Age, HD Duration, Hb, TLC, Platelets, AST, ALT, and S. Albumin were revealed as Mean ± SD; the student t-test was used to analyze data. While Sex, Cause of ESRD, Blood Transfusion, Liver Ultrasound, Hepatomegaly, Splenomegaly, Ascites, HBVs-Ag, HCV-Ab, HEV-IgG, and HEV-RNA were represented as F (%) frequency and percent; the data were analyzed by X2 test. * p-value <0.05 is significant, ** p-value <0.01 is greatly significant. OR; Odd Ratio, CI; Confidence Interval, p-value calculated depend on Logistic Regression analysis among the normal distribution data, while multi-nominal Regression in categorical parameters.

The association of HEV-IgG seropositivity with different parameters was assessed and the risk of these parameters on HEV infection was explored. Blood transfusion was not associated with HEV-Ab seropositivity. There was no association between HEV-Ab seropositivity and either of HCV-Ab seropositivity or HBsAg (Table3).

Regarding the stage of liver disease, a significant association (p-value < 0.001) of HEV seropositivity with a mild increase in liver echogenicity in 40.0% of HEV IgG positive patients was observed; with 11.5 folds risk in the positive HEV-IgG patients (95% C. I=2.711 - 48.777, p-value < 0.001) **(Table 3).**

Hepatitis E virus seropositivity was found to be significantly associated (p-value < 0.01) with moderate increase in liver echogenicity in 60.0% of HEV IgG positive patients with 7.0 folds risk (95% C. I=2.088 - 23.468, p-value = 0.01) (Table 3).

HEV RT- PCR:

Only one patient among 84 patients on maintenance HD showed positive HEV by RT-PCR indicating active viremia **(Figure 2).**

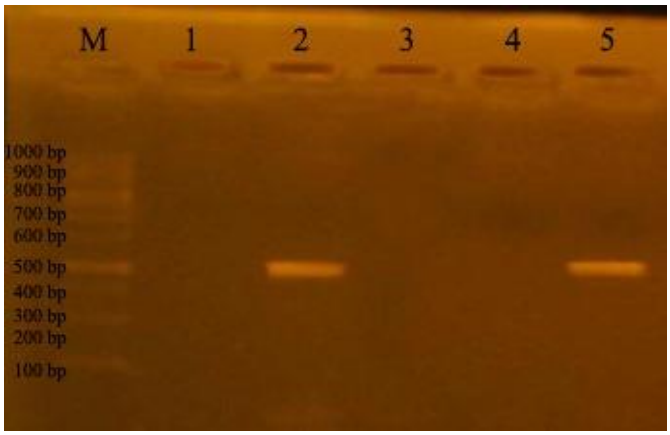

Figure 2: Representative agarose gel electrophoresis of HEV RT-PCR products. The PCR product length is 488 bp. M, 100-bp DNA ladder, HEV-negative serum samples were identified in (lanes1&3) and HEV-positive serum samples were identified in lane (2). for negative control (lane 4) while positive control plasmid HEV-DNA PCR (lane5).

DISCUSSION

The epidemiologies of HCV and HBV in HD patients were well studied and documented; however, the data about HEV infection are limited and vary greatly among different studies.

HEV infection is transmitted typically by the fecal-oral route and is usually self-limited. However, some studies suggested that specific genotypes may be associated with chronic hepatitis, especially in immunocompromised individuals [15, 16]. These include patients with faults of cellular and humoral immunity, like cases with end-stage renal failure requiring renal replacement therapy [17]. Other studies suggested other routes of transmission e.g., vertical transmission from the mother to the child, and parenteral transmission e.g., by blood transfusion [18, 19].

The current study involved 84 HD patients who were screened for this infection, as they have both factors fulfilled; being immunocompromised and highly exposed to parenteral infections by the regular IV access with each HD session and possibly by occasional blood transfusions.

The present study revealed that the prevalence of anti-HEV IgG positivity in HD cases was 6%. However, a prevalence of 67.7% was reported in two Egyptian villages in a study by Stoszek *et al*. [20]. The prevalence of HEV in Egypt in low socioeconomic regions is high. This was confirmed by another study performed on 2,428 Egyptian pregnant females in the Nile delta which found anti-HEV Ab prevalence of 84.3% among them [21].

Moreover, other investigators who studied HEV prevalence in HD patients found a low prevalence of this virus. Fabrizi *et al*. [22] reported a prevalence of 3% among 204 patients. While other investigators reported a higher prevalence among 60 HD patients reaching up to 30% [23]. This demonstrates that the prevalence of HEV infection is highly variable in different populations in different regions with diverse disease states.

The risk of multiple factors in HEV infection was explored. Out of five cases that were positive for HEV-Ab, 4casesreceived blood transfusion, and only one patient did not have blood transfusion during the previous year, our study reported that there was no marked connection between HEV seropositivity and blood transfusion **(Table 3).** This result was previously documented by **Scotto *et al.*** [24], who concluded that blood transfusion was not associated with HEV infection in HD patients. However, Mitsui *et al*. [17] argued that HEV infection could be related to blood transfusion in HD patients.

The association of HEV with other blood-borne viruses was noted in only one out of five positive patients for HEV IgG-Ab, who had concomitant HCV infection and none of the patients had an infection with HBV. This observation indicates that HEV seropositivity is probably not associated with HBV or HCV infection. Also, it does not confirm the hypothesis of the parenteral route of HEV transmission. While some authors did not exclude the possibility of the parenteral mode of transmission of HEV [25], others did not find any association between HEV infection and blood transfusion or blood-borne viruses [26].

Hepatitis E Virus (HEV RT-PCR) assay was positive in only one patient, who was negative for HEV-IgG Ab, denoting an acute infection. Interestingly, the IgG antibodies were absent. This could also be explained by the patient's inability to mount an efficient immune response with detectable antibodies due to the immunocompromised state.

Hepatitis E Virus seropositivity was significantly associated with a mild and moderate increase in liver

echogenicity **(Table 3);** however, none of the patients with a severe increase in echogenicity was positive for HEV-Ab. These results, nevertheless, need to be verified on a large scale, as the number of HEV-Ab-positive patients in our study was too low to make any definite conclusion.

Scotto *et al.* [24] reported a negative correlation between chronic liver disease and HEV infection. Furthermore, none of the subjects in that study, whether HD or renal transplant recipients, remained chronically viremic. Legrand-Abravanel *et al*. [27] did not detect any evidence of chronic HEV infection in over 500 HD patients.

Other studies, however, suggested that HEV infection may develop into a chronic infection, particularly in cases with impaired immunity after organ donation [28, 29], hematological malignancies [30], and HIV [6]. In this respect, the immunodeficient state in HD patients could theoretically predispose patients with HEV infection to chronic liver disease, especially in the presence of other predisposing factors [31].

LIMITATIONS

The small number of HD patients under study for a short period of follow-up makes it difficult to get a clear conclusion about the seroconversion of HEV or new HEV RT-PCR positivity. Again, the determination of HEV IgM may reveal acute infection and correlate it with PCR-positive patients.

CONCLUSION

The prevalence of HEV infection is highly variable in different populations in different regions with diverse disease states.

- Blood transfusion was not associated with HEV infection in HD cases.
- This observation indicates that HEV seropositivity is probably not associated with HBV or HCV infection. Also, it does not confirm the hypothesis of a parenteral route of HEV transmission.

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Conflict of interest: No.

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