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Measurement of HEV Specific RNA Transcripts in Peripheral Blood of Haemodialysis Patients and Normal Individuals Using RTPCR and Comparison to Their Serological Findings

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ABSTRACT

Introduction:*Hepatitis E virus (HEV) is a major cause of acute viral hepatitis, which cause chronicity in immunocompromised patients, patients undergoing hemodialysis. There is Limited Indian data exist on the diagnostic utility of HEV RNA detection compared to serology in this high-risk group.*

Method:*This case–control study was done at a tertiary care center after ethical approval. A total of 80 patients on maintenance hemodialysis (HD group) and 277 age- and gender-matched healthy controls were included for the study. HEV infection was assessed by anti-HEV antibody detection using ELISA and HEV RNA detection by reverse transcription polymerase chain reaction (RT-PCR). Clinical and biochemical parameters were also checked.*

Result:*HEV RNA was detected in 6 (7.5%) HD patients and 7 (2.5%) controls (p=0.285). Anti-HEV antibodies were present in 5 (6.3%) HD patients and 8 (2.9%) controls (p=0.178). A significant correlation was observed between RNA and antibody positivity (p<0.001). There was no associations found with gender-wise differences or liver function parameters.*

Conclusion:*Molecular detection of HEV RNA showed higher prevalence in HD patients compared to controls, although not statistically significant. Combined serological and molecular testing enhances diagnostic accuracy in this vulnerable group.*

Keywords: *Hepatitis E virus; Hemodialysis; HEV RNA; Anti-HEV antibodies; RT-PCR; Serology*

INTRODUCTION

Hepatitis E virus (HEV) is a non-enveloped, single-stranded, positive-sense RNA virus belonging to the Hepeviridae family.[1] It is an important cause of acute viral hepatitis worldwide, with a high burden in developing countries due to poor sanitation and contaminated water supplies.[2] While HEV infection is mostly self-limiting in immunocompetent individuals, it can lead to severe and chronic disease in immunocompromised populations, including solid organ transplant recipients, HIV-positive individuals, and patients undergoing long-term hemodialysis.[3]

Patients on maintenance hemodialysis are at an increased risk of acquiring blood-borne viral infections due to repeated vascular access, prolonged hospital contact, and potential exposure to contaminated equipment or blood products.[4] Although HEV is primarily transmitted via the fecal-oral route, parenteral transmission has been documented, raising concerns about its spread in healthcare settings.[5]

The diagnosis of HEV infection has traditionally relied on the detection of HEV-specific antibodies (anti-HEV IgM and IgG) by enzyme-linked immunosorbent assay (ELISA). However, serological tests may have limitations in sensitivity and specificity, particularly in immunocompromised patients, who may exhibit delayed or absent antibody responses.[6] Molecular detection of HEV RNA by reverse transcription polymerase chain reaction (RT-PCR) offers a more direct and sensitive method for confirming active infection, assessing viral replication, and differentiating between acute and past exposure.[7]

Despite growing awareness, there is limited data on the prevalence and diagnostic agreement between HEV RNA detection and serological findings among hemodialysis patients in India. Understanding this relationship is crucial to identifying subclinical or chronic infections, improving infection control measures, and guiding patient management.[8]

The present study aims to measure HEV-specific RNA transcripts in the peripheral blood of hemodialysis patients and healthy controls using RT-PCR, and to compare these molecular results with corresponding serological findings. This approach will help assess the diagnostic utility of molecular testing in high-risk populations and contribute to the epidemiological understanding of HEV infection dynamics in healthcare-associated settings.

METHODOLOGY

This case–control study was done in the Department of Microbiology in a tertiary care centre, after getting approval from the Institutional Ethics Committee. Written informed consent was taken from all study participants.

The study included 80 patients undergoing maintenance hemodialysis (HD group) and 277 age- and gender-matched healthy volunteers (control group). Hemodialysis patients were recruited from the dialysis unit of tertiary care hoispotal], and healthy controls were recruited from the general population and hospital staff after screening for major illnesses.

Patients with age >18 years or older who had been on maintenance hemodialysis for a minimum duration of three months were included in this study. Patients with history or current diagnosis of acute hepatitis, co-infection with hepatitis A, hepatitis B, hepatitis C, or HIV, or patients not gave informed consent were excluded from the study.

Demographic details (age, gender) and relevant clinical data were recorded using a structured proforma. For each participant, 5 mL of peripheral venous blood was collected under aseptic precautions. The samples were divided into two aliquots:

1. **Serology:** Serum was separated and stored at –20°C until testing for anti-HEV antibodies (IgM and/or IgG) using a commercial enzyme-linked immunosorbent assay (ELISA) kit following the manufacturer's instructions.
2. **Molecular detection:** Whole blood or plasma samples were processed for extraction of viral RNA.

Viral RNA was extracted using a commercially available RNA extraction kit according to the manufacturer's protocol. Reverse transcription PCR (RT-PCR) targeting conserved regions of the HEV genome was performed to detect HEV-specific RNA transcripts. The amplification products were analyzed by agarose gel electrophoresis and visualized under UV illumination. Appropriate positive and negative controls were included in each PCR run to ensure validity.

For HD patients, liver function parameters including serum glutamic oxaloacetic transaminase (SGOT/AST), alkaline phosphatase (ALK-PHOS), total and direct bilirubin, albumin, and total protein were measured using automated analyzers in the clinical biochemistry laboratory.

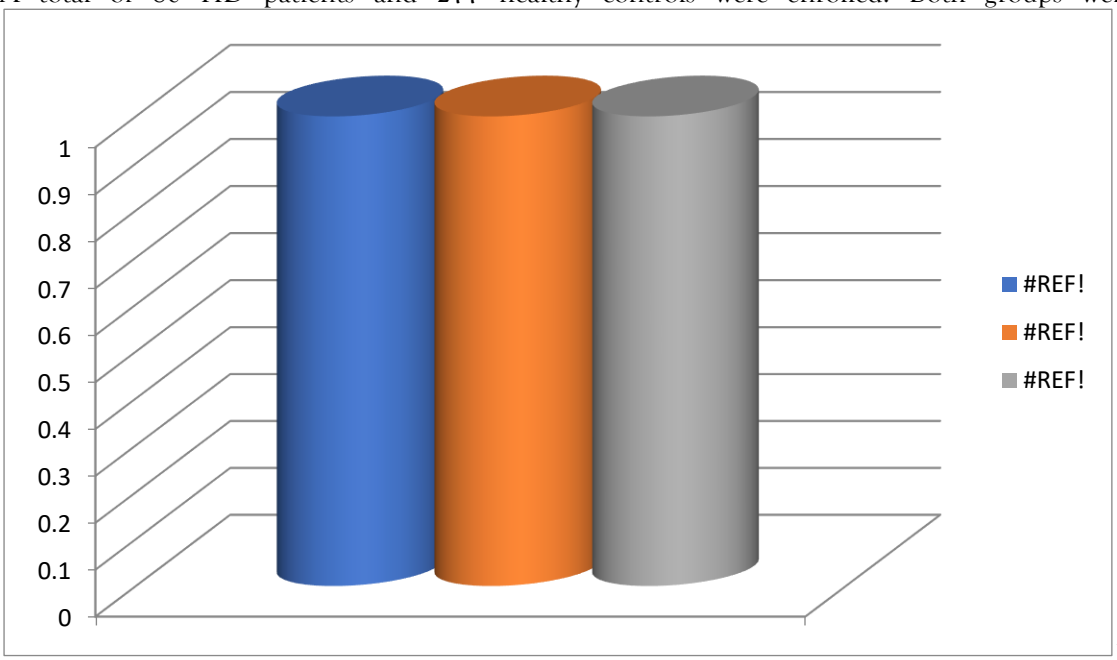
Data were entered in Microsoft Excel and analyzed using SPSS version 25. Continuous variables were presented as mean ± standard deviation (SD), and categorical variables were presented as frequency and percentage n(%). Comparisons between HD patients and controls were made using the independent samples t-test for continuous variables and the Chi-square test or Fisher's exact test for categorical variables. Agreement between HEV RNA and anti-HEV antibody results was evaluated using Kappa statistics. A p-value <0.05 was considered statistically significant.

RESULT:

Table 1. Demographic profile of Hemodialysis patients and healthy controls

Variable	HD patients (n=80)	Controls (n=277)	p-value
Age (years), mean ± SD	45.4 ± 13.9	45.8 ± 12.6	0.801
Male	58 (72.5)	187 (67.5)	0.571
Female	22 (27.5)	90 (32.5)	

A total of 80 HD patients and 277 healthy controls were enrolled. Both groups were com



parable in terms of mean age and gender distribution, with no statistically significant differences.

Table 2. HEV RNA positivity in Hemodialysis patients and healthy controls

HEV RNA status	HD patients (n=80)	Controls (n=277)	p-value
Positive	6 (7.5)	7 (2.5)	0.285
Negative	74 (92.5)	270 (97.5)	

HEV RNA was detected in 7.5% of HD patients compared to 2.5% of controls. Although the prevalence was higher among HD patients, the difference did not reach statistical significance.

Table 3. Prevalence of anti-HEV antibodies in the study population

Anti-HEV Ab status	HD patients (n=80)	Controls (n=277)	p-value
Positive	5 (6.3)	8 (2.9)	0.178
Negative	75 (93.7)	269 (97.1)	

Anti-HEV antibodies were detected in 6.3% of HD patients and 2.9% of healthy controls. The difference was not statistically significant.

Table 4. Cross-tabulation of HEV RNA and Anti-HEV Ab results

Group	RNA+/Ab+	RNA+/Ab–	RNA–/Ab+	RNA–/Ab–	p-value
HD patients (n=80)	5 (6.3)	1 (1.3)	0 (0.0)	74 (92.4)	<0.001
Controls (n=277)	2 (0.7)	0 (0.0)	6 (2.2)	269 (97.1)	

There was a statistically significant correlation between HEV RNA and anti-HEV antibody status in both groups, as confirmed by Kappa analysis ($p < 0.001$).

Table 5. Distribution of HEV markers according to gender

Marker	Male (n=245)	Female (n=112)	p-value
RNA positive	7 (2.9)	1 (0.9)	0.680
Anti-HEV Ab positive	11 (4.5)	2 (1.8)	0.360

No significant association was found between gender and either HEV RNA positivity or anti-HEV antibody prevalence.

Table 6. Association of liver function profile with HEV RNA and Anti-HEV antibodies in HD patients

LFT Parameter	RNA positive n (%)	RNA negative n (%)	p-value	Ab positive n (%)	Ab negative n (%)	p-value
SGOT normal	5 (6.5)	72 (93.5)	0.211	5 (6.5)	72 (93.5)	1.000
SGOT abnormal	1 (33.3)	2 (66.7)		0 (0.0)	3 (100)	
ALK-PHOS normal	6 (7.8)	71 (92.2)	1.000	5 (6.5)	72 (93.5)	1.000

ALK-PHOS abnormal	0 (0.0)	3 (100)		0 (0.0)	3 (100)	
Total bilirubin normal	6 (8.5)	65 (91.5)	1.000	5 (7.0)	66 (93.0)	1.000
Total bilirubin abnormal	0 (0.0)	9 (100)		0 (0.0)	9 (100)	
Direct bilirubin normal	4 (6.7)	56 (93.3)	0.637	3 (5.0)	57 (95.0)	0.594
Direct bilirubin abnormal	2 (10.0)	18 (90.0)		2 (10.0)	18 (90.0)	
Albumin normal	5 (6.3)	74 (93.7)	0.075	5 (6.3)	74 (93.7)	1.000
Albumin abnormal	1 (100)	0 (0.0)		0 (0.0)	1 (100)	
Protein normal	3 (4.5)	64 (95.5)	0.051	4 (6.0)	63 (94.0)	1.000
Protein abnormal	3 (23.1)	10 (76.9)		1 (7.7)	12 (92.3)	

No liver function parameter showed a significant association with either HEV RNA detection or anti-HEV antibody presence in HD patients.

DISCUSSION:

In the present study, the demographic profile of hemodialysis patients showed a mean age of 45.4 ± 13.9 years with a male predominance of 72.5%, which was comparable to the control group. These findings are consistent with the report of Togay and Akyüz (2023)[9], who observed that more than half of their hemodialysis cohort were males, although their study population was slightly older, with the majority being above 50 years. Similarly, Hameed et al. (2024)[10] emphasized that older age and compromised nutritional status were more frequently observed among hemodialysis patients compared to healthy volunteers, supporting the observation that dialysis populations tend to have distinct demographic and nutritional patterns.

With respect to viral markers, HEV RNA was detected in 7.5% of hemodialysis patients in our study compared to 2.5% among healthy controls, though the difference was statistically insignificant. This is similar to findings from Kevorkyan et al.[11] (2023), who also reported HEV infection among hemodialysis patients in Bulgaria but noted no ongoing viremia, and with the meta-analysis by Buescher et al.[12] (2021), which showed that while seroprevalence may be elevated in immunocompromised populations, viremia rates remain relatively low. The prevalence of anti-HEV antibodies in our study was 6.3% in hemodialysis patients versus 2.9% in controls, again showing a higher frequency but without statistical significance, similar to the trends noted by Kevorkyan et al.[11] (2023).

Cross-tabulation of HEV RNA and anti-HEV antibody results in our cohort showed that most positive patients were RNA+/Ab+, while none were RNA–/Ab+, showing fewer individuals with past exposure alone. This differs from the study by De Araújo et al.[13] (2023), who show that among patients with chronic liver disease, several were antibody positive but RNA negative, reflecting prior exposure without active replication. Likewise, Cao et al.[14] (2023) reported a high HEV IgG seroprevalence among Vietnamese blood donors without evidence of RNA positivity, show that antibody detection mostly outweighs RNA positivity in general populations.

In our studythe difference in HEV RNA or antibody positivity between males and females was statistically insignificant. In contrast to our findings , result from Schemmerer et al.[15] (2022) in Germany and Niederhauser et al.[16] (2024) in Switzerland, show men were more frequently HEV positive than women, showing that geographic and exposure-related factors may modulate gender-specific risk.

In present study liver function profiles in hemodialysis patients did not show any significant association with HEV RNA or antibody positivity. This is similar to the study by Kevorkyan et al.[11] (2023), who also found no RNA positivity despite a notable seroprevalence in dialysis patients. Their work showed that risk factors such as vascular access type, duration of dialysis, and water source might be cause of HEV exposure than routine biochemical markers.

Our findings highlight that while the prevalence of HEV RNA and antibodies was higher among hemodialysis patients than healthy controls, the differences were not statistically significant, consistent with earlier observations in similar cohorts. Recent evidence suggests that molecular assays remain more

reliable than serological tests for detecting active infection, especially in immunocompromised groups such as dialysis patients, where antibody responses may be blunted. A multicentric study from Pakistan emphasized the role of demographic and nutritional vulnerabilities in modulating HEV risk among hemodialysis populations, while a 2025 European investigation reinforced the need for ongoing surveillance given that subclinical HEV infections may contribute to transfusion-related risks. Together, these findings underline the importance of integrating molecular diagnostics with serological screening for improved detection and prevention strategies in this high-risk group[17-19].

Taken together, our findings show that while hemodialysis patients may have a slightly higher prevalence of HEV exposure than healthy controls, active viremia is uncommon, and demographic or biochemical parameters do not appear to strongly influence HEV status. Continuous surveillance and consideration of environmental risk factors require in this vulnerable population.

CONCLUSION:

In this study, HD patients and healthy controls were similar in age and gender distribution. HEV RNA and anti-HEV antibodies were detected more frequently among HD patients compared to controls, though the differences were not statistically significant. A significant correlation was seen between HEV RNA and antibody positivity across groups. No association was found between gender or liver function parameters and HEV markers. Overall, HEV exposure seen more in HD patients, but there was no significant clinical or biochemical correlation.

DECLARATIONS

Conflicts of interest: There is no any conflict of interest associated with this study
Consent to participate: There is consent to participate.
Consent for publication: There is consent for the publication of this paper.
Authors' contributions: Author equally contributed the work.

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