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Original article

Hepatitis C Virus Seroprevalence and Genotypic Diversity Among Haemodialysis Patients in a District Hospital: A Comprehensive Study in Telangana, India

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Abstract:
Background: *Hepatitis C virus (HCV) infection poses a significant threat to public health globally, particularly among patients undergoing haemodialysis. The prevalence and genotypic diversity of HCV in this population warrants investigation to guide targeted interventions.*
Objectives: *This prospective study aimed to assess the prevalence of HCV infection and characterize the distribution of HCV genotypes among haemodialysis patients in District Hospital, Khammam, Telangana, India.*
Materials and Methods: *Serum samples were collected from 280 patients on haemodialysis. HCV antigen and antibody detection was done using enzyme-linked immunosorbent assay (ELISA). All ELISA-positive samples were subjected to polymerase chain reaction (PCR) for viral load quantification and genotyping. Sangers sequencing was employed for untypeable genotypes.*
Results: *27 (9.7%) samples were ELISA positive. Out of these, 14 samples had a quantifiable viral load. RT PCR was done and genotypes 1a, 1b, and 3 were identified. Sangers sequencing was employed for untypeable genotypes.*
Discussion: *The predominance of genotypes 1a and 1b underscores the need for tailored treatment strategies, while the presence of genotype 3 highlights genetic diversity. Screening and genotype-specific interventions are crucial to mitigate HCV especially in haemodialysis patients who have an increased of exposure.*
Conclusion: *This study provides valuable insights into the epidemiology and genotypic diversity of HCV in the study region. This aids genotype-based intervention to address challenges posed by HCV infection in hemodialysis settings. Addressing this accurately also prevents further transmission from affected individuals into the community.*
Keywords: *Hepatitis C virus (HCV): Haemodialysis; Genotyping: Seroprevalence: Sangers sequencing: Public health.*

Introduction

HCV infection is a major public health concern with a staggering global prevalence. According to the World Health Organization (WHO), an estimated 71 million people worldwide are chronically infected with HCV. The consequences of HCV infection are profound and include liver-related morbidity and mortality. According to the Dialysis Outcomes and Practice Patterns Study (DOPPS), the global prevalence of HCV in hemodialysis patients is approximately 13.5%, with variations ranging from 2.6% to 22.9% among different countries.[1] This finding indicates the variability in the risk of HCV transmission in healthcare settings, including dialysis units.[1,2] In the Indian context, the burden of hepatitis infections is substantial, contributing significantly to the country's overall disease burden. The World Hepatitis Alliance reports that nearly 40 million people in India are affected by chronic hepatitis infections, encompassing both hepatitis B (HBV) and hepatitis C virus (HCV). These infections pose dual challenges, given their potential to progress silently over time, leading to severe liver diseases such as

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cirrhosis and hepatocellular carcinoma. In India, recent studies have shown the prevalence of HCV in hemodialysis patients to range from 8% to 19.23%.[3] Factors contributing to this prevalence include the number of blood transfusions, the duration of hemodialysis, and the presence of other risk factors, such as hepatitis B or HIV coinfection and prior transplant history.[3-5] HCV infection poses a specific threat to patients undergoing hemodialysis. Prolonged vascular exposure and frequent blood transfusions inherent in haemodialysis increase the vulnerability of patients to blood-borne infections. Among these, HCV stands out as a formidable adversary.[6]

HCV, a single-stranded RNA virus, exhibits remarkable genetic diversity, leading to the classification of distinct genotypes and numerous subtypes. Globally, seven major genotypes (designated 1-7) with multiple subtypes have been identified. The distribution of these genotypes varies geographically, with specific genotypes prevalent in certain regions. Understanding the genetic landscape of HCV is crucial, as it directly influences disease progression, treatment response, and the overall burden of HCV-related complications.[7] Genotypes 1 and 3 are widely distributed globally and are commonly associated with chronic infections. Genotype 1 is particularly prevalent in North America and Europe, while genotype 3 is frequently found in Southeast Asia. Genotype 2 is more common in West Africa and North America, while genotype 4 is predominant in the Middle East and Central Africa. Genotypes 5, 6, and 7 are relatively less common but have distinct regional distributions. Genotypes 5 and 6 are found in South Africa and Southeast Asia, respectively. Genotype 7 is a more recent discovery with a limited prevalence in Central Africa.[7] The choice of HCV genotype significantly influences the severity of liver disease. Genotypes 1 and 4 are often associated with more advanced liver fibrosis and a greater risk of hepatocellular carcinoma (HCC) than genotypes 2 and 3 are. Genotype 3, in particular, has been linked to a greater likelihood of developing steatosis (fatty liver), insulin resistance, and a more rapid progression to advanced liver disease.[8] The diversity of HCV genotypes profoundly affects the response to antiviral therapy. Historically, patients with genotypes 1 and 4 exhibited lower sustained virological response (SVR) rates than patients with genotypes 2 and 3. Recent advancements in direct-acting antiviral (DAA) therapies have significantly improved treatment outcomes across all genotypes, but the choice of treatment regimen may still vary based on genotype considerations.[9]

Hence, HCV genotyping is paramount in tailoring treatment regimens for optimal efficacy, aiding in risk stratification, predicting disease progression, epidemiological surveillance, public health planning and supporting research efforts to elucidate the genetic variation in HCV for the development of more effective antiviral drugs and potential vaccines. The present study aimed to assess the HCV genotype pattern in patients undergoing hemodialysis.

Materials and Methods

Study population

The study was conducted in District Hospital Khammam, Telangana from January 2022 to December 2022. Patients on haemodialysis and HCV treatment were included. Pediatric patients were excluded from the study. Detailed clinical history was gathered through a confidential questionnaire and written informed consent was obtained from every patient.

ELISA, RT–PCR and genotyping

Blood samples were collected from each patient in separate EDTA and plain tubes before heparin administration. ELISA was used to detect the presence of HCV antigens and anti-HCV antibodies. The ELISA (J. Mithra 4gen Ag-Ab) kit was used. Viral load quantification was performed by PCR (Trunat), ensuring the availability of same-day results. The remaining plasma aliquots were stored at -80°C for subsequent RT–PCR analysis. RT–PCR genotyping was performed using PathoDetect HCV Genotyping Kit (Mylab Life Solutions, India) to distinguish genotypes 2, 3, 4, 5, and 6 and subtypes 1a and 1b. The untypable genotypes were subjected to subsequent next-generation sequencing at Huwel Life Sciences Pvt Ltd., Hyderabad, India. The schematic representation of the methodology is represented in the Fig 1.

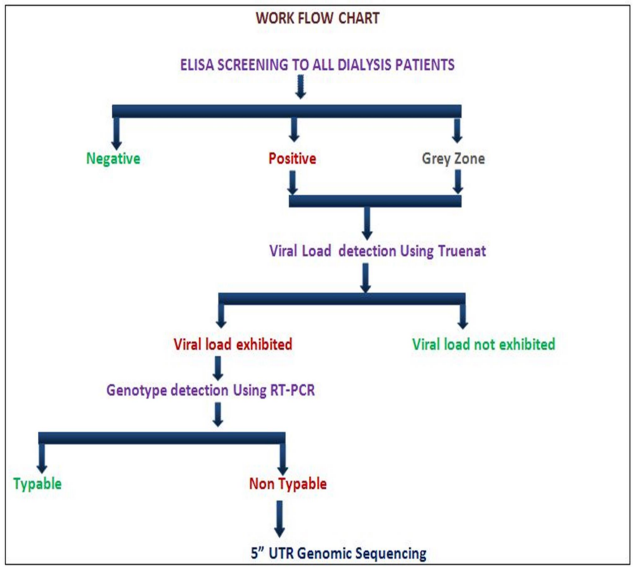

Fig 1. Schematic representation of the workflow.

Ethical considerations and data analysis

All tests were conducted following the manufacturer's instructions and validated protocols. Ethics approval was obtained from District Headquarters Hospital, Khammam, and the Meenakshi Academy of Higher Education and Research (MAHER). Data analysis was performed using descriptive statistics.

Results

ELISA and viral load testing

In the study, 280 patients undergoing haemodialysis who were treatment-naive for HCV were enrolled. Of these participants, 27 serum samples tested positive in the ELISA HCV Ag and anti-HCV antibodies screening assay. Among these 27 ELISA-positive samples, 12 (45%) samples showed detectable levels of viral RNA in the Trunat test (**Fig 2**). Among the positive samples, 21 (78%) were from male patients and 6 (22%) were from female patients. Further age distribution analysis revealed that 7 samples were from individuals aged 18-40 years, 14 samples were from those aged 41-59 years, and 6 samples were from participants aged 60 years and above (**Fig 3**).

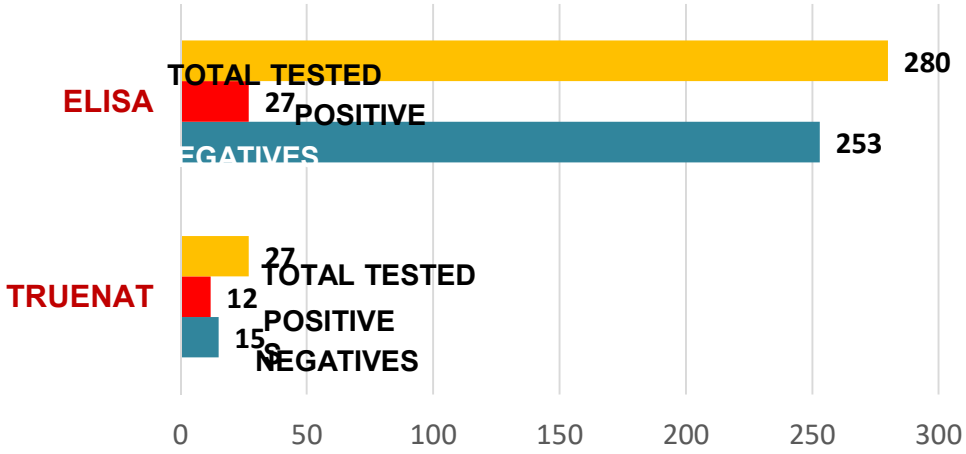

Fig 2. Comparison of HCV infection detection methods using ELISA and Trunat tests.

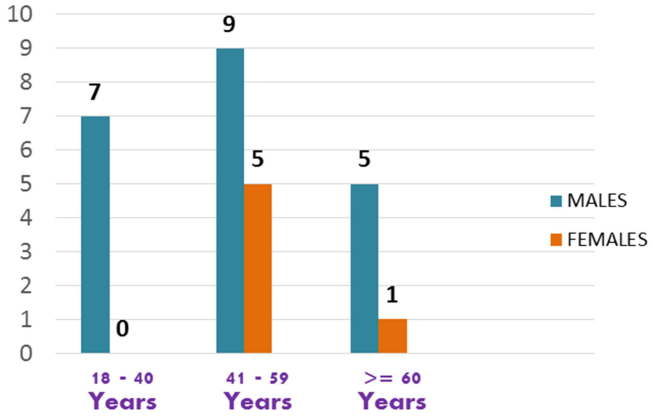

Fig 3. Age and gender-wise distribution of ELISA samples

Genotyping

The genotypic analysis aimed to delineate the distribution of hepatitis C virus (HCV) genotypes among hemodialysis patients. The initial genotyping analysis of the collected samples identified genotype 1a in 4 patients, genotype 1b in 1 patient, and genotype 3 in 1 patient. 6 samples initially yielded Untypable genotypes, suggesting potential genetic variations or limitations in the accuracy of conventional genotyping methods. To resolve this ambiguity and for precise characterization of the HCV genotypes, Sangers sequencing was employed. Upon next-generation sequencing of the Untypable genotypes, the results revealed genotype 1a in 4 patients, genotype 1b in 1 patient, and genotype 3d in 1 patient. This detailed analysis not only confirmed the presence of genotypes 1a, 1b, and 3 but also provided insights into the specific subtypes within these genotypes (Table 1).

Table 1: Distribution of samples based on testing methodologies employed.

METHOD	**ELISA (n=280)**		**VIRAL LOAD IN ELISA-POSITIVE SAMPLES (n=27)**		**GENOTYPING-RT PCR IN VIRAL LOAD POSITIVE SAMPLES (n=12)**				**GENOTYPING-SANGERS SEQUENCING IN GENOTYPES UNTYPABLE BY RT PCR (n=6)**		
	POSITIVE	NEGATIVE	DETECTED	NOT DETECTED	1a	1b	3	UT	1a	1b	3d
	27 (9.7%)	253 (90.3%)	12 (45%)	15 (55%)	4 (34%)	1 (8%)	1 (8%)	6 (50%)	4 (66%)	1 (17%)	1 (17%)

Discussion

In the study, we observed a 9.7% positivity rate for HCV antibodies and antigen using ELISA among hemodialysis patients, which is consistent with previous findings sequencing indicating a high prevalence of HCV infection in this population. [10,11] However, our study also revealed that 45% of ELISA-positive samples had detectable viral RNA levels, highlighting the importance of molecular testing for confirming active HCV infection. This finding aligns with the study by Prakash et al. (2014), which reported a similar need for complementary molecular methods to confirm HCV infection. [12] Furthermore, our observation of a higher proportion of positive samples among males is consistent with previous studies indicating a male predominance in HCV infection among dialysis patients. [13,14] Similarly, the age distribution pattern observed in our study, with a higher prevalence of HCV infection among middle-aged individuals, is consistent with findings sequencing from other studies, suggesting that age may be a significant risk factor for HCV infection among hemodialysis patients. [15,16] Our genotyping results revealed the presence of genotype 1a, 1b, and 3 among HCV-positive hemodialysis patients, which is consistent with previous studies conducted in different geographical regions. [17,18] However, it is noteworthy that the distribution of HCV genotypes may vary geographically, reflecting distinct transmission patterns and population dynamics [19.] Therefore, understanding the regional diversity of HCV genotypes is essential for optimizing treatment strategies and guiding public health interventions structure to local epidemiological profiles. The presence of Genotype 3, albeit in a smaller proportion, adds to the genetic diversity of HCV strains circulating among hemodialysis patients in South India. Genotype 3 has been associated with specific clinical manifestations, including an increased risk of steatosis and faster progression to advanced liver disease, emphasizing the need for genotype-specific characterization [20]. However, the identification of untypable genotypes in a subset of samples highlights potential limitations in conventional genotyping methods, as reported in studies by Waqar et al. (2014) and Ali et al. (2014). [21, 22] The emergence of Untypable genotypes highlights the importance of advanced molecular techniques such as next-generation sequencing in resolving genotype ambiguity and elucidating viral diversity. [23, 24] The use of Sangers sequencing to resolve ambiguities in genotyping and identify specific subtypes within genotypes, as demonstrated in the present study, is supported by previous research emphasizing the utility of Sanger sequencing in characterizing HCV diversity. [25] The comparison of our findings sequencing with previous studies underscores the importance of employing advanced molecular techniques like Sangers sequencing for accurate genotypic characterization in HCV-infected populations. Sangers sequencing provides comprehensive genomic analysis, facilitating precise identification of viral subtypes and variants, which is crucial for understanding viral evolution, transmission dynamics and guiding therapeutic decision-making. The findings sequencing of this study can guide clinical management and public health strategies in Hemodialysis settings sequencing. Genotype-specific treatment approaches are warranted, considering the differential responses of HCV genotypes to antiviral therapy and the potential for treatment failure or resistance mutations [26-30]. Additionally, targeted screening programs focusing on high-risk populations, such as Hemodialysis patients, coupled with rigorous infection control measures are essential for curbing the transmission of HCV and reducing the burden of HCV-related morbidity and mortality in India.

Conclusion

In conclusion, the present study highlights a significant prevalence of hepatitis C virus (HCV) infection among patients undergoing hemodialysis. The predominance of HCV genotypes 1a and 1b underscores the importance of regional genotype profiling to tailor treatment strategies effectively. The detection of genotype 3, albeit in a smaller proportion, underscores the genetic diversity of circulating HCV strains and emphasizes the necessity for genotype-specific characterization for optimized clinical management. Furthermore, the emergence of Untypable genotypes underscores the limitations of conventional genotyping methods and emphasizes the importance of advanced molecular techniques like Sangers sequencing for comprehensive genomic

analysis. Sangers sequencing not only resolves genotype ambiguity but also elucidates viral diversity, providing insights into viral evolution and transmission dynamics. Moreover, targeted screening programs focusing on high-risk populations, such as hemodialysis patients, are crucial for early detection and intervention. Coupled with rigorous infection control measures, these strategies can effectively curb the transmission of HCV and reduce the burden of HCV-related morbidity and mortality in India. Future applications of this research include the implementation of genotype-specific treatment algorithms in clinical settings sequencing, the integration of Sangers sequencing into routine diagnostic protocols for HCV genotyping, and the development of targeted public health interventions tailored to regional epidemiological profiles. Additionally, ongoing surveillance efforts are essential to monitor the prevalence and distribution of HCV genotypes and to adapt therapeutic strategies accordingly. Overall, this study contributes valuable insights into the management and control of HCV infection among hemodialysis patients, paving the way for improved clinical outcomes and public health initiatives in India.

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