Seroprevalence and Quantitative Assessment of Hepatitis C Viral Load in Chronic Hemodialysis Patients in a Tertiary Care Hospital in Udaipur Rajasthan

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ABSTRACT:

Background: Hepatitis C virus (HCV) is a persistent blood-borne infection with a specific affinity for liver tissue. Among individuals undergoing hemodialysis (HD), the incidence of HCV transmission is notably higher than in the general population. Evaluating the seroprevalence and genetic diversity of HCV in this vulnerable cohort is essential for estimating infection burden, enhancing surveillance, and formulating optimized treatment strategies in clinical settings. Therefore, this study aimed to investigate the Seroprevalence and quantitative assessment of HCV among HD patients at a tertiary healthcare center.

Materials and Methods: This cross-sectional observational study included individuals undergoing maintenance HD due to chronic kidney disease (CKD). Data collection involved demographic details, CKD etiology, associated comorbidities, dialysis duration, as well as biochemical and hematological parameters. At baseline, all enrolled patients were screened for anti-HCV antibodies using an HCV enzyme-linked immunosorbent assay (ELISA). Those who initially tested negative were periodically monitored for seroconversion. By the study’s conclusion, all anti-HCV antibody-negative samples and all antibody-positive specimens were analyzed using real-time polymerase chain reaction (RT-PCR) followed by genotyping.

Results: After applying predefined inclusion and exclusion criteria, 145 patients were enrolled in the study, comprising 85 males (58.62%) and 60 females (41.38%). Participants ranged in age from 13 to 89 years, with a mean age of 48.97 years (SD: 18.37 years). Anti-HCV antibodies were detected in 52 individuals (27 during initial screening and 25 upon follow-up). RT-PCR analysis confirmed HCV RNA positivity in 32 patients (13 at screening and 19 during follow-up). Among these, genotyping identified HCV subtype 1a in 28 cases, genotype 3b in one case, while 3 samples yielded inconclusive results.

Conclusion: Prolonged dialysis duration was significantly linked to an increased likelihood of HCV infection. Additionally, discrepancies between serological and molecular findings were observed in HCV detection. Genotype 1a was identified as the most prevalent HCV strain among HD patients in this tertiary care facility.

INTRODUCTION

Hepatitis C virus (HCV) infection remains a significant global health concern, with an estimated 58 million individuals suffering from chronic infection and approximately 1.5 million fatalities annually. Among patients undergoing hemodialysis (HD), key risk factors contributing to HCV transmission include blood transfusions, renal transplantation, and dialysis treatment [1,2]. Additional contributors to HCV acquisition in HD settings encompass the utilization of non-sterile needles, cumulative blood transfusion volume, recurrent transfusions, inadequate adherence to universal safety protocols, extended vascular access, direct exposure to infected individuals and contaminated medical equipment, breaches in infection control measures—including inadequate disinfection of medical instruments and environmental surfaces—poor hand hygiene, insufficient use of protective gear, a high burden of comorbidities, and frequent medical interventions [1]. Individuals on long-term HD therapy exhibit a heightened susceptibility to HCV infection [3]. The prevalence of HCV in this population varies widely, with estimates ranging from 6% to 60% globally, while studies conducted in India have reported rates between 4.3% and 45% [4,5].

HCV is a hepatotropic, enveloped RNA virus with a positive-sense, single-stranded genome, classified under the Flaviviridae family. Its genome spans approximately 9,600 nucleotides. Structurally, HCV consists of four core proteins—core (C), envelope 1 (E1), envelope 2 (E2), and transmembrane (p7). Moreover, six nonstructural proteins (NS2 to NS5B) play a crucial role in viral replication and serve as primary targets for antiviral therapeutics. The virus is categorized into seven major genotypes, further divided into 86 subtypes based on phylogenetic analysis of nearly complete genome sequences. These genotypes are designated as HCV genotype 1 through genotype 7 [6]. Regardless of economic conditions, genotypes 1 and 3 are the most frequently detected worldwide, whereas genotypes 4 and 5 are predominantly found in lower-income regions. With 83.4 million cases—accounting for 46.2% of all HCV infections—genotype 1 remains the most prevalent, with East Asia harboring nearly one-third of these cases. Notably, genotype 1 is the predominant variant among HD patients in southern India [7,8]. The second most prevalent genotype, affecting 54.3 million individuals (30.1%), is genotype 3. Together, genotypes 2, 4, and 6 contribute to 22.8% of cases, while the remaining genotypes constitute less than 1% [8].

HCV genotyping continues to be valuable in personalized treatment, disease monitoring, and public health interventions [9]. Several molecular diagnostic approaches exist for HCV genotyping, with gene sequencing considered the gold standard. Other commonly employed methods include DNA hybridization-based assays, such as the line probe assay (LiPA), polymerase chain reaction-restricted fragment length polymorphism (PCR-RFLP), type-specific PCR, and nested PCR. This study focuses on assessing the seroprevalence of HCV and quantitative assessment plus characterizing its genotypic distribution among HD patients in a tertiary care hospital setting in Udaipur, Rajasthan, India.

MATERIAL AND METHODS

This study was designed as a cross-sectional observational investigation. The research was conducted at a Tertiary Care Hospital in Udaipur, Rajasthan, India. Written informed consent was obtained from all study participants. Inclusion criteria encompassed individuals undergoing maintenance hemodialysis (HD) for chronic kidney disease (CKD) for a minimum duration of six months, those who tested negative for HCV antibodies during screening (to assess incidence rates), and those who provided written informed consent. Patients who declined participation, those receiving HD for acute renal failure (ARF), individuals with negative HCV antibody status who were lost to follow-up, and those on HD for less than six months were excluded from the study.

Patient information, including demographic details, CKD etiology, existing complications, HD duration, and the number of transfused blood units, was retrieved from medical records. Additionally, a history of HCV risk factors and familial HCV infection among immediate relatives was documented. This information was systematically recorded in a structured patient proforma.

For HCV diagnosis, whole blood samples were collected via venipuncture using plain/clot activator tubes and EDTA gel separator vials. The samples were promptly transported to the laboratory for anti-HCV antibody screening, following the testing algorithm recommended by the Centers for Disease Control and Prevention (CDC) [10]. At the study's initiation, all participants were screened for anti-HCV antibodies in serum using enzyme-linked immunosorbent assay (ELISA). Patients who initially tested negative underwent repeat screening every six months. By the study's conclusion, EDTA blood samples from all participants who remained anti-HCV negative were pooled into groups of five and tested for HCV RNA, following the methodology established by Saldanha et al. [11]. Plasma samples were stored at -70°C for subsequent genotyping.

Plasma samples were subjected to reverse transcription polymerase chain reaction (RT-PCR) analysis targeting the 5' untranslated region (5'UTR) and core regions of HCV RNA. Viral RNA was extracted using the RNA Extraction Kit. The 5’UTR region was selected as the primary target due to its high conservation across HCV genotypes, while co-amplification of the core region enabled differentiation of genotypes 1a, 1b, and 6. The assay identified HCV genotypes by analyzing sequence variations in the 5'UTR and core regions.

Data analysis was performed using the SPSS software (version 23.0). The chi-square test was employed to analyze categorical variables. A p-value of ≤ 0.05 was considered statistically significant.

RESULTS

A total of 145 patients were included in the study, among whom 52 (35.86%) were positive for anti-HCV antibodies, while 93 (64.14%) were negative. HCV RNA was detected in 32 (22.07%) patients, whereas 113 (77.93%) were HCV RNA-negative. Among those with confirmed HCV RNA positivity, genotype 1a was the most prevalent (87.50%), followed by inconclusive results in 9.38%, and a single case (3.13%) of genotype 3b (Table 1). Participants ranged in age from 13 to 89 years, with a mean age of 48.97 years (SD: 18.37 years). The distribution of age groups did not show a statistically significant association with anti-HCV antibody positivity ($P = 0.08$) or HCV RNA status ($P = 0.07$). However, the highest proportion of anti-HCV-positive patients was observed in the 41–60 years age group. Gender distribution showed no significant difference in HCV seropositivity or RNA positivity ($P = 0.94$ and $P = 0.63$, respectively). A family history of chronic kidney disease (CKD) was present in 20.69% of the patients, but no significant association was noted with either anti-HCV antibody or HCV RNA status. Patients residing in rural areas were more frequently HCV RNA-negative, with a statistically significant association ($P < 0.05$). The duration of dialysis showed a strong correlation with both anti-HCV antibody positivity and HCV RNA presence, with a significantly higher prevalence in patients undergoing dialysis for more than 36 months ($P < 0.01$ for both) (Table 1).

Table 1: Basic parameters of patients

Patient detail	**Anti-HCV Ab**		**P value**	**HCV RNA**		**P value**	**HCV Genotype (N=32, 22.07%)**		
	+ve (N=52, 35.86%)	**-ve (N=93, 64.14%)**		**+ve (N=32, 22.07%)**	**-ve (N=113, 77.93%)**		**1a (N=28, 87.50%)**	**3b (N=1, 3.13%)**	**Inconclusive (N=3, 9.38%)**
Age (years)									
<20 (4, 3.45%)	1	3	0.08	0	4	0.07	1	0	0
21-40 (57, 39.31%)	17	40		10	47		9	0	2
41-60 (56, 38.62%)	28	28		19	37		15	1	1
>60 (28, 19.31%)	5	23		3	25		3	0	0
Gender									
Male (85, 58.62%)	31	54	0.94	17	68	0.63	13	0	2
Female (60, 41.38%)	21	39		15	45		15	1	1
Family History									
Yes (30,20.69%)	15	15	0.13	11	19	0.07	11	0	0
No (115,79.31%)	37	78		21	94		17	1	3
Residence									
Rural (80, 55.17%)	23	57	0.08	9	71	<0.05	8	0	2
Urban (65, 44.83%)	29	36		23	42		20	1	1
Duration of Dialysis (months)									
≤12 (61,42.07%)	11	50	<0.01	5	56	<0.01	5	0	0
>12-36(68,46.90%)	25	43		15	53		12	0	3
>36 (16, 11.03%)	16	0		12	4		11	1	0

Hypertensive nephropathy was the most common etiology of CKD (63.45%), and a significant association was observed with both anti-HCV positivity ($P < 0.01$) and HCV RNA positivity ($P < 0.01$). Cortical cysts were also significantly associated with HCV RNA positivity ($P < 0.05$), while other etiologies such as diabetes mellitus, nephrolithiasis, and polycystic kidney disease did not show significant associations. Some etiologies, including acute pyelonephritis and obstructive uropathy, were found only in HCV-negative patients (Table 2).

Table 2: Etiology of CKD in patients

Etiology	**Anti-HCV Ab**		**P value**	**HCV RNA**		**P value**	**HCV Genotype**		
	+ve	**-ve**		**+ve**	**-ve**		**1a**	**3b**	**Inconclusive**
Hypertensive nephropathy (92,63.45%)	48	44	<0.01	31	61	<0.01	27	1	3
Acute pyelonephritis (4, 2.76%)	0	4	-	0	0	-	0	0	0
Cortical cyst (7, 4.83%)	4	3	0.27	4	3	<0.05	3	0	0

Diabetes mellitus (30, 20.69%)	13	17	0.41	10	20	0.11	9	0	1
MODS (2, 1.38%)	1	1	0.69	1	1	0.35	1	0	0
Nephrolithiasis (23, 15.86%)	4	19	0.09	3	20	0.28	3	0	0
Obstructive uropathy (4, 2.76%)	0	4	-	0	0	-	0	0	0
PCKD (8, 5.52%)	5	3	0.12	4	4	0.09	4	0	0
Urinary reflux (2, 1.38%)	1	1	0.69	1	1	0.35	1	0	0
Others (8, 5.52%)	0	8	-	0	8	-	0	0	0
Unknown (19, 13.1%)	4	15	0.25	2	17	0.17	1	0	0

Among hematological abnormalities, anemia was significantly associated with anti-HCV antibody positivity ($P < 0.05$), whereas other blood parameters, such as anisocytosis, neutrophilia, and lymphopenia, did not show a statistically significant correlation with HCV status. Thrombocytopenia and leukocytosis were observed exclusively in HCV-negative patients (Table 3).

Table 3: Blood picture in patients

Blood parameter	**Anti-HCV Ab**		**P value**	**HCV RNA**		**P value**	**HCV Genotype**		
	+ve	**-ve**		**+ve**	**-ve**		**1a**	**3b**	**Inconclusive**
Anemia (44,30.34%)	9	35	<0.05	5	39		3	0	0
Anisocytosis (7,4.83%)	3	4	0.91	2	5	-	1	0	0
Microcytosis (2,1.38%)	1	1	0.69	1	1	-	1	0	0
Neutrophilia (12,8.28%)	3	9	0.39	1	11	0.13	1	0	0
Thrombocytopenia (1, 0.69%)	0	1	-	0	1	0.35	0	0	0
Leukocytosis (5,3.45%)	0	5	-	0	5	0.87	0	0	0
Lymphopenia (7,4.83%)	0	7	-	0	7	0.43	0	0	0

DISCUSSION

Hepatitis C virus (HCV) infection remains a critical public health issue due to the absence of an effective vaccine and its potential to cause severe complications. Consequently, strategies for prevention and early detection are essential. One of the major concerns surrounding HCV transmission is its potential for nosocomial spread, particularly in dialysis centers where stringent infection control measures are imperative.

In this study, there was a higher prevalence among males. This pattern aligns with findings from Roy et al. [12], while Madhavan et al. [5] similarly reported a male predominance in hemodialysis (HD) units. The age distribution of participants was comparable to the age distribution observed in the study by Halle et al. [13].Our study found a higher proportion of patients from rural areas compared to urban settings. This contrasts with a study conducted in Egypt by Kerollos et al., which reported a predominance of urban patients [14]. This disparity may be attributed to factors such as increased HCV circulation in urban regions, higher rates of high-risk behaviors and greater healthcare facility density, which increases interactions between infected and susceptible individuals.

Regarding the etiology of ESRD, the most common underlying cause in our study was hypertensive nephropathy, followed by diabetes mellitus and nephrolithiasis. These findings align with those of Kataruka et al., who similarly reported hypertensive nephropathy as the leading cause [15]. However, other studies have identified diabetic nephropathy as the predominant etiology of ESRD [16]. Hypertension and diabetes mellitus are both well-established risk factors for chronic kidney disease (CKD). Hypertension can lead to renal impairment through mechanisms such as tubular atrophy, interstitial fibrosis, and glomerular damage, ultimately progressing to kidney failure.

The majority of patients had undergone HD for 12–36 months, a finding consistent with studies conducted by Madhavan et al. [5] and Kalita et al. [17] in India, as well as international studies by Halle et al. [13]. However, studies from the Middle East have reported a longer HD vintage, with most patients undergoing dialysis for more than 36 months [20,21]. Blood transfusion remains a potential risk factor for HCV transmission among HD patients, despite routine serological screening of blood donors, which has reported an HCV seroprevalence ranging from 0.39% to 5.1% in India [35,36]. Although a statistically significant association between blood transfusion and HCV infection was not observed in our study, it is noteworthy that standard ELISA-based screening may not completely exclude the presence of HCV infection [22].

Previous studies in northern and eastern India have reported HCV genotype 3 as the most prevalent, followed by genotype 1, whereas an equal distribution of genotypes 1 and 3 has been noted in southern and western India [23,24]. In our study, genotyping was performed using line probe assay (LiPA), revealing that 87.5% of HCV RNA-positive cases had genotype 1a, while 3.13% had genotype 3b, and 9.38% were inconclusive. Factors such as potential cross-reactivity, interfering substances, or sample processing errors may contribute to inconclusive results. The predominance of genotype 1a in our study is consistent with findings by Roy et al. [12], who reported a prevalence of 54.1%. This suggests that genotype 1a is the predominant strain in Rajasthan, particularly in the study region.

Our study has some limitations. ELISA-negative samples were pooled before being tested via rRT-PCR, potentially affecting detection sensitivity. Additionally, the study was conducted at a single tertiary care center with a relatively small sample size, limiting the generalizability of findings regarding HCV genotype distribution in the broader population.

CONCLUSION

The detection of HCV infection using nucleic acid-based molecular assays such as RT-PCR has demonstrated substantial clinical utility. The findings of this study indicate that screening via enzyme immunoassays (EIA), such as ELISA, may not be optimal for hemodialysis (HD) units where HCV infection is prevalent, as these methods are prone to false-negative results. Furthermore, the study underscores the importance of understanding the geographical distribution of circulating HCV genotypes and subtypes to enhance the clinical management of dialysis patients. Since treatment efficacy is genotype-dependent, such knowledge could also contribute to the development of future vaccine strategies. It is essential to reinforce quality control and assurance protocols across dialysis units. This includes periodic serological testing for anti-HCV antibodies among all dialysis staff to ensure comprehensive infection control measures are maintained by healthcare professionals statewide.

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