A Cross-Sectional Study to Detect the Prevalence of Hepatitis C Virus Infection among Dialysis Patients in a Tertiary Care Hospital in Bangalore

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Background and study aim: Patients undergoing hemodialysis are at a higher risk of exposure to Hepatitis C Virus (HCV) infection. Therefore, this study aims to detect the prevalence of Hepatitis C Virus infection in hemodialysis patients. **Patients and Methods:** Blood samples collected from 120 dialysis patients were tested for Hepatitis C Virus antibodies (HCV antibodies) by Chemiluminescence Immunosorbent assay (CLIA) and Hepatitis C Virus Ribonucleic acid (HCV RNA) by Real-time Polymerase chain reaction (RT PCR). A P value <0.05 is considered statistically significant. **Results:** By the end of the 1-year study, 7 patients (5.8%) out of 120 patients were positive for both HCV antibodies by CLIA and HCV RNA by PCR. None of the participants were found to be positive only for HCV antibodies or HCV RNA in the present study. 5 positive patients (4.1%) were males and 2 positive patients (1.6%) were females (p<0.05), 3 positive patients (2.5%) belonged to 49-58 years age group and 4 positive patients (3.3%) belonged to 59 years and above age group (p<0.05). In the present study, all 7 (5.8%) HCV-positive patients were above 49 years of age. 5(4.1%) patients underwent dialysis at the rate of 3 sessions per week and 2 patients (1.6%) for 2 sessions per week (p<0.05). 2 positive patients (1.6%) had undergone dialysis for 21-30 months at the time of detection of infection, 3 patients (2.5%) for 31-40 months and remaining 2 (1.6%) patients for more than 40 months (p<0.05). 2 (1.6%) patients were positive for HCV infection at the start of the study and the remaining 5 (4.1%) became positive by the end of the study. **Conclusion:** This study shows a considerably higher prevalence of HCV infection among dialysis patients. This study indicates a high chance of nosocomial exposure to HCV infection during dialysis therapy and recommends screening at regular intervals for early detection and treatment of HCV-positive patients.

INTRODUCTION

Hepatitis C Virus (HCV) is a single-stranded, enveloped RNA virus measuring about 55-65 nanometers in diameter and belongs to the family Flaviviridae. According to the WHO, there are 58 million chronic HCV carriers worldwide with 1.5 million new HCV infections every year and 2,90,000 deaths resulting from HCV infection [1]. About 75-85% of patients infected with the HCV virus become chronic carriers [2]. Chronic Hepatitis C carriers have a 15-20% risk of developing cirrhosis within 20 years [3]. Chronic HCV carriers run 15-20 times higher risk of developing hepatocellular carcinoma (HCC) [4] and account for 30% of total cases of HCC worldwide [5]. A systematic review conducted by SuY et al. found high incidence rates of HCV infection among dialysis patients [6]. The prevalence of HCV in hemodialysis patients is found to be higher than in the general population, with male predominance in studies conducted in tertiary care hospitals in various states across India [7,8].

Hepatitis C Virus (HCV) is a major blood-borne viral infection in patients on dialysis with significant morbidity and mortality. The prevalence of HCV infection is higher in dialysis patients than general population due to underlying impaired immunity, prolonged vascular access, exposure to contaminated fluids, handling by medical staff, frequent blood transfusions, and frequent hospitalization, which increases the risk of exposure to nosocomial HCV infection [9]. The prevalence of HCV infection among dialysis patients ranges from 6% to 60% worldwide, but in India, it ranges between 4.3% and 48% [10]. The presence of HCV infection in dialysis patients is linked to higher mortality rates owing to hepatic and extra-hepatic complications, which include renal manifestations such as cryoglobulinemia, membrane nephropathy, and glomerulonephritis [11]. The high mortality rate among HCV-positive dialysis patients is also linked to the acceleration of atherosclerosis, immune deficiencies, and sepsis [12].

Detection of HCV infection among dialysis patients plays an important role in reducing morbidity and mortality by preventing the development of various hepatic and extra-hepatic complications and in cutting down transmission rates of HCV infection among dialysis patients. The methods that are currently available for detecting HCV infection include serological methods, such as CLIA, and molecular methods, such as RT PCR. CLIA has a sensitivity of 96.1% and a specificity of 91.9% in detecting HCV antibodies [13]. However, there are chances of false negative results by CLIA even with high sensitivity due to a decline in humoral and cellular immunity in dialysis and the absence of antibodies during the initial 2-6 weeks of the window period [14,15]. These disadvantages of CLIA can be overcome by RT PCR, which detects even low levels of HCV RNA during the initial stages of HCV infection [16].

This study aims to detect the incidence and prevalence of HCV infection and associated risk factors among dialysis patients by CLIA and RT PCR methods.

PATIENTS AND METHODS

Study design and source of data

This prospective study was conducted in the Department of Microbiology of M.S. Ramaiah Medical College, Bangalore for a period of 1 year from January 2022 to December 2022.

Sample size estimation

The prevalence of Hepatitis C among renal dialysis patients was reported to be 22% [17]. Assuming similar proportions in our study with an absolute precision of 7.5% and the desired confidence interval of 95% (Alpha error -5%), the required sample size is 117 patients.

Method of Collection of Samples

The requisite minimum sample size for this investigation was established at 117 participants. However, the current study encompassed a total of 120 individuals undergoing dialysis. About 4 mL of blood was collected under aseptic precautions, each in the serum separator (Yellow top) and K2 EDTA (Purple top) vacutainer from the patients who were receiving dialysis therapy in M S Ramaiah Memorial Hospital. These patients were selected based on the following inclusion and exclusion criteria. Informed consent was obtained from all patients after a detailed explanation of the purpose and procedure.

Inclusion Criteria

- CKD patients who were above 18 years of age on dialysis therapy.

Exclusion Criteria

- CKD patients with a history of HCV infection before starting dialysis therapy.
- CKD patients who are co-infected with Hepatitis B Virus (HBV) or Human Immunodeficiency Virus (HIV) as they can act as independent risk factors.

The blood collected in vacutainers was subjected to centrifugation at a speed of 4000 rotations per minute (rpm), and serum and plasma were separately obtained in the yellow-top and the purple-top vacutainers, respectively. The serum and plasma samples were stored at -20.℃

HCV antibody detection

The VITROS Anti-HCV test was performed using the VITROS Anti-HCV Reagent Pack and VITROS Immunodiagnostic products, Anti-HCV calibrator on the VITROS ECIQ Immunodiagnostic system. An immunometric technique was used. In the first stage, HCV antibodies present in the sample bind with HCV

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recombinant antigens coated on the wells. The Unbound sample is removed by washing. In the second stage, horse radish peroxidase (HRP) labeled antibody conjugate binds to any human IgG captured on the well in the first stage. The unbound conjugate was removed by washing. The bound HRP conjugate was measured by a luminescent reaction. The amount of HRP conjugate bound was indicative of the level of HCV antibodies present in the sample. The HCV reactivity of tested serum samples was decided based on VITROS ECIQ CLIA kit literature as – values below 0.9 as non-reactive, values between 0.9 to 0.99 as intermediate reactive and retested using fresh serum collected from the patient and reclassified accordingly and values above or equal to 1 as reactive. In this study, we didn't get any intermediate reactive values. The serum samples were then stored at -20℃ for further reference.

HCV RNA detection by PCR using the Thermo Fisher Quant Studio 3 Real-Time PCR Machine

Steps include-

1) Nucleic acid extraction HCV RNA extraction from plasma samples was done immediately using a BIOBEE viral DNA extraction kit. 250 microliters of plasma were mixed with 250 microliters of lysis buffer, 5 microliters of carrier RNA, and 10 microliters of Proteinase in the sterile microcentrifuge tube and incubated at room temperature for 5 minutes. The tube is then centrifuged at 8000 rotations per minute (rpm) for 5 minutes to get lysate. 400 microliters of this lysate are mixed with 400 microliters of binding buffer in a new 1.5 mL. Microcentrifuge tube and then centrifuged at 8000 rpm for 30 seconds. After discarding the flow-through, 450 microliters of wash buffer 1, is added and centrifuged at 8000 rpm for 30 seconds. The process is repeated with wash buffer 2 also. After discarding the flow through, the spin column is air-dried at 8000 rpm for 3 minutes. Then the spin column is shifted to a new 1.5 mL. A Microcentrifuge tube and 50 microliters of elution buffer are added and centrifuged at 8000 rpm for 1 minute. The lysate collected in the microcentrifuge tube will have viral RNA if present. The extracted samples (templates) were stored at -80℃. HCV RNA detection from those templates is done using the NEODX HCV RNA qualitative RT PCR detection kit .

2) Master Mix preparation (120 reactions) (Table 1 and 2) – Thaw the contents of the kit at room temperature till equilibrated and the reaction mix is prepared for 100 and 20 reactions as below. According to the NeoDx HCV PCR Detection Kit used in the current study, the cDNA is available inbuilt in the kit and does not require additional preparation.

3) Template addition- 15 microliters of reaction mix are prepared for each of 120 PCR reaction tubes with 1 tube each for negative and positive controls to which 10 microliters each of sample, negative and positive control is added to respective tubes and centrifuged and transferred to PCR room where tubes are placed in the sample holder and programmed as below (Table 3) and the result is generated and data is interpreted for each run (Table 4 and 5).

Follow-up – All the participants were tested for HCV antibodies by CLIA and HCV RNA by PCR at the beginning and at the end of a 1-year study and results were noted down and positive patients were treated with direct-acting antivirals (DAAs) and were put on separate dialysis machines dedicated for HCV positive patients.

Statistical Method

The Sample size was estimated using the n-master version 2.0 software developed by CMC Vellore. Descriptive statistics of HCV positives were analyzed and summarized in terms of percentage. Statistical data were generated using IBM SPSS Statistical Software. Statistical significance between categorical variables was tested using the chi-square test of independence, and continuous variables using the Paired T-test. A p-value <0.05 is considered significant.

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RESULTS

Demographic characteristics of the patients (Table 6(

The incidence and prevalence rate of HCV in dialysis patients in this study, by CLIA and RT PCR methods, is calculated as follows

Incidence rate = number of new cases over time/total population* 100 patient-years

Incidence rate = 5/120*100=4.1% per 100 patient-years

Prevalence rate = number of cases (existing and new) during specified time/total number of cases * 100

Prevalence rate = 7(2+5)/120=0.58*100=5.8%. The HCV antibody and HCV RNA of the dialysis patients were measured at the beginning and at the end of the study to compare the prevalence rate of HCV infection in that year which is depicted in Table 7.

Master Mix preparation (total 120 reactions)

Table 1 – for 100 reactions (1st batch)

Reagents	**For 100 reactions**
2x master mix	1500 microliters
20 x primer and probe mix	150 microliters
Nuclease-free d H2O	350 microliters
Total	2000 microliters

Table 2 – For 20 reactions

Reagents	**For 20 reactions**
2x master mix	300 microliters
20x primer and probe mix	30 microliters
Nuclease-free d H2O	70 microliters
Total	400 microliters

Table 3 – Steps of programming PCR instrument

Sl.no	**Steps**	**Temperature**	**Time**	**Cycle**
1	Reverse transcription	50℃	15 min	**1**
2	Initial denaturation	95℃	2 min	1
3	Denaturation	95℃	15 sec	45
4	Annealing, Extension, and Fluorescence	58℃	30 sec	

Table 4 -Result interpretation for HCV

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For HCV	For Internal control gene	Assay result
Ct <40	**Ct</=40**	**Positive**
Ct>/=40	**Ct</=40**	**Negative**
Ct>/=40	**Ct>/=40**	**Invalid**

Ct – cycle threshold

Invalid – (Repeat extraction and detection)

Table 5 – Data interpretation for HCV

HCV (HEX/VIC)	IC (TEXAS RED)	Result
+	+	HCV detected
-	+	HCV not detected
-	+	Invalid (repeat test)

HEX/VIC – HCV genes tested, IC – Internal control.

Table 6 – Patient demographic characteristics

Variables (N=120)	Positives (n%)	Negatives (n%)	P value
Gender of the patients			
Males	5 (4.1%)	50 (41.6%)	0.04
Females	2 (1.6%)	63 (52.5%)	
Age of the patients			
19-28 years	0	13 (10.8%)	0.001
29-38 years	0	24 (20%)	
39-48 years	0	31 (25.8%)	
49-58 years	3 (2.5%)	27 (22.5%)	
59 years and above	4 (3.3%)	18 (15%)	
Rate of the dialysis			
2 sessions per week	2 (1.6%)	68 (56.6%)	0.02
3 sessions per week	5 (4.1%)	45 (37.5%)	
Duration of dialysis			
0-10months	0	13 (10.8%)	0.0007

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11-20 months	0	30 (25.0%)	
21-30 months	2 (1.6%)	23 (19.2%)	
31-40 months	3 (2.5%)	25 (20.8%)	
40 months and above	2 (1.6%)	22 (18.3%)	

Table 7 – Prevalence of HCV infection among dialysis patients by CLIA and RT PCR.

Variables (N=120)	**Positives (n %) (Existing+New)**	**Negatives (n %)**	**P value**
HCV antibodies by CLIA			
At the start of the study (Existing cases)	2 (1.6%)	118 (98.4%)	0.01
At the end of the study (Existing + New cases)	7 (5.8%)	113 (94.2%)	
HCV RNA by PCR			
At the start of the study (Existing cases)	2 (1.6%)	118 (98.4%)	0.01
At the end of the study (Existing + New cases)	7 (5.8%)	113 (94.2%)	

DISCUSSION

Hepatitis C Virus infection is a chronic blood-borne viral infection in dialysis patients with significant morbidity and mortality. The Mortality rate is particularly higher due to the increased rate of progression to cirrhosis and liver failure among HCV-infected dialysis patients. An association of higher cardiovascular mortality is also seen in HCV-infected dialysis patients [18]. The present study calculates the statistical association between categorical variables using the Chi-squared test of Independence and between continuous variables using the paired t-test. The present study calculated the incidence of HCV positives among dialysis patients for a period of 1 year and found the annual incidence rate to be 4.1% per 100 patient years. Various other studies have also found the annual incidence rate of HCV infections in dialysis facilities to range from 1.1% to 3.6% per 100 patient years, accounting for more than 20,000 cases annually worldwide [19,20]. The present study shows an HCV prevalence rate of 5.8% among dialysis patients by both CLIA and RT-PCR methods. A seroprevalence study conducted in a tertiary care hospital in Bangalore by Priyanka N et al. also showed an HCV prevalence of 11% in patients undergoing hemodialysis [21]. A study conducted by AI Shukri et al also showed that the seroprevalence of HCV infection was 5.7% among hemodialysis patients by electrochemiluminescence test. Only 31% of the HCV antibody positives had HCV RNA tested by PCR, indicating past exposure and

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cure among dialysis patients [22]. Another study conducted in a tertiary care hospital in South India by A. Madhavan et al also showed a similar HCV prevalence of 8% among dialysis patients tested by PCR where HCV antibodies were found in only 1 sample and HCV RNA was detected in 8 samples among a total of 100 samples, indicating a high false negativity rate by serological methods like ELISA, especially during the window period of the HCV infection which is overcome by PCR which detects even low levels of HCV RNA even during the initial period of the infection [23]. A study conducted in a tertiary care hospital in South India by Rajasekaran C et al showed an HCV prevalence of 13.9% among hemodialysis patients by PCR and it also shows a high rate of false negativity by ELISA and recommends screening of samples by PCR in the early stages of HCV infection to prevent nosocomial spread of the virus among dialysis patients [24]. Another study conducted by Lok AS et al has also shown that antibody detection methods alone may not detect all the cases in the acute phase of HCV infection because of the longer window period in these immunocompromised patients leading to higher false negative rates and confirmation by RT PCR is required before ruling out as negative [25]. Various other studies have also shown that the prevalence of HCV among dialysis patients ranges between 2.7%-41% [26,27,28].

In the present study, HCV positivity is commonly seen in males (p=0.04), in older patients above 50 years of age (p=0.001), in those who receive dialysis therapy 3 times a week (p=0.02), and those who are on dialysis therapy for a longer duration of more than 20 months (p=0.0007). The study conducted by Joukar et al showed a significant statistical association of HCV infection with gender and duration of dialysis ($p<0.05$) but no statistical association with age and frequency of dialysis [29]. The present study contrasts with the findings of Kerollos K.M.N et al, where the duration of hemodialysis was shown to be an insignificant risk factor [30]. The present study is concordant with the study conducted by Chizoba et al, where the duration of dialysis increased the risk of HCV infection among dialysis patients [31]. Rajasekaran et al [24] also found a significant statistical association between HCV infection and duration of hemodialysis but found no association with the age and gender of the patient. The present study is concordant with the findings of the study conducted by Tsung Hui-Hu et al, where the HCV seropositive rates increased with age and duration of the dialysis [32].

In this study, all HCV-positive patients with confirmed molecular detection were isolated immediately and put on separate dialysis machines exclusively for HCV patients to prevent transmission among other HCV-negative dialysis patients. Various studies have shown reduced HCV transmission in dialysis units with the use of dedicated machines and the practice of hygiene measures such as hand hygiene, use of gloves, periodic disinfection of the equipment, and proper aseptic precautions by health care personnel handling those patients [33].

In this study, all HCV-positive patients were started on direct-acting antiviral therapy (DAA) to prevent the progression of infection and risk of developing complications such as liver cirrhosis and hepatocellular carcinoma, as the DAA's are confirmed to be effective and less toxic than traditional ribavirin and interferon [34]. However, a study conducted by Dina G Abdallah et al has shown that the risk of development of HCC is independent of DAA-achieved HCV sustained virologic response and urged for careful follow-up for HCC [35]. In the present study, 2 HCV positive patients (1.6%) were found to be probably non-responders to DAA therapy as HCV RNA levels remained detectable by PCR throughout 1 year follow up at regular intervals of 3 months and were not followed up later and 5 new HCV positive patients (4.1%) who were put on DAA therapy were not followed up post DAA therapy due to limited study duration. A study conducted by Elhammadi et al. found that the non-response to DAA therapy was associated with older age ($p<0.001$), higher BMI ($p<0.001$), and male gender (p=0.002). It also showed a significant association with Diabetes and other biochemical parameters, such as low platelet count, and high Alanine aminotransferase (ALT) levels [36]. However, the current study did not focus on risk factors associated with non-response to DAA therapy, as the study was mainly on the incidence and

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prevalence rate of HCV infection among dialysis patients.

CONCLUSION

This study shows that dialysis is a potential risk factor for HCV infection in chronic kidney disease patients, especially those who are undergoing dialysis frequently for a longer duration. Hence, regular screening of these high-risk category patients for HCV infection by CLIA and confirmation by RT PCR is essential to reduce transmission rates as well as to reduce morbidity and mortality. This study also showed the importance of isolation and the practice of strict aseptic precautions by healthcare personnel to prevent HCV infection in dialysis units, as the prevalence of HCV infection is still high in dialysis units of various institutes in the country. The limitations of the study are that the study was conducted at a single center, limiting the generalizability of the findings. The specific patient population and the practices at this center might not be representative of other healthcare settings. Therefore, the result may not apply to other populations or healthcare settings. Further research should consider multi-center trials to address this limitation and enhance the external validity of the study. Another potential limitation of this study is the lack of adjustment for potential confounding factors such as the history of blood transfusion or other comorbid conditions, such as Diabetes mellitus due to limited data availability which could influence the relationship between HCV infection and dialysis, which could lead to potential overestimation of HCV infection in dialysis patients. Further studies should consider including such confounding factors in their analysis.

List of abbreviations:

HCV – Hepatitis C Virus

CKD – chronic kidney disease

RNA – Ribonucleic acid

CLIA – Chemiluminescent Immunosorbent Assay

RT-PCR- Real-time Polymerase chain reaction

HCC – Hepatocellular carcinoma

ELISA – Enzyme-linked immunosorbent assay.

DAAs – Direct Acting Antivirals.

Data availability

The data generated or analyzed during the study are included in the article .

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Ethical approval:

The study was approved by the ethics committee of M S Ramaiah Medical College in January 2021. The ethical clearance certificate number is MSRMC/EC/PG-10/01-2021. Informed consent was obtained from all participants in the study

Author contribution: We declare that all listed authors have made substantial contributions to

All the following three parts of the manuscript:

- Research design, or acquisition, analysis, or interpretation of data.
- Drafting the paper or revising it critically
- Approving the submitted version

We also declare that no one who qualifies for authorship has been excluded from the list of authors.

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Conflict of interest

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

HIGHLIGHTS

- Hepatitis C Viral infection is a major cause of morbidity and mortality among dialysis patients**.**

- Risk factors include male gender, old age, increased frequency, and long duration of dialysis**.**
- Maintenance of strict aseptic conditions and isolation of HCV-positive patients on separate dedicated machines play a role in cutting down nosocomial HCV transmission**.**

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