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Seroprevalence of Hepatitis C Virus Infection among Hemodialysis Patients in A Tertiary Care Hospital in South India

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Abstract

Hepatitis C virus (HCV) infection in hemodialysis patients is mainly associated with insufficient precautionary measures. Compared with the general population, the prevalence of HCV infection is high among hemodialysis patients. HCV infection in these patients is typically associated with increased morbidity and mortality; however, the early detection of HCV may retard disease progression and reduce morbidity and mortality. In this study, we sought to estimate the seroprevalence of HCV infection among patients undergoing hemodialysis in a tertiary care hospital in Chennai, Tamil Nadu, South India. This was a prospective, observational, cross-sectional study that included 82 hemodialysis patients. Blood samples were collected from each patient, and the serum was separated for detection of the presence of HCV antibodies, HCV core antigen, and HCV RNA using an enzyme-linked immunosorbent assay, enzyme immunoassay, and polymerase chain reaction, respectively. Relevant socio-demographic, clinical, and laboratory information was also obtained. Among samples collected from the 82 patients, seven were found to be positive for HCV antibodies, whereas nine and eleven samples were found to be positive for HCV core antigen and HCV RNA, respectively. Long durations of renal failure and hemodialysis were identified as being positively associated with HCV infection in these hemodialysis patients, and a statistically significant reduction in hemoglobin levels and an increase in creatinine levels were detected among the HCV-positive patients. The overall seroprevalence of HCV infection in hemodialysis patients was found to be 13.4%. Given that high rate false-negative reactions were detected using ELISA and EIA, screening of HCV RNA during the early stages of HCV infection can contribute to preventing its nosocomial spread in these patients. Moreover, it is imperative that stringent universal precautionary measures are implemented in all health care settings and hemodialysis units to minimize the risk of HCV transmission.

Keywords: Seroprevalence, Hepatitis C, Haemodialysis, ELISA

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INTRODUCTION

Hepatitis C virus (HCV) is a single-stranded, enveloped, positive-sense RNA virus that causes hepatitis and can lead to serious liver complications, including cirrhosis and hepatocellular carcinoma, particularly in patients undergoing hemodialysis (HD), in whom immunity is typically low.[1]

Whereas in the general population, the prevalence of HCV infection ranges from 0.5% to 1.5%, in patients undergoing HD, the prevalence can exceed 50% in both developed and developing countries.[2-7] A range of risk factors have been identified for the high prevalence of HCV infection in HD patients, the most important of which are an increase in the number of blood transfusions, an increase in the duration of HD, and improper sterilization or internal contamination of dialysis equipment.[8] Given the associated high rates of morbidity and mortality, HCV infection in HD patients is recognized as a significant health problem.[9]

The detection of HCV infection in HD patients poses considerable challenge, as the poor immune response of these patients means that they have a longer window of infection.[10] The earliest infection-related marker to appear in the blood is HCV RNA, which occurs at approximately 1 to 3 weeks after onset, followed by the appearance of HCV core antigen at 2 to 6 weeks. However, it is only at 8 to 12 weeks after exposure to HCV that HCV antibodies are generally detected in the blood.[11] With respect to the diagnosis of HCV infection; many laboratories preferentially perform HCV antibody detection. However, the main disadvantage of this procedure is that when used independently it fails to detect all HCV-infected patients who are in the early stages of infection, and thus may lead to high false-negative rates.[12]

The gold standard method for the early diagnosis of HCV infection is PCR-based HCV RNA detection; however, this approach tends to be limited by its higher costs and low availability.[13,14] An alternative method for detecting HCV infection during its early stages is the detection of HCV core antigen in the blood.[15,16]

In India, although numerous studies have been conducted to determine the seroprevalence of HCV and to assess the efficacy of different serological tests among patients undergoing HD, either the data remain scarce or the tests conducted are based exclusively on the detection of HCV antibodies.

AIM

To estimate the seroprevalence of HCV infection among hemodialysis patients attending a tertiary care hospital in Chennai, Tamil Nadu, South India

Objective

To assess the efficacy of different serological tests in diagnosing HCV infection and also to identify the risk factors for HCV infection in patients undergoing hemodialysis.

MATERIALS AND METHODS

The study was initiated after having initially gained approval from the Institutional Human Ethics Committee (IEC No: 40102015).

Study design

This was a prospective, observational, cross-sectional study

Study Duration

6 months (July 2016 to December 2016).

Number of samples

Blood samples were collected from 82 patients undergoing HD in a tertiary care hospital in Chennai, South India.

Inclusion criteria
(a) Both genders were included, and the age range of participants was set to 18–60 years
(b) Those willing to give written, informed consent to participate in the study

Exclusion criteria
(a) Patients who have K/C/O HCV infection and were receiving treatment
(b) Pregnant women
(c) Patients co-infected with HIV

Details regarding the study and study procedures were clearly explained to the patients in their native language, and written informed consent were obtained. In no part of this

manuscript is the personal identity of the patients revealed.

Relevant socio-demographic, clinical, and laboratory information was obtained from all patients, and having collected the necessary information, detailed physical examinations were performed.

Sample collection and processing

Samples of blood (5 mL) were collected from patients under aseptic conditions and transferred to collection tubes containing EDTA. Serum was subsequently separated and stored at -80°C until further use. All the samples were subjected to the following diagnostic tests:

1. HCV RNA detection using polymerase chain reaction (PCR)
2. HCV core antigen detection using an enzyme immunoassay (EIA)
3. HCV antibody detection using a 3rd generation enzyme-linked immunosorbent assay (ELISA)

All tests were performed in accordance with the manufacturer's instructions.

Statistical analysis

Statistical analysis was performed using SPSS 20.0 software. For all continuous variables, the results are presented as mean values ± standard deviation, and for the categorical variables, data are expressed as percentages. Continuous and categorical variables were compared using Student's t-tests and chi-square tests, respectively. A probability value (P value) of less than 0.05 was considered statistically significant.

RESULTS

A total of 82 patients were included in this study, among whom 65 (79%) were males and 17 (21%) were females. A majority of the patients [47 (57%)] were in the 21–40-year age group and the mean age of patients was 36 ± 10.32 years. Details of a percentage analysis of participant age groups are presented in Figure 1.

Most common risk factors for HCV infection in HD patients were found to be hypertension (34%), followed by diabetes (12%). The percentage analysis for the risk factors of the study participants is given below in Figure 2.

Analysis of the duration of renal failure revealed that in 57% of the patients, the disease had been present for more than 24 months. Similarly, analysis of the duration and frequency of HD revealed that a majority (72%) of the patients had been receiving HD for more than 24 months, and 90% were undergoing seven to nine HD sessions per month. Furthermore, approximately 67% of the patients had a history of changing dialysis center. Co-infection with HBV was found in 4% of the patients. Data obtained from a frequency analysis of the duration of CKD and HD are presented in Figure 3.

The sensitivity and specificity of the various diagnostic tests in detecting the HCV infection is given below in Figure 4.

Of the 82 patients, 11 (13.4%) were found to be HCV-positive, among whom 10 (91%) were male and one (9%) was female. A majority (82%) of the HCV-positive patients were in the 21–40-year
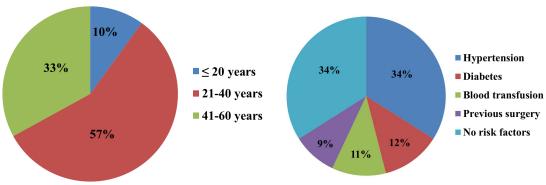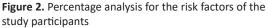

Figure 1. Percentage analysis of the age groups of the study participants

Figure 2. Percentage analysis for the risk factors of the study participants

age group, among whom the mean age was 38 ± 13.36 years. The summary of the seroprevalence of HCV by various diagnostic methods is presented below in Table 1.

Analysis of the risk factors of HCV infection revealed that HCV positive-patients were more likely to have a history of diabetes, blood transfusion, and surgery, although the associations were not significant. The mean duration of renal failure and HD were found to be significantly longer among the HCV-positive patients, and even though these patients frequently changed dialysis center, this does not appear to have any significant association with HCV infection. A comparison of the different characteristics of HCV-positive and -negative patients are shown below in Table 2.

Analysis of different biochemical parameters revealed a statistically significant reduction in hemoglobin levels and an increase in creatinine levels among HCV-positive patients. Although the levels of AST and ALT were normal

in HCV-positive patients, they were found to have a positive association with HCV infection. In both HCV-positive and -negative patients, the levels of urea was found to be particularly high, although the differences were not statistically significant. Other parameters, such as total bilirubin and total protein, were found to be within the normal range in both groups. A comparison of the assessed biochemical parameters in HCV-positive and -negative patients are shown in Table 3.

DISCUSSION

Hepatitis C virus (HCV) infection occurs mainly as a consequence of failures to comply with universal precautionary measures.[17] Among hemodialysis (HD) patients, HCV infection is associated with an increased risk of progression to end-stage renal disease and cardiovascular events.[18] Poor compliance with HCV-antiviral therapy, the lack of an effective vaccine, and adverse complications have contributed to HCV infection becoming a major global health problem. Hence, identifying HCV infection in HD patients at the earliest stage possible and the timely initiation of treatment may contribute to reducing progression and also the incidence of morbidity and mortality.

Table 1. Seroprevalence of HCV by various diagnostic methods

No.	Tests	Frequency (n=82)	Percentage (%)
1.	ELISA - Positive for HCV antibody	7	8.5
2.	EIA - Positive for both HCV core antigen	9	11
3.	PCR - Positive for HCV RNA (Gold standard)	11	13.4

Seroprevalence of HCV

In the HD patient cohort examined in this study, we detected an overall HCV prevalence of 13.4%, which is comparable to the proportions
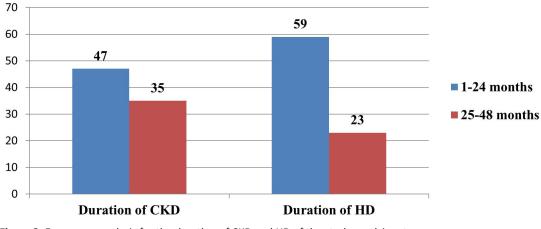

Figure 3. Frequency analysis for the duration of CKD and HD of the study participants
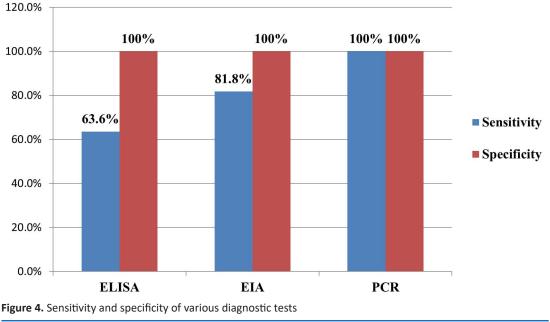
reported in studies conducted in other regions of India. Table 4 presents a list of the HCV seroprevalence studies conducted on HD patients in different regions of India.

Studies on the prevalence of HCV infection among HD patients in other countries have reported prevalences ranging from 4.8% to 39% (Table 5), which is accordingly broadly consistent with our findings in the present study.

A majority of the HCV prevalence studies conducted in both India and other countries have been based on the detection of HCV antibodies

Table 2. Comparison of various characteristics of HCV positive and HCV negative patients

No.	Characteristics	HCV Positive (N=11)	HCV Negative (N=71)	P value
1.	Age			
	≤ 20 years	1 (9%)	7 (9%)	0.17
	21-40 years	9 (82%)	38 (54%)	
	41-60 years	1 (9%)	26 (37%)	
2.	Gender			
	Male	10 (91%)	55 (77%)	0.44
	Female	1 (9%)	16 (23%)	
3.	Risk factors			
	Hypertension	3 (27.3%)	25 (35%)	0.09
	Diabetes	3 (27.3%)	7 (10%)	
	Blood transfusion	3 (27.3%)	6 (8.5%)	
	Surgery in the past	1 (9.05%)	6 (8.5%)	
	No risk factors	1 (9.05%)	27 (38%)	
4.	Duration of renal failure (in months), Mean ± SD	29 ± 7.16	19 ± 13.06	0.01
5.	Duration of HD (in months), Mean ± SD	28 ± 7.62	18 ± 13.39	0.02
6.	Change in dialysis centre			
	Yes	9 (82%)	46 (65%)	0.26
	No	2 (18%)	25 (35%)	

Figure 4. Sensitivity and specificity of various diagnostic tests

Table 3. Comparison of various biochemical parameters among HCV positive and HCV negative patients

No.	Parameters	HCV Positive (N=11) Mean ± SD	HCV Negative (N=71) Mean ± SD	P value	Normal range
1.	Haemoglobin (g/dl)	10.1 ± 1.18	9.1 ± 1.48	0.03*	12-16
2.	Urea (mg/dl)	113 ± 36.38	113 ± 49.44	0.97	14-23
3.	Creatinine (mg/dl)	7.2 ± 4.72	4.6 ± 3.60	0.04*	0.7-1.3
4.	Total Bilirubin (mg/dl)	0.4 ± 0.09	0.5 ± 0.25	0.12	0.1-1.2
5.	AST (U/L)	20 ± 8.84	26 ± 9.16	0.05*	8-48
6.	ALT (U/L)	14 ± 3.26	24 ± 11.45	0.007*	7-55
7.	Total Protein (g/dl)	6.2 ± 0.60	6.3 ± 0.46	0.68	6-8.3

Table 4. Seroprevalence studies of HCV infection in HD patients done in India

Author	Place	Year	Prevalence (%)	Method
Chadha MS et al.[19]	Maharashtra	1993	12.1	ELISA/PCR
Jaiswal SP et al.[20]	Madhya Pradesh	1996	41.9	ELISA
Gosavi MS et al.[21]	Maharashtra	1997	27.8	ELISA
Chandra M et al.[22]	Andhra Pradesh	2004	46	ELISA
Reddy AK et al.[23]	Andhra Pradesh	2005	13.2	EIA
Medhi S et al.[24]	Delhi	2008	17.2	EIA
Jasuja S et al.[25]	Delhi	2009	27.7	ELISA/PCR
Surendra kumar P et al.[26]	Tamilnadu	2011	12.4	Enhanced chemiluminescence
Prakash S et al.[27]	Uttar Pradesh	2012	6.9	ELISA/PCR
Jamil M et al.[28]	Meghalaya	2016	1.38	ELISA
Subramanian G et al.[29]	Gujarat	2016	2.7	ELISA
Anitha madhavan et al.[30]	Kerala	2018	8	ELISA/PCR
Dhar A et al.[31]	Jammu	2016	31.4	ELISA/PCR
Present study	Tamilnadu	2016	13.4	ELISA/EIA/PCR

using ELISA. However, when used alone, antibody detection may be ineffective in detecting early HCV infection. As an alternative, PCR can detect HCV during the early stages of infection, although the high cost of analysis tends to limit its use. Consequently, we believe that detection of the HCV core antigen using EIA would enable a sufficiently reliable early diagnosis of HCV infection, such that measures can be taken to reduce the spread of infection and prevent nosocomial dissemination.

To date, there have been few studies on the seroprevalence of HCV based on the detection of HCV core antigen using EIA. Among those studies that have been conducted, Reddy et al.[23] and Medhi et al.[24] used HCV core antigen EIA for the detection of HCV infection in patients undergoing HD and reported prevalences of 13.2% and 17.2%, respectively, which are comparable to

seroprevalence of 13.4% detected in the present study.

The data presented in Table 4 indicate that during the period from 1993 to 2010, the seroprevalence of HCV in HD patients ranged from 12.1% to 46%,[19-25] whereas studies conducted after 2010 reveal a general decline in prevalence to 12.4%.[26-30] One exception in this regard, however, is a study conducted in Jammu in 2016, which reported a considerably higher prevalence of 31.4%.[31] We speculate that this wide divergence in prevalence could be attributed to a number of contributory factors, including different geographical locations, a different study duration, or differences in the sensitivity and specificity of the methods used for detecting HCV infection. In this latter regard, prior to 2010, such studies tended to rely heavily on 1st generation ELISAs,

Table 5. Seroprevalence studies of HCV infection in HD patients done in other countries

Author	Place	Year	Prevalence (%)	Method
Muller GY et al.[32]	South America	1992	39	ELISA
Soni PN et al.[33]	South Africa	1993	4.8	ELISA
Fabrizi et al.[34]	Italy	1994	20	ELISA
Hinrichsen S et al.[35]	Europe	1996	7	ELISA
Seck SM et al.[36]	West Africa	2011	5.6	ELISA/PCR
Present study	Tamilnadu, South India	2016	13.4	ELISA/PCR

which were characterized by comparatively low sensitivity and specificity. These assays have, however, been superseded by 2nd and 3rd generation ELISAs, the sensitivities and specificities of which are considerably enhanced.

In the present study, we used a combination of three diagnostic approaches, on the basis of which, we detected prevalences ranging from 8.5% to 13.4%. HCV antibody detection using a 3rd generation ELISA revealed an 8.5% prevalence of HCV infection among HD patients, with 63.6% sensitivity and 100% specificity. Comparatively, the prevalence of HCV infection detected using an HCV core antigen EIA was 11%, with 81.8% sensitivity and 100% specificity. The sensitivity of a test is defined as its ability to yield a positive result for a subject who has a particular disease. Given that the sensitivities of both ELISA and EIA are comparatively low, there is a high likelihood of obtaining false negative results when using these techniques. In general, PCR assays are considered to be the most sensitive tests for detecting HCV infection.

In this study, we indeed obtained high false negative rates of 36.4% and 18.2% using ELISA and EIA, respectively, and hence if HCV is suspected, it is highly recommended that HCV RNA is obtained from all patients undergoing HD.

CONCLUSION

Using the gold standard PCR test, we detected an overall seroprevalence of HCV infection of 13.4% among HD patients in a tertiary care hospital in South India. The most important risk factors associated with the acquisition of HCV infection among HD patients were identified as a prolonged duration of renal failure and a long duration of HD. We detected a significant reduction and increase in the levels of hemoglobin and creatinine, respectively, among HCV-positive patients. Analysis based on ELISA was found to be moderately sensitive and highly specific in detecting HCV infection, whereas EIA showed both high sensitivity and specificity. Hence, given that seroconversion to HCV antibodies does not occur in all HD patients, on account of their poor immune response, the detection of antibodies based exclusively on ELISA would be insufficiently sensitive for establishing a diagnosis of HCV infection; Consequently, in cases in which PCR tests are either unavailable or unaffordable. EIA would be a reasonable alternative as a diagnostic test for detecting HCV infection. In addition, providing relevant education and creating awareness among health care providers regarding the stringent screening of blood and blood products, adopting strict universal precautionary measures in HD units, proper management of HD machines, and appropriate waste management may contribute to minimizing the risk of the nosocomial spread of HCV infection.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

None.

DATA AVAILABILITY:

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

The study was approved by the Institutional Ethics Committee, Madras Medical College, Chennai, India, with IEC No: 40102015.

INFORMED CONSENT

Written informed consent was obtained from the participants before enrolling in the study.

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