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ORIGINAL ARTICLE

Prevalence of hepatitis C among haemodialysis patients in a tertiary care hospital in south India

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ABSTRACT

Background and Objectives: Hepatitis C is the most common hepatotropic viral infection that affects patients on maintenance hemodialysis. Most of the laboratories in India depend on HCV antibody detection by ELISA. PCR based studies on detection of HCV RNA among haemodialysis patients are very scanty in India. The current study was undertaken to find the prevalence of HCV among haemodialysis patients by ELISA and PCR.

Materials and Methods: This prospective study was conducted from January to May 2018 in a total of 100 samples. Patients more than 18 years of age, who had undergone at least 15 sessions of dialysis were enrolled in the study. All samples were screened for HCV antibody by ELISA and HCV RNA by PCR. Data regarding age and gender of the patients, history of blood transfusion, duration of hemodialysis, total bilirubin levels were collected from medical records.

Results: Among the 100 samples, only one was positive for HCV antibody by ELISA. Eight samples were positive for HCV RNA by PCR. In this study 62.5% of the HCV positives had a previous history of blood transfusion. Duration of dialysis was more among the HCV positive group but there was no statistical significance.

Conclusion: This is the first study from the southern state of Kerala in India showing the prevalence of HCV among hemodialysis patients by PCR. Our study showed an overall HCV prevalence of 8% by PCR. All the PCR positive samples were negative by 3rd generation ELISA which is an alarming finding and further justifies the need for PCR for detecting HCV.

Keywords: Hepatitis C; Enzyme linked immunosorbant assay; Polymerase chain reaction; Haemodialysis patients

INTRODUCTION

The global prevalence of hepatitis C is estimated to be 3% with 12.5 million people in India alone infected with the virus (1). Persons living with HCV (Hepatitis C Virus) infection are at risk for developing cirrhosis and hepatocellular carcinoma (2). HCV is a single stranded RNA virus belonging to the family flaviviridae of genus hepacivirus. It is the most common chronic blood borne infection in the world. It is also the most common hepatotropic viral infection that affects patients on maintenance hemodialysis (MHD). The prevalence of HCV in MHD patients ranges from 6- 60% whereas in India various studies show a prevalence of 4.3% to 45% (3). A number of risk factors have been identified for high incidence of HCV infection in HD (Haemodialysis) patients; the most important ones being the number of blood transfusions, duration of the hemodialysis treatment, and nosocomial transmissions due to inadequate infection-control measures (4). HCV infection in HD

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patients has been associated with high morbidity and mortality (5).

CDC (Centre of Disease Control) recommends screening for HCV antibody should be performed routinely in patients at increased risk of infection. Most of the laboratories in India depend on HCV antibody detection by ELISA (Enzyme Linked Immuno Sorbant Assay). Antibody detection methods alone may fail to detect all the cases in the acute phase of the disease. The window period in HD patients may be longer due to the immunocompromised state and this can lead to higher false-negative rates when the antibody detection method alone is used for diagnosis (6). A reactive or indeterminate/equivocal antibody test should be followed by HCV RNA testing to determine occult infections (7). HCV RNA detection by PCR is regarded as the gold standard method for diagnosing HCV infection in haemodialysis patients but it is limited by cost and availability (8). Real time PCR assay was also introduced for monitoring of viral load in infected patients (9). Another method for diagnosing HCV infection is detection of HCV core antigen at the early stage of infection when HCV antibodies have not been produced (10).

Most of the studies in India on prevalence of HCV among HD patients have been done based on antibody detection methods. High prevalence of HCV infection in dialysis settings can result in severe consequences. The main objective of this study was to find the prevalence of HCV among haemodialysis patients by ELISA and PCR in all the samples. Although studies have been conducted in various parts of India showing the prevalence of HCV among HD patients, this is the first such study from the southern state of Kerala.

MATERIALS AND METHODS

This prospective descriptive study was conducted from January to May 2018 in Government Medical College, Alleppey. A Total of 100 samples were collected from two different hemodialysis units in Alleppey, Kerala, India.

Inclusion criteria. Patients > 18 years of age who have undergone at least 15 sessions of Hemodialysis.

Exclusion criteria. i) Patients who have undergone less than 15 sessions of hemodialysis; ii) Patients less than 18 years of age; iii) Patients who were not willing to participate in the study; iv) Patients who were HCV Positive prior to initiating dialysis.

Both hemodialysis units had two routine HD unit areas with 5 machines in each area. Both units have dedicated dialysis machines for HCV positive patients. All patients were tested for HBsAg, Anti HCV and HIV prior to initiating dialysis. Data regarding age and gender of the patients, history of blood transfusion, duration of hemodialysis, total bilirubin levels were collected from medical records.

Under strict aseptic precautions 5 ml blood was collected from the cubital fossa by venipuncture by phlebotomist. The serum was separated and subjected to initial screening for HCV antibody by ELISA. Sera for ELISA and PCR were stored at -20°C and -70°C respectively till the tests were carried out. ELISA was carried out using 3rd generation HCV Microlisakit (J.Mitra HCV Microlisa Kit, India). All the serum samples were also tested by Nested PCR for the detection of HCV RNA. The RNA was extracted from all serum samples with RNA extraction kit (NucleospinRNA, Macherey – Nagel, Germany). Extracted RNA was reverse transcribed and amplified with nested primers (11).

Primers used were as follows:

Ist Set Primers

F-5'ACTGTCTTCACGCAGAAAGCGTCTAGCCAT-3'

R-5'CGAGACCTCCCGGGGCACTCGCAAGCACCC-3'

2nd Set primers

F-5'ACGCAGAAAGCGTCTAGCCATGGCGTTAGT-3'

R-5'TCCCGGGGCACTCGCAAGCACCCTATCAGG-3'

The PCR products (5 µl) were subjected to electrophoresis in 3% agarose (Invitrogen, USA) and the band size was assessed by direct comparison with a 100-bp DNA marker (Takara, Japan). For each run of PCR an EC (Extraction control), PC (Positive Control) and NC (Negative Control) were used to rule out contamination.

Ethical approval. The study was approved by the Institutional Review committee of Government Medical College, Alleppey and informed consent was ob-

tained from patients.

Statistical analysis. Statistical Analysis was done using IBM SPSS 20 (SPSS Inc., Chicago, USA). For all the continuous variables the results are either given in mean ± standard deviation and for categorical variables as percentage. To compare the mean difference of numerical variable between HCV infected and Non Infected groups ,Mann Whitney U test was applied. To Test the Statistical Significant Association between Categorical Variables, chi square test applied. Probability value (p value) less than 0.05 was considered as statistically significant.

RESULTS

Out of the 100 patients ,77 were males and 23 were females. HCV antibody detection and HCV PCR was done in all the 100 patients. Among the 100 samples, only one was HCV antibody positive by ELISA. HCV RNA was detected in eight samples by PCR accounting for an overall prevalence of 8%. Sample which was positive by ELISA was found to be negative by PCR. Among the eight positive patients, five had history of blood transfusion and one was a post kidney transplant patient.

Analysis of risk factors in patients with HCV infection is shown in Table 1. Duration of dialysis and total serum bilirubin level was found to be more among the HCV positives than the HCV negative patients but there was no statistically significant association.

DISCUSSION

Lack of an effective vaccine and the increased risk of serious complications, have made prevention and early detection of HCV extremely important. One of the main concerns in HCV transmission is its potential for nosocomial spread. Stringent universal precautions in the dialysis units and availability of isolated area and separate dialysis machines for infected patients will lead to reduced cross-contamination and nosocomial infection among patients (12).

HCV prevalence among hemodialysis patients varies widely in different parts of the world. Studies have shown a prevalence in HCV of 8-36% in North America, 25-39% in South America, 1-36% in Europe, 17-51% in Asia, 1.2-10% in New Zealand and Australia and 7-85% in South Africa (13-18). Indian studies from 1990 to 2009 have shown HCV prevalence among hemodialysis patients varying from 12.1-46%. Chadha et al. reported 12.1% in 1993, Gosavi et al. reported 27.8% in 1997, Jasuja et al. reported 27.7% in 2009 and Chandra et al. reported a prevalence of 46% in 2004 (19-22). Studies in India in the last decade have shown a prevalence of HCV among haemodialysis patients ranging from 1.38-12.4%. Details are shown in Table 2.

Our study showed an overall prevalence of 8% by PCR. This is concordant with the studies conducted in the last decade showing prevalence ranging from 1.38-12.4% (23-26). Most of the Indian studies have relied on ELISA for detecting HCV. Data regarding prevalence of HCV by PCR is scanty. Reddy et al. and Medhi et al. used HCV core antigen ELISA for

Table 1. Comparison of risk factors in patients on hemodialysis with and without HCV infection

Variables	HCV Infected	Non -Infected	P- value
Gender, No. (%)			
Male	5 (62.5)	72 (78.3)	0.50
Female	3 (37.5)	20 (21.7)	
Blood Transfusion, No (%)			
Yes	5 (62.5)	43 (46.7)	0.47
No	3 (37.5)	49 (53.3)	
Kidney Transplantation, No (%)			
Yes	1 (12.5)	4 (4.3)	0.34
No-	7 (87.5)	88 (95.7)	
Age in Years, Mean ± SD	58.25 ± 16.35	52.52 ± 14.96	0.162
Duration of dialysis, Months, Mean ± SD	26.25 ± 12.57	22.08 ± 18.72	0.161
Total Serum Bilirubin mg/dl, Mean ± SD	1.0 ± 0.21	.80 ± .34	0.63

Table 2. HCV prevalence in various Indian studies

Author	Place	Year	Total No of patients	HCV Prevalence (%)	Method	Reference
Prakash et al.	Lucknow (North India)	2012	186	6.9	HCV ELISA and PCR	23
Kumar et al.	Coimbatore (South India)	2011	145	12.4	Enhanced Chemiluminescence	24
Jamil et al.	Meghalaya (East India)	2016	507	1.38	HCV ELISA	25
Subramanian et al.	Gujarat (Western India)	2016	910	2.7%	HCV ELISA	26

detecting HCV among hemodialysis patients and reported a prevalence of 13.23% and 17.2% respectively (27-28). The wide variation in the prevalence data is due to the sensitivity and specificity of the testing method. In the 1990s the studies were mainly based on first generation ELISA which had poor sensitivity and specificity. This was replaced by second and third generation ELISA for HCV with improved sensitivity and specificity. Third Generation ELISA has given excellent accuracy in other studies with 0-0.23% false-negative rates (29). Hinrichsen et al reported that seroconversion to HCV antibodies does not occur in all hemodialysis patients (30). Similarly Bukh et al. had reported the presence of HCV RNA in eight dialysis patients who were seronegative (31). In our study all the eight PCR positive samples were seronegative by third generation ELISA resulting in a high false negative rate for ELISA. The only sample which was positive for HCV antibody was found to be negative for HCV RNA by PCR. These findings highlight the need for HCV RNA PCR for detection of HCV.

In our study 62.5% of the HCV positives were males and the average age of the infected patients was 58.25 ± 16.35 years (Mean ± SD). This was concordant with the findings of Joukar et al. who reported that majority of the HCV positives were males and the majority of the positives were in the 50-70 year age group (32). Studies have shown that a previous history of blood transfusion increases the risk of acquiring HCV infection (33-34). In our study 62.5% of the HCV positives had a previous history of blood transfusion but there was no statistical significance. Similar findings were also reported by Kumar et al. (24). Most studies agree that the duration of dialysis is closely related to the development of HCV (35). In our study the duration of dialysis was more among the HCV infected group when compared to the HCV non infected group. Total serum bilirubin level was found to be normal in the HCV infected group and HCV non infected group. Among the 100 patients in our study, five had a history of kidney transplantation and one of the five patients was found to be positive for HCV RNA. However there was no statistical significance between HCV positivity and a history of kidney transplantation. Similar findings were also reported by Tajbakhsh et al. in Iran (36).

It is a well known fact that haemodialysis patients are at increased risk of developing Hepatitis C infection. Studies have shown evidence for the nosocomial transmission of Hepatitis C among haemodialysis patients (37). Prolonged vascular access, potential for exposure to infected patients and contaminated equipment are the other risk factors for acquiring HCV among HD patients. Outbreak investigations have revealed breaches in infection control mainly involving cleaning and disinfection of equipment and environmental surfaces, adherence to hand hygiene and use of gloves, and preparation and administration of medications as some of the factors to be associated with HCV transmission (38). Eventhough the practice of using dedicated machines or institution of isolation precautions for HCV-infected patients is an excellent initiative, if its done without correcting the underlying breaches in infection control then it would not effectively reduce transmission of HCV (39). In our study duration of dialysis was not an independent risk factor for developing HCV infection. Among the eight HCV positive patients, five had a history of blood transfusion. Eventhough there was no statistically significant association, HCV may have been acquired through blood transfusion. It is worth noting that in our hospital, although blood for transfusion is screened for HCV, only antibody testing by ELISA is done and absence of antibody cannot completely rule out HCV infection.

CONCLUSION

This is the first study from the southern state of Kerala in India showing the prevalence of HCV

among hemodialysis patients by PCR. All the eight PCR positive samples were negative by 3rd generation ELISA which is an alarming finding and further emphasizes the need to use PCR as a screening tool in this population. In our study there was no independent risk factor associated with development of HCV among HD patients. Haemodialysis patients should be routinely screened for HCV infection, preferably using PCR. More studies based on molecular testing are needed in India to show the true prevalence of HCV infection among haemodialysis patients. This study had a few limitations. PCR positive samples could not be send for sequencing and genotyping due to financial constraints.

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