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Detection of hepatitis C virus (HCV) RNA by PCR among hemodialysis patients attending a tertiary care centre, Mysuru, South India

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***Abstract*---**The infection rate of Hepatitis C virus in chronic hemodialysis (HD) patients is comparitively higher than the normal population. Hepatocellular carcinoma and cirrhosis are the major complications that have been frequently reported among the patients with HCV infection who were on hemodialysis. Hence, continuous monitoring of HCV infection in hemodialysis settings at health care centres may aim towards the assessment and prevention of device induced infection. Hence this current study was aimed to detect the HCV RNA using PCR among hemodialysis patients attending JSS, a tertiary care centre situated in Mysuru, South India.

***Keywords*---**HCV infection, hemodialysis, PCR.

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Introduction

Hepatitis C virus is the most common causative agent that causes chronic hepatitis, liver cirrhosis, hepatocellular carcinoma (HCC). The incidence rate of Hepatitis C virus globally accounts for about 3% and India accounts for about 12.5 million people. Dialysis patients are at high risk in obtaining HCV infection. One of the possible causes can be due to the cross contamination. The prevalence rate of HCV infection in dialysis patients ranges from 5 – 35% and is associated with mortality and morbidity significantly, but still remains an infection of public health concern[2-5]. Other causes for this prevalence rates may be due to poor infection control practices during the invasive procedure which can contribute to the risk in increase of seroconversion[6]. Long term complications such as cirrhosis and hepatocellular carcinoma have been frequently reported among the patients with HCV infection who were on hemodialysis. Hence, continuous monitoring of HCV infection in hemodialysis settings at health care centres may aim towards the assessment and prevention of device induced infection. At present, anti-HCV test is commonly used screening assay[7], but antibodies against HCV cannot be detected. The virus cannot be detected in first 3-4 weeks of post infection[8-10]. Anti-HCV test positive does not assure the viremia and PCR assay plays a major role in detection of HCV infection [11,12]. Polymerase chain reaction helps in early detection and plays a crucial role in outbreak investigation. Hence this study was aimed to detect the HCV RNA using PCR in comparison with CLIA among the patients undergoing hemodialysis in JSS, a tertiary care centre situated in Mysuru, South India.

Patients and Methods

This laboratory based prospective study included a total of 100 adult patients who were on hemodialysis. Of these 100 patients, 76% were males and 24% were females, with the mean age 52.75 ± 15 years. All the patients included in the study corresponds to chronic hemodialysis patients at our centre who were being dialyzed atleast 2 times a week. The dialyzer membranes used were disposable and single used. The duration of hemodialysis was more than 30 months in majority of patients. History of blood transfusion, hypertension, diabetes, chronic kidney disorder, liver function and renal function parameters of the HCV positive patients were studied (table 1).

Anti HCV antibodies was determined using CLIA (Chemiluminisence immune assay) and HCV viral RNA was detected using Reverse transcriptase Real time PCR (FluoroAMP® Hepatitis C Virus RNA Real Time PCR Kit). Briefly, 2ml of venous blood sample was collected in vacutainer from the included patients and serum was separated and stored at -20^{o}C until RNA extraction. RNA extraction was done from collected serum samples by using standard protocol. The extracted isolates was subjected to Real Time Polymerase Chain Reaction (RT PCR) for qualitative detection of HCV RNA according to the manufacturers protocol. Through the research on reference values, the lower detection limit of this diagnostic kit is determined to be 25 IU/mL, and the Ct reference value of internal control is determined to be 38.

Statistical Analysis

Statistical analysis was performed using open epi software for windows. Data was expressed as mean ± standard deviation or frequencies (%) as appropriate. If the P value is less than 0.05 was considered as statistically significant.

Ethical Considerations

Ethical clearance certificate was taken from the (Institutional ethics review board) IRB, JSSAHER, Mysuru before initiation of the study.

Results

A total of 100 serum samples were subjected for screening of anti-HCV by CLIA and the same samples were further processed for RNA extraction for detection of HCV using Real time PCR. Of 100 samples that were screened using CLIA 4(4%) were positive & 96(96%) were negative (Table 3) and 7 (7%) detected the presence of HCV RNA & 93(93%) did not detect HCV RNA through Real time PCR (Table 2 & 3).

Discussion

Hepatitis C viral infection is a growing health issue in public. Worldwide, HCV infects approximately 180 million people[13]. The incidence rate of HCV infection in chronic dialysis patients is more than that of the common population. The rate of occurrence of HCV infection in hemodialysis patients is 7.8% in the United States[14], 5.2% in Germany[15], 10% in Japan and 5.9% in India[16]. The HCV is the common cause of parenteral hepatitis. In many parts of the world, it is a major cause of chronic hepatitis and primary hepatocellular carcinoma. Across Asia and Africa's developing countries, while infection with hepatitis B virus (HBV) is the most common cause of chronic liver disease, HCV is increasingly emerging in these populations as an equally significant infection.[17]

In this current study, of 100 cases 76(76%) of them are male & 24(24%) are female. Maximum number of patients were from the age group 46-55 years(32 patients) with the mean age of 52.75.This is comparable with study conducted by Maryam Moini , MazyarZiyaeyan*et al(2013)* from Iran showed the mean age of patients is 52.86[18]. The HCV infection is significantly higher in hemodialysis patients when compared with general public, so it is recommended to screen HCV infection in hemodialysis patients. Anti-HCV assay are widely used method for HCV detection though it does not help in true status. The utility of antibody detection assays are simple, cost effective, and improve the specificity and sensitivity of the assay. Anti-HCV test and the limitations of this assay inefficient to distingush between the current active infection and previous infection. Molecular methods such as polymerase chain reaction assay is more efficient than anti-HCV for detection of HCV virus. Hepatitis C virus can detect in human serum within 2 weeks of infection. The virus will disappears in recovering patients and persists in chronic patients in this cases HCV antibody testing and PCR was recommended for detection of virus. Molecular methods has its own drawbacks due to cost effect and needs experts to perform this assays[19].In our study, Among

100 samples 7(7%) were positive for HCV RNA by PCR method. This is concordance to the study conducted by Maryam Moini, MazyarZiyaeyan*et al(2013)*from Iran showed 6% positivity. Another study conducted by AtiehMakhlough and MohammadrezaMahdavi, *et al (2017) from* Iran showed 6.8% positivity[18,20]. The rate occurrence of HCV infection in this present study was compared to many other studies due to several elements which includes blood unit HCV screening, erythropoietin prescription for the treatment of anemia in patients undergoing hemodialysis, use of a dedicated hemodialysis system, improvement of hygiene in hemodialysis units such as dialysis of HCV-positive patients in separate wards and improvement of HCV awareness in medical professionals[19].In the present analysis, in HCV positive and negative cases, the values of, ALT, and ALP were insignificantly different, but AST values were substantially different between the 2 classes, which indicated that in HCV positive cases liver enzymes were significantly higher. Most newly diagnosed individuals with HCV (60%-70%) are asymptomatic. Later, HCV infection continues to become a chronic infection in around 75 percent of cases; eventually, chronic HCV infection leads to progressive liver diseases such as end-stage liver disease, cirrhosis or liver cancer in 15 percent-25 percent of patients; but more frequently, they have indolent coarsity. In addition, the liver enzyme values in patients undergoing hemodialysis have lower specificity than normal individuals; and the serum values of liver enzymes have not been directly correlated with the degree of liver damage and HCV titer[20]. History of the positive cases who detected the presence of HCV RNA through PCR was obtained from the hospital information system & existing medical records 80% of the cases had the history of blood transfusion so the possible positivity for HCV among these patients might be blood transfusion. None of our subjects had the history of renal transplantation. Co-morbidities among the 7 positive cases were further assessed, of which 5 subjects have hypertension, 4 subjects have diabetes and 5 subjects have CKD & 4 subject have hypertension, diabetes and also CKD. In current the study, only 2 cases are positive for anti-HCV & HCV RNA. 2 cases are PCR negative but positive for anti- HCV. Special attention should be paid to anti-HCV positive & HCV negative RNA. Their results may be associated with fluctuating viremia, prior HCV exposure, or may be biologically false positive. The negative results of triple HCV RNA for the patient's plasma exclude the first possibility. While they do not bear an epidemiological risk, they are unprotected.They might also be accounted for by an earlier, spontaneously resolving acute hepatitis C infection. However, recovery of patients on dialysis following acute hepatitis C is very rare compared with the general population. Specific antibodies can disappear after virus clear and thus survive for many years, and could be found very rarely at low concentration. 5 cases are positive for PCR but negative for anti-HCV. It can either be attributed to a gap between the infection and the seroconversion or to the immunocompromised status of the patient or might be an evidence of acute HCV Hepatitis infection [20,21].

Conclusion

Hepatitis C virus transmits easily from one person to another; early detection of virus plays a vital role in prevention of cross-infections in haemodialysis settings and helps in proper diagnosis and treatment. PCR was found to be promising

method in early detection of HCV RNA amongst the patients undergoing haemodialysis.

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Conflicts of interest

Authors report no potential conflicts of interest relevant to this article.

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Table 1: Co-morbidities studied among HCV positive patients

SAMPLES	HYPERTENSION	DIABETES	CHRONIC KIDNEY DISEASE (CKD)
1	NO	NO	NO
2	NO	NO	NO
3	YES	NO	YES
4	YES	YES	YES
5	YES	YES	YES
6	YES	YES	YES
7	YES	YES	YES

Table 2: Results of Anti HCV through CLIA

	FREQUENCY	PERCENTAGE
POSITIVE	4	4%
NEGATIVE	96	96%
TOTAL	100	100%

Table 3: Results of HCV RNA PCR

	FREQUENCY	PERCENTAGE
POSITIVE	7	7%
NEGATIVE	93	93%
TOTAL	100	100%

Conflict Of Interest: All authors report no conflict of interests relevant to this article.

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