Detection and Genotyping of Hepatitis C Virus Among Dialysis Patients with Chronic Kidney Disease in A Tertiary Care Teaching Hopsital

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Abstract

Background: Hepatitis C virus (HCV) infection in patients undergoing dialysis is an emerging major condition globally. This parenterally transmitted infection lead to persistent infection, progressive liver disease, cirrhosis, hepatocellular carcinoma. The present study was undertaken to determine the prevalence of HCV infection and genotypes prevalent in these patients.

Materials and Methods: A total of 100 chronic kidney disease patients (CKD) on dialysis were studied. All the patients were tested for anti-HCV antibodies by immunochromatography (ICT), Enzyme linked immunosorbent assay (ELISA) and HCV-RNA by Real-time Polymerase Chain Reaction (RT-PCR) and efficacy of tests were evaluated. Detailed history regarding demography, duration of dialysis, number of dialysis centres, blood transfusion were collected.

Results: The overall HCV prevalence was 9 % with genotype 1 most prevalent in these patients. Total of 9 patients were HCV positive. 6 (67%) were positive for both RT-PCRand ELISA. One patient (11%) was positive for HCV by RT- PCR and negative by ELISA while two (22%) patients were negative for RT- PCR and positive for ELISA.

Conclusion: PCR is accredited as a specific, reliable method recommended for establishing exact, final diagnosis of these patients. Third generation ELISA are easy to use, cost-effective and having low variability. Best way is to combine both Immunoenzymatic and molecular method for detecting HCV and preventing further HCV transmission in these patients. Longer duration of dialysis and multiple dialysis centres visit were the major risk factors for HCV positivity. Adopting strict universal precautions are essential in reducing HCV transmission.

Keywords: HCV, Dialysis, CKD, ICT, ELISA, PCR

INTRODUCTION

Hepatitis C virus (HCV) infection is an important emerging public health issue. HCV is more commonly associated with chronic active hepatitis [1]. But acute stage of the disease remains unnoticed due to the paucity of symptoms. It follows a variable course with some patients developing fibrosis, cirrhosis and hepato-cellular carcinoma while others having minimal or no significant liver disease[2]. Main sources of HCV infection include injection drug abuse, chronic dialysis, organ transplantation, blood product transfusion, occupational exposure, unprotected sexual contact and vertical transmission[3].

In dialysis patients, HCV infection is common. This is one of the most important cause of liver disease in patients on renal replacement therapy. Chronic kidney disease (CKD) is found in approximately 10 % of general population. Dialysis is commonly used as renal replacement therapy for end stage renal disease (ESRD) patients. Dialysis patients have a considerably increased HCV prevalence compared to general population. This is due to prolonged vascular access,

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frequent hospitalization and blood transfusion, contaminated dialysis equipment and their co-morbid condition. In patients on dialysis the HCV prevalence varies between 1% to 85 % globally[12] and 3% -45% in India [4].

Dialysis duration, frequency of blood transfusions and interpersonal horizontal route of transmission are contributing factors for HCV infection in dialysis patients. Higher prevalence is seen in haemo-dialysis (HD) patients compared to peritoneal dialysis (PD) patients. The reasons include domiciliary location of therapy, no vascular access and lesser requirement of blood transfusions in peritoneal dialysis.

The diagnosis of HCV infection is based mainly on two categories of laboratory tests. They include serologic assays to detect HCV antibodies and tests to quantify HCV RNA. Both assays are done to minimize the false- positive and false- negative results. The most sensitive tool to detect HCV infection is antibody detection test. HCV-RNA detection by Polymerase Chain Reaction (PCR) is immensely accepted as a gold standard procedure for the diagnosis of current HCV infection. Designing the therapeutic strategies depends on both genotyping and viral load assessment. Genotyping of HCV is a strong predictor of response to anti- HCV treatment. So, there is a need not only to find out HCV prevalence but also to find out the genotypes of the virus prevalent in dialysis patients.

Hence primary objective of our study is to find out the prevalence of hepatitis C virus infection in dialysis patients, to evaluate the rapid immunochromatographic (ICT) assay with Enzyme linked immunosorbent assay (ELISA) in anti – HCV antibody detection, to detect HCV –RNA by quantitative PCR and evaluate ELISA in comparison to PCR.

MATERIALS AND METHODS

The present study was conducted at the Microbiology department, Tirunelveli Medical College, Tirunelveli for period of eight months to detect the prevalence of anti –HCV antibody by rapid immunochromatographic test [ICT] , Enzyme linked immunosorbent assay[ELISA] and also to detect HCV -RNA by Real time Polymerase chain reaction [RT-PCR] among Chronic kidney disease (CKD) patients on dialysis. A total of 100 chronic kidney disease individuals on dialysis were subjected here as study group, all cases were recruited through geriatric department.

In this study patients with age more than 12 years, Patients with chronic kidney disease on dialysis for more than three months were included and children less than 12 years of age, acute kidney injury individuals were excluded. Ethical committee clearance was obtained from the college ethical committee before the commencement of the study. Informed consent was obtained from reliable informants of patients and patients who participated in the study.

A filled up proforma regarding patients name, age, sex, area, details of any co- morbid condition, History of previous hospitalization for surgery, dental procedures, any injections, any drug abuse history, acupuncture, tattooing, history of past blood transfusions, history of anemia, sharing of needles, razors, brushes, history of jaundice, history of spouse HCV, organ transplant were collected .Regarding dialysis, type of dialysis, hemodialysis –number, duration, frequency, nature, A-V fistula made, re-usage of dialyzer, previously visited dialysis center and all these details were collected.

A sum of 100 non-repetitive serum samples were obtained from the study group. Around 4 ml of venous blood sample was collected from study cases under aseptic precautions in labelled disposable tubes. Samples were immediately tested for HCV antibody using rapid immunochromatographic method and later stored for ELISA, PCR and genotyping at -20 °C in a deep freezer until testing in separate aliquots. All 100 samples were assayed for parameters of HCV with rapid immunochromatography (ICT), ELISA (anti-HCV) and PCR (HCV-RNA). All the 100 samples were tested by rapid immunochromatography card test (SD BIOLINE anti-HCV antibody).

Hepatitis C virus real-time PCR kit constitutes a ready to-use system for the detection of HCV genotypes using polymerase chain reaction (PCR). It contains reagents and enzymes for the specific amplification of 185bp region of the HCV genome, and for the direct detection of the specific amplicon in fluorescence FAM channel. All genotypes are FAM labeled and external positive controls (HCV – QS1) are supplied to assist the reaction.

All the results obtained from the study were analysed statistically for their completeness, consistency and accuracy by the parameters like mean and percentages. Kappa value was calculated to measure the degree of agreement between three diagnostic methods. The results of rapid ICT, ELISA and RT-PCR were compared by Mcnemar's χ^2 test and confirmed by 'Z' test of proportions. The above statistical procedures were performed by IBM SPSS Statistics 20. Significant probability value of less than 0.05 was important statistically.

RESULTS

The selected 100 study subjects were analysed based on age and sex among which out of 100 patients, 65 were males. Of this, 3 (5%) were in the age group of less than 20 years,9 (14%) were in third decade and 26(40%) were in fourth decade and 20 (30%) ,4(6%) ,3(5%) were in fifth, sixth, seventh decade respectively. Remaining 35 were females. Of this 3(8%) were in the age group of less than20 years and 7 (20%) were of third decade and

8(23%),8(23%),7(20%),2(6%) were in the fourth, fifth, sixth, seventh decade respectively.

Out of 100 cases,51 % cases were hypertensive (HTN)and 24% were diabetic (DM). These are followed by 13 % cases constituting Chronic glomerulonephritis (CGN) and then by congenital and obstructive causes and other unknown causes (7 %). Least number of cases(5%) leading to CKD were due to Chronic interstitial nephritis(CIN) as per the present study.

Out of 100 cases studied,57 % of cases were undergoing hemodialysis only and 30% of cases underwent peritoneal dialysis initially followed by hemodialysis and 13 % cases were on peritoneal dialysis alone.

Coming to main part of study all the 100 samples were tested by three diagnostic tests-rapid ICT, ELISA and Real-time PCR for Hepatitis C infection (anti-HCV, HCV RNA). Out of this ELISA was positive for 8% of samples while 7% were positive for ICT. Real time PCR also showed 7% positivity.

Table :1 Correlation between rapid ICT and ELISA in HCV antibody detection

ICT	ELISA		TOTAL
	Positive	Negative	
Positive	7	0	7
Negative	1	92	93
Total	8	92	100

The rapid immunochromatographic card test was evaluated for its sensitivity and specificity against ELISA ,a reference test , From the above table, sensitivity of rapid ICT was 87.5% when evaluated against ELISA, a reference test. Specificity was 100 % compared to ELISA and positive predictive value of ICT was also 100%. Negative predictive value of ICT was 98.92%. The kappa value measuring the degree of agreement between ICT and Elisa was 0.928.

Table 2: Evaluation of ICT with PCR as reference

ICT	PCR		TOTAL
	Positive	Negative	
Positive	5	2	9
Negative	2	91	93
Total	7	93	100

The above table showed that only 5 cases were positive for both PCR and ICT. 2 cases were positive for ICT only. Another 2 cases were positive for PCR alone .91 cases were negative for both ICT and PCR. Detection of anti-HCV positive cases by rapid immunochromatographic card test was evaluated for its sensitivity and specificity against PCR,a gold standard test. With sensitivity of 71.4%, specificity = $= 91/93 \times 100 = 97.8\%$, Positive predictive value o f 71.4% and negative predictive value of 97.8%. The kappa value denoting the degree of agreement between ICT and PCR was 0.693.

Table 3: Evaluation of ELISA with PCR as reference

ELISA	PCR		TOTAL
	Positive	Negative	
Positive	6	2	8
Negative	1	91	92
Total	7	93	100

Finally, we analysed the detection of HCV positive cases by Elisa test was evaluated for its sensitivity and specificity against PCR as gold standard test. The sensitivity was 85.7%, Specificity was 97.5%, Positive predictive value was 75%, negative predictive value was 98.9%. The kappa value measuring the degree of agreement between Elisa and PCR was 0.794.

The above table showed the presence of both ELISA and RT-PCR positive in 6 % of cases. ELISA positive and RT-PCR negative (Anti-HCV positive and HCV RNA negative) were reported in 2 % of cases. ELISA negative and RT-PCR positive was seen in 1 % of total cases. The net number of infected persons was 9(9 %). Therefore, the overall prevalence rate was 9 % in this study. Both ELISA and RT-PCR were negative (anti-HCV and HCV RNA negative) in 91% cases of study group.

In our study majority of positive cases were in the fourth decade. Total of 9 positive samples tested by Elisa and PCR, 4 (44%) were in the age group of 31-40 years. This is followed by a incidence little more in third decade. The mean age among positive cases was 30.9 years. Sex wise distribution showed that out of total 9 positive cases ,6(67%) was male and 3(33%) were females. Male to female ratio among positive cases 2:1.

Among HCV infected cases, about 67% were under HD group and 33% were from peritoneal dialysis initially followed by hemodialysis. None of the cases were infected from peritoneal dialysis alone in infected group.P value was derived and significant statistically representing hemodialysis patients have higher positivity rate.

Around 7 (77%) cases among HCV positive group had duration of dialysis of more than 20 months. Among HCV noninfected cases only 12% had longer duration of dialysis. The mean average duration of dialysis in infected cases was about 23 months. The mean duration of dialysis in non-infected cases was 11 months. The difference between them was 12 months. Association of duration of dialysis and HCV positivity had significance statistically (P<0.05) by students t test.

Association of blood transfusion in HCV positive and HCV negative cases. Among positive group,1 (11%) case had blood transfusion more than 6 times ,2 cases (22%) had transfusion history of 3-6 times, one (11%) case had transfusion of less than 3 times and 5 cases (55%) never had transfusion. Among negative group,23(26%) cases had multiple blood transfusion of more than 3 times. One case had transfusion of more than 6 times .67 cases (74%) never had transfusion.

Table 4: Distribution of genotypes in PCR positive cases

GENOTYPES	PCR POSITIVE (n=7)	PERCENTAGE
Type 1	5	72
Mixed(1and 2)	1	14
Untypable	1	14

The above table:4 showed the HCV genotypes prevalence in the positive cases.Five cases (72%)had type 1 ,one case(14%) had mixed genotype of 1 and 2.One case(14%) had untypable genotype .None of the cases had genotypes 3,4,5 or 6 in the current study.

DISCUSSION

HCV is linked to CKD in several ways –some forms of renal disease are precipitated by HCV and ESRD patients are at rising risk of HCV acquisition. Nowadays, the spread of HCV among dialysis patients is declining but its prevalence remains high mainly in developing countries. This study documented the HCV prevalence among CKD patients. With this background, this study entails in demonstrating the efficiency of ICT, ELISA in comparison with Real time PCR in detection of HCV. This is one of the first study in identifying the HCV genotypes prevalent in this area and may act as an aid in designing therapeutic strategies in order to cure liver damage and reduce extra- hepatic complications of HCV, including progression of CKD to late stages in near future.

In the present study of 100 chronic kidney disease patients on dialysis, males were predominant than females in the ratio of 1.85:1. .In a similar study conducted in Calicut by Shabana Razmin et al [5] revealed around 83% of males and 17% of females among dialysis group . Male predominance was mainly due to their exposure to various risk factors like tattooing, blood donations, community participation.

In this study regarding the age of patients undergoing renal replacement therapy, majority (63%) of the patients were in the age group of 31-40 yrs of age. The mean age in the present study was 39.4 years. Similar findings were observed in studies done by Murthy et al[6] in Vellore and by Razmin et al[5] reported mean age of study group around 34 years and 42 years respectively and most affected age group 31-50 years.

In the present study with 100 CKD patients, the total infected cases were 9 and all were on hemodialysis. None of the cases had HCV infection in patients with peritoneal dialysis alone. Similar results showing higher prevalence of HCV in hemodialysis (HD) patients compared to peritoneal dialysis (PD) reported by Conway et al[8]. In contrast Huang et al[7] had reported equal prevalence of HCV infection at about 10%-15% in Taiwan for both HD and PD patients. The reason for higher prevalence in HD is mainly due to prolonged vascular access, increased frequency of blood transfusions and hospitalization, multiple invasive procedures, nosocomial transmission via contaminated dialysis equipments and their co-morbid condition. Lower prevalence of HCV in PD patients is mainly due to lower requirement of blood transfusion, absence of a vascular access site, domociliary location of therapy, less number of visits to hospital.

In the current study, hypertension (HTN) accounted for half of the cases in study group (51%) followed by Diabetes mellietus (DM) 24% and chronic glomerulonephritis (13%). Other causes include congenital conditions like (vesicoureteric reflex, posterior urethral valve), obstructive causes (calculi, tumour causing hydro uretero nephrosis) and chronic interstitial nephritis. In a Taiwanese study by Lee et al reported, Diabetes mellietus is the primary cause contributing to end stage renal disease ESRD[9]. Hypertension and CKD relationship is cyclical. Elevated blood pressure (BP)cause damage to the blood vessels within the kidney leading to impairment infiltration of fluid and waste which in turn leads to increased fluid volume in the blood causing increased BP.

Comparison of rapid ICT, ELISA and RT-PCR in detection of HCV:

The positivity rate of rapid ICT was 7% and that of ELISA was 8%. The positivity rate of RT-PCR was 7%. Over all positivity rate and prevalence of HCV

infection was 9 % in the current study. Similar results were obtained by Kanagapriya et al[10] in which seroprevalence was5.8% in Tirunelveli in 2011 out of 121 patients on HD. In contrast very high prevalence of 34.6% and 45% was reported from an Jordanian and Syrian study done by Salma Bdour et al[11] and Abdul Karim et al respectively[12]. Geographical location, socio economic factors and health care procedure related to adoption of universal precaution policy, dialysis unit hygiene, isolation of HD machine, proper sterilization of equipment's and dialyser reuse are the factors which influence the prevalence of HCV. Several studies reported HCV RNA positive with anti-HCV negativity among dialysis patients varies from 0%-12%.

There was some variation between serologic assay and virologic assay in the present study also. About 1 % of patients were anti-HCV negative but had HCV RNA detected by RT-PCR. The reason was mainly due to the fact that these uremic patients are in partial state of immunosuppression and failed to mount an efficient antibody mediated immune response to viral antigen and dysfunction in cell mediated immunity. Also the expression of antigen and antibody in these immunocompromised patients was very inconsistent, irregular and fluctuating.

A study by Pawlotsky et al[13] reported the HCV RNA positivity among anti-HCV negative cases in dialysis population and they concluded that the window period was longer in these patients. In contrast to present study, Zero false negative rate for serology was reported by Garinis et al[14]. In the present study, out of total positive of 9 cases,2 cases were positive for anti-HCV serology by ELISA but negative for HCV RNA detected by Real time –PCR. The possible explanations related to this may be either due to past resolved infection or patients may be in the state of intermittent viraemia or occult infection. In contrast to the current study,Garinis et al reported that the anti-HCV positivity correlated with that of HCV RNA by RT-PCR in a Greece study in 1999[14].

In the current study, the sensitivity of third generation Elisa was 85.7% when evaluated against RT-PCR, a reference test. Specificity of Elisa was 97.85%compared to RT-PCR. The positive predictive value and negative predictive value were 75% and 98.9% respectively. Similar report was given by Prakash et al [15] in Lucknow in 2014 where the

sensitivity and specificity of Elisa was 80% and 97.78% whereas the positive and negative predictive values were 76.19 %and 98.21% respectively. The kappa value agreement between ELISA and PCR was 79% in this study. Similar results were obtained by a Moreira et al in which the kappa value agreement between them was 71% [16].ELISA is used as a screening test for serological detection of anti-HCV for its sensitivity and ease of performance. But in dialysis patients, due to poor immunological reactivity, adequate antibodies are not formed. This leads to false negative ELISA and hence there is a need for molecular detection methods.

Evaluation of ICT with RT-PCR and ELISA:

In the present study, sensitivity of rapid ICT was 71%, specificity 98 %, positive predictive value 71% and negative predictive value 98% in comparison with RT-PCR, a gold standard test. A study done by Ali et al reported 100% concordance results among dialysis group patients tested by rapid ICT and RT-PCR test. This is in contrast to our study. A study from Lahore in 2010 by Khan et al [17] reported low senstitivity of rapid ICT test. The sensitivity and negative predictive values were very low and were 66% and 43% respectively. In the present study, there was only a small difference between ICT and Elisa test results, that of 7% and 8% positivity respectively.

Similar to present study, a study demonstrated small differences between ICT and Elisa technique results for anti-HCV (1% and 3% positivity respectively) by Tajeldin et al[18] in 2015. ICT is a rapid test with low sensitivity. It can be used in low resource setting as a screening tool. But needs further confirmation with ELISA or RIBA or molecular methods.

In the present study, males constitute most of the positive cases compared to females in the ratio of 2:1. Similar findings was studied by Nayle Maria Oliveria da Silva et al[19] in Southern Brazil that males 63% were infected with HCV in comparison to females. This study was in similar agreement to the fact that male population is affected more in comparison to females. This is contributed mainly by exposure status to varying risk factors due to their life style and due to their cultural attributes. Also, one study reports the clearance of HCV virus in females more due to the effect of estrogen hormones. Regarding age in this study, half of the infected case belongs to 31–40-year

age group. The mean age of males in the present study was 39.4 years and that of females same of 39.4 years. Over all mean age was 39.4 years. Similar results were obtained by Ummate et al[133] in Nigeria in2014 reported the mean age of study group was 39.9 years. *B*haumik et al [20]reported the age group between 40-60 years is significant among both study and infected cases group in 2012 in Tripura. In contrast to present study, a study by Prasad KP Babu et al reported the higher prevalence of HCV in the age group of 60 -70 years among dialysis patients[21].

In the current study, HCV infected cases had longer duration of dialysis treatment compared to non-infected cases. The mean duration of dialysis of infected cases was about 23 months. 66 % of infected cases had dialysis duration of 21 -30 months. Similar results were obtained by Chawla et al[22] in which duration of dialysis was reported as one of the major contributing factor HCV infections. In a study by Chawla the mean duration of dialysis was 8 months in HCV positive cases.

The present study also reported that increased number of hemodialysis sessions was significantly associated with increased HCV positivity.67 % of the HCV positive patients in this study had number of dialysis sessions above 110.The mean number of sessions among positive cases was 122. A study by Khasia Anwar et al in 2016 reported the significant association of frequency of dialysis and increased number of sessions in relation to HCV positivity[23].

In the current study, out of 9 infected cases, one case had multiple transfusion of more than six times. 2 more had blood transfusions three to six times and one case had transfusion less than 3 times and 5 positive cases never had a transfusion. Das et al [24] in 2015 from Ludhiana reported that there is no significant association between HCV reactivity and blood transfusion. This study was similar to present study.

Among the infected, one case had renal transplantation done Similarly among HCV negative cases, one case had undergone renal transplantation. There is no significant correlation between organ transplantation and HCV positivity. Similar result was obtained by Hegde et al in Mangalore who found out insignificant association between HCV positivity and organ transplantation in contrast to this study

Genotypes prevalent in PCR positive cases

In the present study, most prevalent genotype is type 1 accounting for 72% cases.14% had mixed genotypes (type 1,2) and another 14 % had untypable genotypes. Similar results were observed in a Japanian study[26] in which genotype 1 was the most prevalent (74 %) among dialysis population.[1,2] Mixed genotype accounted for 5 cases (12%) out of 40 HCV RNA postitive cases in a study by Hairul Aini et al in 2012 [27] stated that all chronic dialysis patients had an equal chance of getting single or mixed genotype infection. No risk factors were associated with mixed genotype in the study. Mixed genotypes were prevalent in 5.5% cases in a Pakistanian study by Sadia Butt et al.[28]

CONCLUSION

The current study determines HCV infection prevalence in chronic kidney disease patients on dialysis. Rapid Immuno chromatographic test is easy to perform and interpret in low resource settings. This test is less sensitive and needs further confirmation. ELISA test is considered as a diagnostic tool for anti-HCV detection because of its ease of use, cost effectiveness and low variability. False negative ELISA is due to poor immunological reactivity in these patients. PCR is the "gold standard" for RNA detection of Hepatitis C in patients undergoing dialysis. But PCR may be negative in ELISA positive cases due to fluctuating viraemia.

The best way for screening dialysis patients is to combine immune-enzymatic method and molecular method for synergistic effect in detecting HCV and this further helps in preventing HCV transmission. Stringent adherence to universal precautions, isolation of hemodialysis machines, ideal nursing practices and proper sterilization of equipments and periodical viral parameters monitoring are essential in reducing HCV transmission. Genotyping of patients on dialysis may be helpful in formulating prevention and treatment strategies.

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