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Original Article

Prevalence and genotyping pattern of hepatitis C virus among patients on maintenance hemodialysis at five centers in Pune, India

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ABSTRACT

Background: Worldwide prevalence of Hepatitis C virus (HCV) infection hemodialysis (HD) ranges from 1 to 84.6% with serious complications. Assessment of prevalence, risk factors, and genotyping of HCV infection in patient on HD was carried out at Pune, India.

Methods: A total of 250 patients on HD from five HD centers were recruited and tested for anti-HCV antibody using third-generation enzyme-linked immunosorbent assay (ELISA). Qualitative HCV RNA detection was carried out by nested reverse transcriptase polymerase chain reaction (RT-PCR). Genotyping and sequencing were carried out using the BigDye Terminator cycle sequencing ready reaction kit.

Results: Mean age of patients was 47.3 years. Forty-seven cases out of a total of 250 were reactor for HCV antibody. Overall prevalence rate was 18.8% ranging from 6.7% to 35.6% in the five centers. Of total, 44.1% of females and 13.5% of males were HCV infected. The mean duration of HD in HCV-infected patients was 6.03 years. Prevalence was higher in patients aged > 5 years on HD with higher number of blood transfusions. Thirty-six cases were positive for HCV RNA. Only one HCV RNA was detected among the 203 anti-HCV negative samples. Discordance between antibody and HCV RNA positivity was noted. Seventeen infected cases had changed dialysis centers four times. Thirteen cases were HBsAg positive, of which six cases were coinfecting with HCV. Thirty-seven samples were genotyped. **Conclusion:** The predominant genotype was 1a (54.1%) followed by 1b (43.2%) and 3a (2.7%). Highest prevalence of HCV (35.6%) and intracenter PNI of 99.3% of genotype 1b (84.6%) in center 3 indicates a possible nosocomial transmission.

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Introduction

Hepatitis C virus (HCV) infection has an approximate prevalence of about 3% globally, i.e., 180 million people. In India, 12.5 million people are HCV infected. Patients on hemodialysis (HD) are at an increased risk for acquiring HCV infection due to cross contamination in dialysis units. Prevalence of HCV Infection among patients undergoing HD has been reported to be significantly higher. Worldwide data indicate a prevalence rate of 1–84.6% in HD centers in different countries, and in developed countries, this prevalence ranges from 5 to 35%. Significant morbidity and mortality among such patients remain a matter of great public health concern.^{1–4} Since long-term complications such as cirrhosis and hepatocellular carcinoma are reported more frequently in HCV-infected HD patients, monitoring of HCV infection assumes importance in a HD setting aimed toward profiling, assessment, and prevention of device-induced infection.

The heterogenic nature of the RNA genome of HCV is a well-recognized entity. Accordingly, HCV has been classified into six major genotypes and more than 100 subtypes worldwide. HCV genotyping and viral load assay is an important tool in the study of epidemiology, pathogenesis, and therapeutic response to antiviral medication.^{3,5,6}

We undertook this study to evaluate the prevalence of HCV infection in patients on maintenance HD, to assess different risk factors to HCV infection, and to determine circulating HCV genotypes in five HD centers at Pune, in the western region of India.

Material and methods

Patients

Two hundred fifty patients on maintenance HD were screened from five HD centers in Pune, India, that included two government and three private hospitals, for a period of one year from February 2014. Informed consent from patients and ethical clearance was obtained from institutional ethical committee. Demographic data such as age, sex, marital status, contact details, and history of travel and relocation were recorded. Among clinical parameters, history of fever, jaundice, organomegaly, incidence of complications, changes in treatment centers, and so forth were sought. Previous laboratory reports with special reference to hepatitis workup, HCV serology, HCV RNA estimation, and genotyping were recorded. To assess different risk factors, we collated and analyzed data to include total duration and number of HD cycles, HCV infection status, total number of transfusions, use of dedicated HD machines, and change in HD centers for HCV-infected patients. Clinical profile of the patients on HD included chronic glomerulonephritis, chronic pyelonephritis, diabetic and hypertensive nephropathy, and malignancy. Five milliliters of blood was collected aseptically in two vacutainers containing clot activator (Becton Dickinson, USA). Serum samples were stored at –70°C in aliquots. Anti-HCV antibody detection was carried out in duplicate using third-generation enzyme immunoassay (SD HCV, Standard Diagnostics Inc).

Testing for Hepatitis B surface Antigen (HBsAg) to detect hepatitis B infection was carried out using sandwich enzyme-linked immunosorbent assay (ELISA) (HEPALISA, J. Mitra & Co. Pvt. Ltd, India).

HCV RNA detections and genotype analysis

A two-step nested reverse transcriptase polymerase chain reactions (RT-PCRs) was carried out on 47 anti-HCV antibody-reactive samples for the qualitative detection of HCV RNA. Briefly, RNA extraction was carried out using QIAamp (QIAGEN) viral RNA mini kit. Nested RT-PCR (Thermal cycler Applied Biosystems 9700) was carried out with primers targeting the core region (405 nucleotides), namely, external forward CC1: 5'ACTGCCTGATAGGGTGCTTGC3'; external reverse CC2: 5'ATG TAC CCC ATG AGG TCG GC3'; internal forward CC3: 5'AGG TCT CGT AGA CCG TGC A3'; internal reverse CC4: 5'CAC GTT AGG GTA TCG ATG AC3' as described previously.^{7,8} PCR products were separated on 2% agarose gel, and 405-bp-sized bands were considered positive for HCV (Fig. 1). Appropriate internal controls were used to rule out inhibitory activities during amplification. PCR products were purified with a gel extraction kit (QIAGEN, Valencia, California). Cycle sequencing was carried out on purified templates using the BigDye Terminator cycle sequencing ready reaction kit (Applied Biosystems). This was followed by analysis on an automated sequencer (ABI PRISM 310 genetic analyzer; Applied Biosystems).

HCV RNA detection was also performed for the 203 anti-HCV-negative samples. Owing to paucity of resources, mini-pool nucleic acid testing (NAT) was used. For that, samples were pooled (25 pools of 8 samples each) and tested. Only one pool of the samples tested positive. RNA detection was carried out separately for all 8 samples of this pool, and a single sample was detected positive. This sample was included in the HCV RNA-positive group for sequencing and further analysis. Representative HCV sequences were retrieved from NCBI database and selected for comparison with HCV sequences from this study. The phylogenetic status was assessed using the software MEGA 5.⁹ Jukes-Cantor distance was utilized using the neighbor joining algorithm for the analysis in MEGA.

Unpaired Student's t-test was used as test of significance for quantitative variables and Chi-square test for qualitative

Fig. 1 – Gel documentation picture in 2.0% agarose gel with 1-kb DNA ladder. Lane S-1 to S-4 positive for HCV with 405-bp band and lane PC as positive control.

Fig. 2 – Age distribution of patients on HD (n = 250).

variables. Yate's correction was applied to the Chi-square test wherever frequency of variable was less than 5. All tests were two tailed, and a p value < 0.05 was taken as significant.

Results

A total of 250 patients (207 males and 43 females) were recruited for the study from 5 dialysis centers in Pune to ensure adequate sample size and sufficient HCV isolates necessary for prevalence studies and genotyping and phylogenetic analyses collectively and at individual centers. Mean age of cases in the study was 47.3 years, and 60% of the cases were between the age group of 41–60 years (Fig. 2).

Anti-HCV positivity in dialysis patients

Of the 250 total patients, 47 samples were detected positive for anti-HCV antibodies, indicating an overall positivity of 18.8%. This rate varied from 6.7% to 35.6% in the 5 study centers (Table 1). The anti-HCV positivity was significantly higher in females (44.1%, 19/43) than in males (13.5%, 28/207). Thirteen cases were Hepatitis B Virus (HBV) infected, of which 6 cases were coinfecting with HCV.

HCV RNA positivity and genotypes

HCV RNA was detected in 36 of 47 anti-HCV-positive cases indicating a 76% positive correlation (Table 1). This indicated

that these patients were currently infected with HCV. Because prolonged persistence of antibody after the viral clearance is known, RNA-negative individuals had either cleared the virus or had undetectable HCV replication. In patients in whom HCV RNA was detected, liver enzymes and bilirubin were elevated marginally, indicating active liver disease.

Among 203 anti-HCV-negative cases, one detected positive for HCV RNA. This could be either due to window period between the infection and seroconversion or immunocompromised status of the patient. In all, the total number of HCV-infected cases was 48 (antibody positive + HCV RNA positive) with a marginal increase in the prevalence to 19.2%.

Core gene sequence-based genotype analysis of 37 HCV isolates showed predominance of genotypes 1a, 1b, and 3a in the patients from the 5 centers (Table 1). Of these, genotype 1a was most predominant (54.1%), followed by genotype 1b (40.5%) and then genotype 3a (2.7%). Genotype 1a was more prevalent in 3 of 5 centers, with centerwise occurrence given as, center 1: 75%, center 2: 100%, and center 4: 70%. Other HCV isolates belonged to either genotype 1b or 3a. The center 3 had predominance of genotype 1b virus (84.6%).

A phylogenetic analysis of the HCV isolates showed centerwise clustering of the isolates, suggesting that the patients were infected during the course of dialysis. Interestingly, in the center 3, 1b (84.6%) isolates showed 99.3% nucleotide similarity. This strongly suggested nosocomial common source transmission of HCV, and the same center showed highest (35.6%) prevalence of HCV infection too (Fig. 3).

Table 1 – Centrewise distribution of HCV infectivity status and prevalent genotypes of patients on HD (n = 250).

Center number	Number of HD patients	Anti-HCV antibody		HCV RNA-positive cases	Genotype distribution (%)		
		Positive reactor	% Infected		1a	1b	3a
Center 1	55	9	16.4	8	6 (75)	2 (25)	0
Center 2	45	7	15.6	6	6 (100)	0	0
Center 3	45	16	35.6	13	1 (7.7)	11 (84.6)	1 (7.7)
Center 4	90	14	15.6	10	7 (70)	3 (30)	0
Center 5	15	1	6.7	0	0	0	0
Total	250	47	18.8	37	20 (54.1)	15 (40.5)	1 (2.7)

Fig. 3 – Phylogenetic tree of HCV genotypes in different HD centers (n = 37). Phylogenetic analysis of core region sequences of 37 HCV isolates. The isolates are numbered as C, followed by the center number (1–5) and isolate number, e.g., C1ID09, and prototype sequences used are numbered as HCV, followed by the genotype number such as HCV1A1.

With the duration/frequency of dialysis, number of blood transfusions received, and number of dialysis centers visited, it can be said that there was a significant correlation between the HCV positivity and duration of HD in years ($p = 0.014$). Prevalence of HCV positivity in patients who were on HD for more than five years was 70.2% (33/47) (Table 2). Mean duration of HD in HCV-positive cases was found to be 6.03 years. In addition, patients undergoing >700 dialysis cycles were found to be at greater risk of HCV infection ($p = 0.035$) (Table 2). Thirty five of 47 (74.5%) patients were HCV infected, who were exposed to >700 dialysis cycles.

One hundred and fourteen patients undergoing maintenance dialysis had been transfused blood or blood products, and 32 (28.1%) were found to be HCV infected. Of the 136 patients with a negative history for blood transfusion, only 15 (11%) were found to be HCV infected. Thirteen HCV-infected

patients received more than 15 transfusions, and increasing numbers of blood transfusions were found to be associated with higher HCV positivity ($p < 0.01$) (Table 3).

Seventeen patients visited 4 dialysis centers, 12 patients visited 3 centers, and 10 patients visited 2 centers, whereas 8 patients visited only a single center (Table 3). With increasing frequency of changes in the HD centers, HCV infection was found to be significantly increasing as analyzed from the HCV positivity in patients undergoing dialysis in one center against patients who have visited up to 4 centers ($p < 0.01$) (Table 3).

Discussion

Nosocomial transmission of HCV is being brought under control in developed nations but continues to account for a

Table 2 – HCV infection status vis-a-vis the number and duration of HD cycles (n = 250).

Number of HD cycles	Anti-HCV antibody status		Duration of HD	Anti-HCV antibody status	
	Positive reactor	% HCV infected		Positive reactor	% HCV infected
300–700	12	25.5	<5 years	14	30
700–1400	35	74.5	>5 years	33	70.2
Total	47	100	Total	47	100

Table 3 – Distribution of HCV-infected patients according to the number of blood transfusions and change in HD centers.

Number of blood transfusions ^a	Number of HCV-infected patients ^b	% HCV infected	Number of changes in HD center	Number of HCV-infected patients	% HCV infected
1–5	4	8.5	1	8	17.1
6–10	6	12.8	2	10	21.2
11–15	9	19.1	3	12	25.5
>15	13	27.6	4	17	36.2

^a Total number transfused: 114.^b Total HCV infected: 32.

significant percentage of the new cases in developing countries. Higher incidence of HCV infection in patients on maintenance HD has been attributed to lack of stringent universal precautions. In the absence of an effective vaccine and emergence of serious complications, namely cirrhosis (5–20%) and hepatocellular carcinoma (1–4%), prevention and early detection of HCV infection assumes greater importance. Patients with chronic kidney disease (CKD) in conjunction with liver diseases are particularly at risk of developing such HCV-associated complications.^{10,11}

Limited data on age, gender, and racial and ethnic distributions are available. Gender variation in the prevalence of acute HCV remains controversial.^{11,12}

Nosocomial transmission of HCV is being brought under control in developed nations but continues to account for a significant percentage of the new cases in developing countries. In several studies, hemodialysis has been shown to be associated with the emergence of acute HCV infection.^{3,13}

Dialysis outcomes and practice patterns study carried out worldwide indicates significant variation among different dialysis units as far as prevalence of HCV infection is concerned and varies among countries from 2.6 to 22.9% with a mean HCV facility prevalence of 13.5%. A direct relation between the incidence and prevalence of infection in the dialysis units has been suggested by an earlier report. Studies indicate that a prevalence of 19% or lower had an approximate annual incidence of 2.5%, whereas a prevalence >60% was encountered when the incidence was around 35%.^{13,14}

Serological testing for HCV infection is presently recommended for HD patients, and the infection is primarily assessed by seroconversion. We used third-generation anti-HCV ELISA in our study and found overall 18.8% positivity (47/250), which varied from 6.7% to 35.6% in the 5 HD centers. Worldwide, wide variations have been reported, ranging from 5% in Northern Europe to 30–50% in Japan and Poland, 30.25% in Saudi Arabia, 39% in Brazil, and 5–30% in USA.^{3,4} Several studies from India have reported different prevalence rates of HCV infections in HD patients, such as 24–28% by Arankalle et al¹⁵ and Gosavi et al¹⁶ and, 4.3% by a study from Delhi.¹⁷ Chandra et al (2004) have reported a very high prevalence (46%) among CKD and renal transplant patients from Hyderabad.¹⁸ Constant monitoring of patients on maintenance hemodialysis and isolation of HCV-infected patients leading to significant decrease in the HCV seroconversion rate was shown by a group in tertiary hospital from New Delhi.¹⁹

In our study, all the 5 centers adhered to the use of dedicated machines for patients with HCV infection. However, there was overall 19.2% anti-HCV positivity among HD

patients (one additional HCV RNA positive among seronegative samples). Among 47 anti-HCV antibody positive samples, 78.7% (37/47) were HCV RNA positive, whereas only 1 of the 203 anti-HCV-negative samples was found to be HCV RNA positive (0.49%). A previous study from India has shown a comparatively higher (79.16%, 38/48) RNA positivity and lower anti-HCV antibody positivity (27.07%, 13/48) in patients on maintenance dialysis.²⁰ These results confirm inconsistent antibody response in patients on chronic HD and suggest that antibody-based tests carried out in isolation are liable to underestimate or miss the magnitude of hepatitis C infection.²¹ Use of RT-PCR for HCV RNA detection is not recommended as the primary test for routine screening. Nonetheless, for confirmation of infection, RT-PCR should still be considered as and when the patient tests reactive for anti-HCV or if liver enzyme levels are repeatedly beyond the normal ranges. It should also be considered in those who are anti-HCV nonreactor, wherein other possible etiological causes have been excluded.^{3,22}

Knowledge of a patient's genotype is important because it directs the duration and dosage of the antiviral therapy. In India, northern and eastern regions have predominance of genotype 3, followed by genotype 1 infections,²³ whereas southern and western regions have equivalent distributions of genotype 1 and 3.²⁴ Surprisingly, we noted only one genotype 3 isolate, whereas 36 of 37 infections were with either genotype 1a or 1b in the HD patients. This selective predominance of genotype 1a or 1b in HD patients was significantly different from the equivalent distribution of genotype 1 and 3 infections in the general population in this western region of India. This clearly indicated person-to-person transmissions of genotype 1 virus via hemodialysis units.

We endeavored to assess different risk factors responsible for high nosocomial transmission of HCV infection in HD patients. We found a significant association of the number of dialysis cycles, total duration of dialysis, number of dialysis centers used, and number of blood transfusion with the higher incidence of HCV infection. Many recent surveys have suggested the length of time on HD as a risk factor for HCV seropositivity.^{19,20,25,26}

Transfusion of blood and blood products is an important part of the treatment protocol in many HD patients.^{27,28} The use of new-generation tests with increased sensitivity and specificity for HCV detection in screening blood and blood products has resulted in significant decline in transfusion-related HCV infections in both developed and developing countries. However, detection of higher HCV positivity in patients undergoing >15 blood transfusions as compared with

those with 1–5 blood transfusions in the present study indicated lapses in the current screening procedures. In western countries, the incidence and prevalence of HCV infection among patients on HD is on the decline.^{3,22} NAT-based screening and implementation of universal precautions for infection control are probably responsible for the decline in posttransfusion HCV infection.

The efficacy of the therapy is measured by whether a sustained virologic response (SVR) occurs, i.e., undetectable HCV RNA 24 weeks after the cessation of treatment. Patients infected with genotype 1 require 48 weeks of treatment to attain SVR, whereas patients with genotype 2 and 3 have a 70–80% likelihood of a SVR after only 24 weeks of treatment. HCV genotypes 4, 5, and 6 generally respond to treatment as genotype 1 does and need to be managed in the same manner, i.e., 48 weeks of therapy.²⁹

In conclusion, we detected 19.2% HCV positivity in patients undergoing maintenance dialysis. Virus isolates from the same HD centers revealed high sequence similarities indicating common source of transmission. Predominant prevalence of only genotype 1 viruses among these patients further confirmed this. Longer duration and higher number of HD cycles were directly correlated to higher HCV positivity. Overall, our observations suggest that dialysis units in India need to have a closer look and review their strategy to control transmission of HCV in patients undergoing dialysis.

Conflicts of interest

The authors have none to declare.

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