

Retraction

The article titled, "Genotyping pattern of hepatitis C virus among patients on maintenance haemodialysis at tertiary care hospitals of Pune, Maharashtra, India", published in the International Journal of Research in Medical Sciences, Volume 6, Issue 4, 2018, Pages 1435-1439, DOI: <http://dx.doi.org/10.18203/2320-6012.ijrms20181310> is being retracted. We received complaint from one of the co-authors, after publication of the article that corresponding author, Dr. Anubha Patel had submitted the manuscript without informing other co-authors and analysis was not comprehensive. We contacted the corresponding author who could not satisfactorily respond to our queries. Since the author could not satisfactorily defend her paper and contravened the declaration she made while submitting her manuscript, it was decided to retract the article from International Journal of Research in Medical Sciences and not to consider any manuscript submitted by her in future.

Original Research Article

Genotyping pattern of hepatitis C virus among patients on maintenance haemodialysis at tertiary care hospitals of Pune, Maharashtra, India

Anubha Patel^{1*}, Ramanesh Murthy¹, Ashish Baghel², Partha Roy³, Kavita S. Lole⁴

¹Department of Microbiology, ²Department of Community Medicine, Chhattisgarh Institute of Medical Sciences, Bilaspur, Chhattisgarh, India

³Department of Microbiology, Armed Forces Medical College, Pune, Maharashtra, India

⁴Department of Hepatitis Division, National Institute of Virology, Pune, Maharashtra, India

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*Correspondence:

Dr. Anubha Patel,

E-mail: dr.aana99@gmail.com

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ABSTRACT

Background: Increased incidence of HCV infection is noted universally throughout the world in patients undergoing haemodialysis. Genotyping and gene sequencing of Hepatitis C virus in individuals with positive HCV-RNA helps in determining the optimal type, duration and response to therapy. Aim of the study was to determine gene sequencing which is considered as gold standard for determination of genotypes.

Methods: A cross sectional study design was conducted among 250 patients from five dialysis centres in Pune city in a period of one year. Qualitative HCV RNA detection was carried out by nested RT-PCR. Genotyping and sequencing was carried out using the Big-Dye Terminator cycle sequencing ready reaction kit. SPSS 21.0 version software was used to analyze the data.

Results: Out of total 250 patients 47(18.8%) were anti HCV antibody positive and 37 (14.8%) were HCV RNA positive. Out of 47 patients who tested positive for anti-HCV antibody 36 showed presence of HCV RNA (76.6% positive correlation). Out of 203 ELISA negative patients only 1 was found to be positive. Out of total 37 patients predominant genotype was found to be 1a (54.1%) followed by 1b (43.2%) and 3a (2.7%).

Conclusions: Genotyping and gene sequencing in patients with HCV RNA positivity revealed predominant genotype 1a and 1b. Detailed phylogenetic tree analysis revealed clustering of same genotypes in centre likely suggesting common source of infection prevalent in dialysis units and nosocomial transmission of virus.

Keywords: Hepatitis C virus, Hemodialysis, Genotyping pattern, Nosocomial transmission

INTRODUCTION

HCV Infection is major public health problem with an estimated Global Prevalence of approx 3% i.e. 180 million people.¹ In India 12.5 million people are infected with HCV infection.² An estimated 5-20% of HCV infected patient have or will develop cirrhosis, 1-4% of whom will annually develop hepatocellular carcinoma. Well known routes for HCV transmission include

intravenous drug use, blood transfusion, organ transplantation, chronic haemodialysis, occupational exposure among health care workers, unprotected sexual contact and vertical transmission.³

Prevalence of HCV Infection among dialysis patient is much higher than healthy blood donors.⁴ Studies held in dialysis centre from different countries revealed that prevalence ranges from 1-84.6% and there is particular

concern because HCV chronic infection causes significant morbidity and mortality among patient undergoing haemodialysis.¹ Studies in developed countries shows prevalence of 5% to 35% in patients undergoing maintenance haemodialysis.

Significant morbidity and mortality among such patients remains a matter of great public health concern.⁵ 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 towards 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.^{6,7}

In this background this study was conducted to determine circulating HCV genotypes in haemodialysis patients at tertiary care hospitals of Pune, Maharashtra, India.

METHODS

This was a cross sectional study carried out in tertiary care hospitals of Pune from July 2014 to June 2015. The study comprised of 250 patients from five dialysis centres in Pune (2 government hospitals and 3 corporate hospitals) who reported to the nephrology department for haemodialysis. Their clinical and demographical profile as well as several biochemical parameters was recorded. Informed consent was obtained from concerned departments and patients. Blood was collected in a 10ml vacutainer aseptically and serum and plasma were separated using centrifuge at 2000rpm for 5min. The serum and plasma so collected was kept at -70⁰ celsius.

HCV RNA detections and genotype analysis

HCV antibody detection was done using third generation ELISA kit manufactured by SD HCV ELISA. A two-step nested reverse transcription (RT)-PCR 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. PCR products were separated on 2% agarose gel and 405 bp sized bands were considered positive for HCV. Appropriate internal controls were employed 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 Big-dye 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 done for the 203 anti-HCV negative samples. Due to paucity of resources, minipool 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. A representative HCV sequences were retrieved from NCBI database and selected for comparison with HCV sequences from this study. Phylogenetic status was assessed employing the software MEGA 5. 9 Jukes-Cantor distances were utilized employing the neighbour joining algorithm for the analysis in MEGA.

Data collected was compiled in Microsoft Excel software and checked for its completeness and correctness before data was analyzed. Descriptive statistical analysis has been carried out in the present study. Results on categorical measurements are presented in numbers (%). Chi-square test been used to find the significance of study parameters on categorical scale between two or more groups. P-value of <0.05 was considered to be statistically significant. SPSS 21.0 version software was used to analyse the data.

RESULTS

A total of 250 patients (207 males and 43 females) were recruited for the study from 5 dialysis centres in Pune to ensure adequate sample size and sufficient HCV isolates necessary for prevalence studies and genotyping and phylogenetic analysis collectively and at individual centres. Of total 250 patients, 47 samples were detected positive for anti-HCV antibodies indicating an overall positivity of 18.8% (Table 1).

Table 1: Patients positive for anti HCV antibody and HCV RNA (n=250).

Test	Number of Positive Patients	% Positivity
Anti-HCV Antibody	47	18.8%
HCV RNA	37	14.8%

HCV RNA positivity, genotyping and gene sequencing

Out of total 250 patients 47 (18.8%) were anti HCV antibody positive and 37 (14.8%) were HCV RNA positive (Table 1). Out of 47 patients who tested positive for anti-HCV antibody 36 showed presence of HCV RNA

(76.6% positive correlation). All the cases that had HCV RNA also showed presence of anti-HCV antibody by ELISA and so had 100% positive correlation with anti-HCV antibody positive cases. Out of 203 ELISA negative patients only 1 was found to be HCV RNA positive.

Sequencing was carried out using big dye terminator cycle sequencing ready reaction kit (applied biosystems USA). All 37 samples were confirmed for HCV by demonstrating presence of HCV specific core region. Genotypes of 37 patients found positive for HCV RNA on basis of gene sequencing.

Core gene sequence-based genotype analysis of 37 HCV isolates showed predominance of genotypes 1a, 1b and 3a in the patients from the 5 centres. Of these, genotype 1a was most predominant (54.1%), followed by genotype 1b (43.2%) and then genotype 3a (2.7%) (Table 2). Genotype 1a was more prevalent in 3/5 centres, with centre wise occurrence as, Centre 1: 75%, Centre 2:

100% and Centre 4: 70%. Other HCV isolates belonged to either genotype 1b or 3a. The Centre 3 had predominance of genotype 1b virus (84.6%) (Table 3).

Phylogenetic analysis of the HCV isolates showed centre wise clustering of the isolates suggesting that the patients were infected during the course of dialysis. Interestingly, in the centre 3, 1b (84.6%) isolates showed 99.3% nucleotide similarity.

Table 2: Genotype distribution of HCV RNA positive cases (n=37).

Genotype	No. of HCV RNA positive cases	%
1a	20	54.1
1b	16	43.2
3a	1	2.7
Total	37	100

Table 3: Centre wise distribution of genotype.

Dialysis Centre	Total number of Dialysis Patients	Total number of HCV RNA Positive cases	Genotype	Positive Cases	
				No.	%
Centre 1	55	8	1a	6	75%
			1b	2	25%
			3a	-	-
Centre 2	45	6	1a	6	100%
			1b	-	-
			3a	-	-
Centre 3	45	13	1a	1	7.7%
			1b	11	84.6%
			3a	1	7.7%
Centre 4	90	10	1a	7	70%
			1b	3	30%
			3a	-	-
Centre 5	15	0	1a	-	-
			1b	-	-
			3a	-	-
Total	250	37	1a	20	54.1%
			1b	16	43.2%
			3a	1	2.7%

Phylogenetic analysis of core region sequences of 37 HCV isolates were done. The isolates are numbered as C = centre, then (1-5)-centre code. ID number where ID= identity number as C1ID09 and prototype sequences used are numbered as HCV then respective genotype number or institution or area as HCV1a (Figure 1).

DISCUSSION

Out of total patients (18.8%) were anti HCV antibody positive and (14.8%) were HCV RNA positive. Out of 47 patients who tested positive for anti-HCV antibody 36

showed presence of HCV RNA (76.6% positive correlation). This indicated that these patients were currently infected with HCV. All the cases that had HCV RNA also showed presence of anti-HCV antibody by ELISA and so had 100% positive correlation with anti-HCV antibody positive cases. Out of 203 ELISA negative patients only 1 was found to be positive. Since 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 where HCV RNA was detected, liver enzymes and bilirubin were elevated marginally indicating active liver disease.

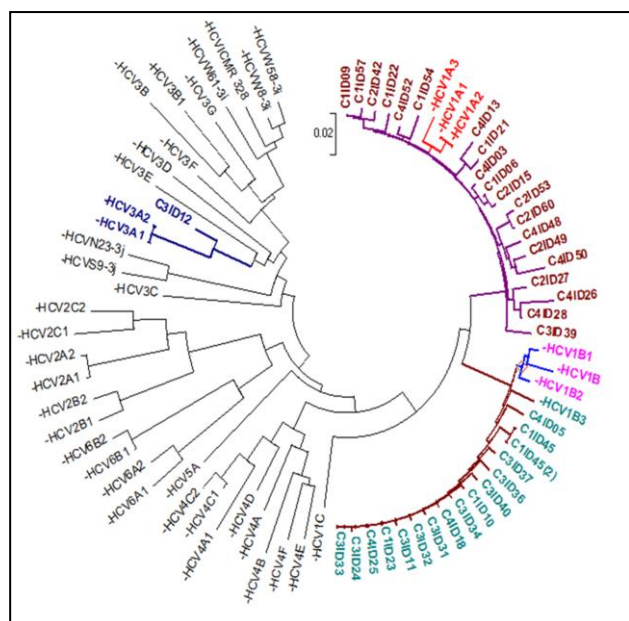


Figure 1: Phylogenetic tree.

Amongst 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 numbers of HCV infected cases were 47. Core gene sequence-based genotype analysis of 37 HCV isolates showed predominance of genotypes 1a, 1b and 3a in the patients from the 5 centres. Of these, genotype 1a was most predominant (54.1%), followed by genotype 1b (43.2%) and then genotype 3a (2.7%). Genotype 1a was more prevalent in 3/5 centres, with centre wise occurrence as, Centre 1: 75%, Centre 2: 100% and Centre 4: 70%. Other HCV isolates belonged to either genotype 1b or 3a. The Centre 3 had predominance of genotype 1b virus (84.6%). Phylogenetic analysis of the HCV isolates showed centre wise clustering of the isolates suggesting that the patients were infected during the course of dialysis. Interestingly, in the centre 3, 1b (84.6%) isolates showed 99.3% nucleotide similarity. This strongly suggested nosocomial common source transmission of HCV and same centre showed highest (35.6%) prevalence of HCV infection too.

In our study, all the 5 centres adhered to the use of dedicated machines for patients with HCV infection. However, there was overall 18.8% anti-HCV positivity amongst HD patients. Amongst 47 anti-HCV antibody positive samples, 76.6% (36/47) were HCV RNA positive, while, only 1 of the 203 anti-HCV negative samples was found to be HCV RNA positive (0.49%). A previously 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.⁸ These results confirm inconsistent antibody response in patients on chronic HD and suggest that antibody based tests carried out in isolation is liable to underestimate or miss the magnitude of hepatitis C

infection.⁹ Use of reverse transcriptase polymerase chain reaction (RT-PCR) for HCV RNA detection is not recommended by 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 non reactor wherein other possible etiological causes have been excluded.^{10,11}

Knowledge of a patient's genotype is important because it directs the duration and dosage of the antiviral therapy. In India, northern, eastern regions have predominance of genotype 3 followed by genotype 1 infections, while southern and western regions have equivalent distributions of genotype 1 and 3. Surprisingly, we noted only one genotype 3 isolate, while 36/37 infections were with either genotype 1a or 1b in the HD patients.^{12,13} This selective predominance of genotype 1a/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 haemodialysis units.

CONCLUSION

Genotyping and gene sequencing in patients with HCV RNA positivity revealed predominant genotype 1a and 1b. Detailed phylogenetic tree analysis revealed clustering of same genotypes in centre likely suggesting common source of infection prevalent in dialysis units and nosocomial transmission of virus. Confirmation of the genotype by sequencing has become a necessity as the treatment varies according to the genotype possessed by the individual with genotype 1, 4, 5 and 6 requiring 48 weeks of treatment and genotype 2 and 3 requiring 24 weeks of treatment with PEG-IFN and ribavirin. Moreover, boceprevir or telaprevir (direct antiviral drugs) must be given in combination with ribavirin and peg interferon for genotype 1 to improve the response. In developing countries with a high endemic background of HCV infection surveillance programs and efforts to increase awareness, improve diagnosis and facilitate treatment of acute HCV will have far reaching implications for the management of chronic HCV, where current disease management and health outcome strategies are less effective. Thus, preventive measures and the adherence to universal precautions for HCV control remains a priority.

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