ORIGINAL ARTICLE

Distribution of HCV genotypes among patients attending south Indian tertiary care centre

Masood Ahmed Tahashildar[1], Morubagal Raghavendra Rao[2], Umme Hani[3], Sowmya G Shivappa[2], Rashmi P Mahale[2], Anil Kumar[4], Ravindran Kumar[2]*

[1]Department of Microbiology, JN Medical College & Dr Prabhakar Kore Basic Science Research Center, KLE Academy of Higher Education and Research, Belagavi-590010 (Karnataka) India, [2]Department of Microbiology, JSS Medical College & Hospital, JSSAHER, Mysuru-570015 (Karnataka) India, [3]Department of Microbiology, Faculty of Life Sciences, JSS Academy of Higher Education and Research, Mysuru-570015 (Karnataka) India, [4]Department of Public Health, J. N. Medical College, KLE Academy of Higher Education and Research, Belagavi-590010 (Karnataka) India

Abstract

Background: Globally Hepatitis C Virus (HCV) is considered to cause one of the major infection burdening public health. Presently HCV is categorized into seven genotypes and 67 subtypes, transmitted through various routes and vary in their pathogenesis. *Aims and Objectives*: Present work aimed to detect the prevalent HCV genotypes among individuals attending tertiary care hospital. *Material and Methods*: A hospital based cross sectional study was carried out. Patient blood samples received for screening of HCV antibodies were included in this study. Serum was subjected to Enzyme-Linked Immunosorbent Assay (ELISA) or Chemiluminescent Immunoassay (CLIA) and samples found to be positive for HCV antibodies were subjected to viral RNA extraction and genotyping. *Results:* Forty-two samples were found to be positive for HCV antibodies out of 19526 samples screened and were further subjected to genotyping. The genotypes identified were genotype-1, genotype-3, and genotype-4. *Conclusion:* Genotype-1 and genotype-3 predominate in this region. However, genotype-4 detection was novel to this region and has never been reported earlier. This study highlights the importance of screening HCV-positive patients for genotyping.

Keywords: Hepatitis C Virus, Genotyping, Hepatitis, Hepatocellular Carcinoma

Introduction

Hepatitis C Virus (HCV) infects more than 180 million of the population globally and emerged as one of the major infection associated with public health burden [1]. HCV is a positive-sense, single-stranded RNA-enveloped virus belonging to flaviviridae family, transmitted through blood and blood-derived products [2]. HCV infection starts with an acute phase that usually goes undetected [3]. Chronic HCV infection can manifest as major life-threatening complications such as cirrhosis of the liver, liver failure, and hepatocellular carcinoma [4]. HCV is transmitted through contact with infected blood and shared used needles particularly among drug users [5]. Healthcare workers may get exposed to infected blood through accidental needle prick. However, individuals on long-term dialysis, children born to HCV-infected mothers, and individuals with multiple sex partners are at very high risk of acquiring HCV infection [5-7].

The occurrence of a significant range of mutations causes a high level of genetic diversity in viral population [8]. This change in the genetic makeup of virus leads to the emanation of novel strains of undulation in the viral population. The chronicity of HCV infection is due to the viral load persistence [9].

Presently, HCV is classified into seven confirmed genotypes and subcategorized into 67 subtypes. Interestingly, a novel genotype has been reported in the region of Punjab, India designated as genotype-8 [10]. Globally, the distribution of HCV genotypes is mixed. However, worldwide HCV genotype-1 and genotype-2 infections are the most common, accounting for 67.0% of infections together. Genotype-2 is widely found in West Africa while the most prevalent genotypes in central, north Africa and Southeast Asia are genotype-4 and genotype-6 (82.9%, 35.3% and 30.8%). Collectively the distribution of genotype-2, genotype-4 and genotype-6 is estimated to cover third of all global HCV cases. [11-12]. In India, genotype-3 is most prevalent in the northwest and east, while genotype-1 is widely found in south India [13].

The knowledge of various genotypes is a preliminary requisite to comprehend the current status of genetic diversity of different strains of HCV, pathology of HCV infection related to particular genotype, the progress of a disease in individuals, modulation in the antiviral therapy and the formulation cum advancement in the development of effective vaccines for prophylactic use for the prevention of infection. This study was carried out to determine the distribution of HCV genotypes among patients attending tertiary care hospital in this region.

Material and Methods

The present study was based on cross sectional study design and was conducted from September 2019 to December 2020, after clearance from the Institutional Ethics Committee. A total of 19526 samples were screened for HCV antibodies by using convenience sampling technique, out of which 42 HCV positive samples were analysed for various HCV genotypes based on the viral RNA quantification.

Screening of HCV antibodies

The blood samples were subjected to centrifugation at 3000 rpm for 5 min and the serum was separated and screened for HCV antibodies by Chemiluminescent Immunoassay (CLIA) (by using Abbott and vitrosECiQ immunodiagnostic equipment) or Enzyme-Linked Immunosorbent Assay (ELISA) (by using HCV microlisa kit, J. Mitra, and Co. Pvt. Ltd) as per manufacturers protocols. Out of 19526 samples, 42 samples were positive for HCV infection which were stored at -80°Cdegree freezer for HCV genotyping.

HCV genotyping

Extraction of viral RNA

Samples positive for HCV antibodies by ELISA or by CLIA were processed for HCV RNA detection by Real-Time Polymerase Chain Reaction (RT-PCR) and RNA extraction was done by using spinster viral nucleic acid kit 1.0 following the kit instructions.

Genotyping of HCV RNA

The extracted RNA samples were subjected to genotype detection by using a HCV genotype diagnostic kit (Lot number: 2020003) based on PCR-Fluorescence probing by Sansure biotech.

Statistical analysis

Statistical analysis was done with the help of biostatistician using Fisher's exact test by using SPSS software version 22 to determine significance of association between HCV genotypes and different study variables. Value of p less than 0.005 was considered as significant in this study.

Results

A total of 19526 samples were screened out of which 42 samples were positive for HCV antibodies and the HCV positivity rate during this period was 0.33%. Out of the 42HCV positive samples which were subjected to HCV genotyping, 23 (54.76%) were positive for genotype-1, 16 (38.09%) were positive for genotype-3, 2 (4.76%) were positive for genotype-4 and in one sample genotype could not be ascertained.

Based on the health and clinical history, the modes of HCV transmission were found to be dialysis in 40.47% (n=17) cases and blood transfusion in 14.28% (n=6) cases. In 33.33% (n=14) cases, mode of transmission could not be traced (Table 1). Thirty-two out of 42 samples were male patients, of which 18 were positive for genotype-1, 11 for genotype-3, 2 for genotype-4 while genotype could not be ascertained for 1 sample. The remaining ten samples were of female patients of which 5 each were positive for genotype-1 and genotype-3 (Table 2).

The occurrence of HCV infection is mainly seen in the age group between 61-70 years followed by 41-50 years. Genotype-1 is prevalent in the age group 61-70 years and 41-50 years. However, genotype-3 predominates in the age group 51-60 years followed by the age group 41-50 years. Age group below 30 and above 70 years showed a very low rate of infectivity to HCV (Table 3).

The statistical analysis done using Fisher's exact test showed that there was no association between different variables and HCV genotypes.

Table 1: Distribution of HCV genotypes with modes of transmission

Modes of transmission	Total	Genotype-1	Genotype-3	Genotype-4	Undetected	*p*
Blood transfusion	6 (14.28%)	4 (17.39%)	2 (12.50%)	-	-	0.605* NS
Dialysis	17 (40.47%)	9 (39.13%)	7 (43.75%)	1 (50%)	-	
Surgery	3 (7.14%)	2 (8.69%)	1 (6.25%)	-	-	
Renal allograft recipient	2 (4.76%)	1 (4.34%)	1 (6.25%)	-	-	
Unknown	14 (33.33%)	7 (30.43%)	5 (31.25%)	1 (50%)	1	
Total	42 (100%)	23 (100%)	16 (100%)	2 (100%)	1	

*NS- Not significant, *statistically significant at p<0.05*

Table 2: Gender wise distribution of HCV genotypes

Gender	Total	Gentype-1	Genotype-3	Genotype-4	Undetected	*p*
Male	32 (76.80%)	18 (78.26%)	11 (68.75%)	2 (100%)	1 (100%)	0.875* NS
Female	10 (23.20%)	5 (21.74%)	5 (31.25%)	-	-	
Total	42 (100%)	23 (100%)	16 (100%)	2 (100%)	1 (100%)	

*NS- Not significant, *statistically significant at p<0.05*

Table 3: Age wise distribution of HCV genotypes

Age range (years)	Total	Genotype-1	Genotype-3	Genotype-4	Undetected	*p*
0-18	1 (2.38%)	1 (4.34%)	-	-	-	0.993* NS
19-30	2 (4.76%)	1 (4.34%)	1 (6.25%)	-	-	
31-40	7 (16.66%)	4 (17.39%)	2 (12.5%)	-	1 (100%)	
41-50	12 (28.57%)	6 (26.08%)	4 (25%)	2 (100%)	-	
51-60	7 (16.66%)	2 (8.69%)	5 (31.25%)	-	-	
61-70	10 (23.80%)	8 (34.78%)	2 (12.5%)	-	-	
71-80	3 (7.14%)	1 (4.34%)	2 (12.5%)	-	-	
Total	42 (100%)	23 (100%)	16 (100%)	2 (100%)	1 (100%)	

*NS- Not significant, *statistically significant at p<0.05*

Discussion

HCV infection is transmitted majorly through parenteral routes, contact with blood and blood-borne products, especially in Intravenous Drug Users (IVDU), blood transfusions, and long-term dialysis [3].

A total of forty-two samples were analysed for HCV genotyping, which revealed 23 samples were positive for HCV genotype-1, 16 for genotype-3, 2 for genotype-4 and one sample was not detectable for any genotype used in the assay. The HCV positivity rate during this period was 0.33% which is similar to Shanmugam *et al.* [14] in 2018 who found seroprevalence rate to be 0.30% in Tamilnadu and Patil *et al*. [15] in 2017 who found the positivity rate to be 0.46% in Maharashtra. However, the HCV positive rate in Punjab reported a higher percentage (3.6%) [16]. The reason for diverse HCV positivity rates in different studies is possibly due to the selection of cases. In the present study, the total number of samples

included was more because of the routine screening for HCV infection before surgical procedures and admission to Intensive Care Unit (ICUs) irrespective of suspicion of HCV infection.

In this study, HCV infection was more common among males (n = 32; 76.1%) than females (n= 10; 23.8%) which is similar to that observed by Patil *et al*. [15] in which males (n = 68; 58.7%) were more prone than females (n=52; 43.3%) for HCV infection. The occurrence of HCV infection was observed more in the age group between 41-50 (28.5%; n= 12) in the present study similar to Yabaji *et al.* [17] who reported 30.8% of HCV positivity among the age group 41-50. But a previous study [18] revealed the prevalence of HCV infection being more in older (>50 of age) population. However, in present work 9 (21.4%) individuals were positive for HCV infection in age group of 61-70 and 2 (4.76%) cases were positive among the age group 71-80.

Genotype-1 (46.2%) is most commonly associated with HCV infection worldwide [19]. In Asia HCV infection is majorly associated with genotype-1, followed by genotype-3. In South Asian regions highest percentage of infection is majorly associated with genotype-1 and genotype-3 [3]. The overall distribution of genotype-1 in India is surprisingly varied, dominated in South India [11, 20, 21]. Interestingly in North India, this genotype is less dominated than genotype-3 [17, 22, 23]. However, the present study shows that genotype-1(54.76%) is majorly prevalent, followed by genotype-3 (38.1%).

Genotype-1 is dominantly associated with dialysis followed by blood transfusion in this region and can be validated with the work done previously by Ramya *et al*. [21] Mysore, Karnataka, and John *et al*. [11] Kerala.

Overall, globally 9.1% positive cases of HCV belong to genotype-2 which are predominantly documented in the West African region. In North America, Europe, and Japan subtypes 2a and 2b were majorly found followed by 2c found in Northern Italy [11]. Transmission of genotype-2 is notably seen in IVDU. In India genotype-2 is negligibly documented [24]. But present study did not found any cases of genotype-2.

Globally around 30% of all HCV cases are related to genotype-3. In south Asia, 66.7% of HCV infections belong to genotype-3 [3]. In the European population high incidence is seen of IVDU. Patients with genotype-3 infection have high chances of developing cirrhosis, fibrosis progression, and a high incidence of development of Hepatocellular Carcinoma (HCC) unlike genotype-1 infection [25]. The incidence of genotype-3 in the Indian population is around 54.4% of overall HCV infections [3]. However, genotype-3 is predominant in North India as reported by Yabija *et al.* [17], Prakash *et al*. [22] and Raghuraman *et al*. [23]. Whereas in South India, studies like John *et al*. [11] and Ramya *et al*. [21] reported genotype-3 as the second most common genotype which is similar to the present study. In this current work, mode of transmission of genotype-3 was notably associated with dialysis among 7 individuals, and blood transfusion among 2 individuals while in remaining five cases the route of transmission was unknown.

In our findings, 2 cases were detected to be positive for genotype-4 and both were males, one with a history of dialysis and another with HCC respectively. Identification of genotype-4 is neoteric in the present study but unusual to previous work done in this region by Ramya *et al*. [21].

However, present study findings can be correlated with the study done in the state of Kerala [11] where two cases of genotype-4 were reported. Genotype-4 is predominantly seen in the Middle East, North and Central Africa. In fact more than 20% of all chronic infections globally and more than 90% of patients among all HCV-positives in Egypt harbour genotype-4 [26]. However, in India the prevalence of genotype-4 is very rare [11]. Surprisingly, males are much more prone to HCV genotype-4 infection than females [26].

Genotype-5 is mainly prevalent in South Africa [27]. Genotype-6 is very diverse genetically, and common in Asia. About 19.9-95.6% of HCV infected population in East Asia present with genotype-6. However, in India genotype-6 is highly prevalent in Northern states. Individuals with genotype-6 HCV infection are at high risk of developing HCC and cirrhosis [28]. Genotype-7 is detected in individuals from the Congo region of Central Africa [29]. Surprisingly, genotype-8 has been reported from the region of Punjab, India [30]. This reveals the possibility of the high rate of mutation in HCV further leading to the emergence of novel strains. In present research, genotype-5 and genotype-6 were not found. However, there was one sample out of forty two that we could not characterize based on genotyping of the kit used. In the present study, the primer targeted genotype-1 to genotype-6 as genotype-7 is now emerging mainly from the Congo region, central Africa. The presence of this new genotype needs to be evaluated globally.

Conclusion

The facts obtained from the study infer that the most common genotype prevalent in this region is genotype-1 followed by genotype-3. The probable modes of transmission in both genotypes based on the clinical history were dialysis and blood transfusion. Presence of genotype-4 is novel in this region which was not detected in a previous study conducted in the region of the Mysore district in Karnataka. This new strain may result in the emanation of new mixed genotype strains. Additionally, it may constitute clinical and epidemiological challenges. This study also strongly recommends the screening of HCV-positive patients for genotyping before therapeutic intervention and imperative management of positive individuals.

Acknowledgement

The authors are humbly thankful to Dr Sheetal U. Harakuni, Professor Department of Microbiology, J N Medical College, KAHER, Belagavi – 590010 and Dr Shridhar Ghagane, Scientist Grade – 2, KAHER's Dr Prabhakar Kore Basic Science Research Center, Belagavi– 590010 for their research guidance in the present study.

References

1. Ryan KJ, C. Ray Gerorge, Nafees Ahmed, Drew WL, Michael Lagunoff, Paul PL, *et al*. Sherris Medical Microbiology, 6th Edition, Chapter 13: Hepatitis Viruses:238.
2. Petruzziello A, Marigliano S, Loquercio G, Cozzolino A, Cacciapuoti C. Global epidemiology of hepatitis C virus infection: An up-date of the distribution and circulation of hepatitis C virus genotypes *World J Gastroenterol* 2016; 22(34):7824.
3. Grebely J, Dore GJ, Kim AY, Lloyd A, Shoukry NH, Prins M, Page K. Genetics of spontaneous clearance of hepatitis C virus infection: A complex topic with much to learn *Hepatology* 2014;60:2127–2128.
4. Bennett JE, Dolin R, Blaser ME, Mandell MD. Hepatitis C. Douglas and Bennett's Principles and Practices of Infectious Diseases (8thed.), Elsiever Saunders, Philadelphia, USA 2010; 15:1904-1927.
5. Kohli A, Shaffer A, Sherman A, Kottilil S. Treatment of hepatitis C: a systematic review. *JAMA* 2014; 312(6):631–640.
6. Nemagoudar P, Nikam P, Nikam S, Nimbal N. To study the activity of paraoxonase-1 and high density lipoprotein-cholesterol in alcoholic liver cirrhosis. *J Krishna Inst Med Sci Univ* 2017; 6(1):66-71.
7. Tambe MP, Patil SP, Dravid M, Bhagwat VR. Investigation of an outbreak of hepatitis' E' in a rural area of Dhule district in Maharashtra. *J Krishna Inst Med Sci Univ* 2015; 4(1): 109-114.
8. Hepatitis C Virus: Epidemiology and Transmission Risks. EMI Guidelines-Appendix 23. Available from: http://www.hpsc.ie/a-z/
9. John M, Oommen S, Jagan OA, George S, Pillai S. A study on the circulating genotypes of hepatitis C virus in a tertiary care hospital in Central Kerala. *Indian J Med Microbiol* 2019;36(4):532-536.
10. Borgia SM, Hedskog C, Parhy B, Hyland RH, Stamm LM, Brainard DM, *et al.* Identification of a novel hepatitis C virus genotype from Punjab, India: expanding classification of hepatitis C virus into 8 genotypes. *J Infect Dis* 2018; 218(11):1722-1729.
11. Simmonds P. Hepatitis C Virus: from molecular virology to antiviral therapy. *Curr Top Microbiol Immunol* 2013; 369:17-49.
12. Panigrahi AK, Roca J, Acharya SK, Jameel S, Panda SK Genotype determination of hepatitis C virus from northern India: identification of a new subtype. *J Med Virol* 1996; 48(2):191-198.
13. Mohd HK, Groeger J, Flaxman AD, Wiersma ST. Global epidemiology of hepatitis C virus infection: new estimates of age-specific antibody to HCV seroprevalence. *Hepatology* 2013; 57(4):1333-1342.
14. Shanmugam RP, Balakrishnan S, Varadhan H, Shanmugam V. Prevalence of hepatitis B and hepatitis C infection from a population-based study in Southern India. *Eur J Gastroenterol Hepatol* 2018; 30(11):1344-1351.
15. Patil SR, Datkhile KD, Ghorpade MV, Patil SS, Kakade SV. Seroprevalence, risk factors and genotype distribution for hepatitis C infection: A study from rural hospital in Maharashtra. *Indian J Med Microbiol* 2017; 35(4):563-567.
16. Sood A, Suryaprasad A, Trickey A, Kanchi S, Midha V, Foster MA, *et at.* The burden of hepatitis C virus infection in Punjab, India: a population-based serosurvey. *PloS One* 2018;13(7):e0200461.
17. Yabaji PM, Shankarkumar A, Shukla A, Bhatia S. Hepatitis C virus infection in a tertiary care hospital in Mumbai, India: Identification of a mixed and novel genotype. *Indian J Med Microbiol* 2018;36(3):352-635.
18. Mengal MA, Abbas F, Mengal MA, Shafee M, Babar S, Mengal MA, *et al.* Passive surveillance of anti-hepatitis C virus antibodies in human subjects of four medical units of Balochistan, Pakistan. *Int J Agric Biol* 2012;14(4):585–589.
19. Messina JP, Humphreys I, Flaxman A, Brown A, Cooke GS, Pybus OG, *et al.* Global distribution and prevalence of hepatitis C virus genotypes. *Hepatology* 2015; 61(1):77-87
20. Ponamgi SP, Rahamathulla S, Kumar YN, Chandra M, Lakshmi N, Habibullah CM, *et al.* Prevalence of hepatitis C virus (HCV) coinfection in HIV infected individuals in south India and characterization of HCV genotypes. *Indian J Med Microbiol* 2009; 27(1): 12-16.
21. Ramya SR, Kulkarni M, Kumar GV. Hepatitis C virus seropositivity and genotyping by RealTime PCR in a tertiary care hospital in Southern India. *BMC Infect Dis* 2014; 14(3):1.
22. Prakash S, Shukla S, Ramakrishna V, Jain A. Distribution of hepatitis C genotypes in Uttar Pradesh, India; rare genotype 4 detected. *J Med Virol* 2018; 90(12):1875-1881.

23. Raghuraman S, Shaji RV, Sridharan G, Radhakrishnan S, Chandy G, Ramakrishna BS, *et al.* Distribution of the different genotypes of HCV among patients attending a tertiary care hospital in south India. *J Clin Virol* 2003; 26(1):61-69.
24. Malhotra P. HCV Genotype 2 is rare in India-ten years' experience at tertiary care center of Northern India. *Japanese J Gastroenterol Hepatol* 2021;5(13):1-3
25. Kattakuzhy S, Levy R, Rosenthal E, Tang L, Wilson E, Kottilil S. Hepatitis C genotype 3 disease. *Hepatol Int* 2016;10(6):861-870
26. Jd Bruijne, J Schinkel, M Prins, SM Koekkoek, SJ Aronson, VM Ballegooijen, *et al.* The emergence of hepatitis C virus genotype 4: phylogenetic analysis reveals three distinct epidemiological profiles. *J Clin Microbiol* 2009;47(12):3832-3838.
27. Daw MA, El-Bouzedi A, Dau AA. Geographic distribution of HCV genotypes in Libya and analysis of risk factors involved in their transmission. *BMC Res Notes* 2015; 8(1):1-9.
28. Mettikanont P, Bunchorntavakul C, Reddy KR. Systematic review: epidemiology and response to direct-acting antiviral therapy in genotype 6 chronic hepatitis C virus infection. *Aliment Pharmacol Ther* 2019; 49(5):492-505.
29. Murphy DG, Sablon E, Chamberland J, Fournier E, Dandavino R, Tremblay CL. Hepatitis C virus genotype 7, a new genotype originating from central Africa. *J Clin Microbiol* 2015; 53(3):967-972.
30. Borgia SM, Hedskog C, Parhy B, Hyland RH, Stamm LM, Brainard DM, *et al.* Identification of a novel hepatitis C virus genotype from Punjab, India: expanding classification of hepatitis C virus into 8 genotypes. *J Infect Dis* 2018; 218(11):1722-1729.

***Author for Correspondence:**

Dr. Morubagal Raghavendra Rao, Department of Microbiology, JSS Medical college & Hospital, JSSAHER, Mysuru Email: getdrraghurao@yahoo.com Cell: 9902555558

How to cite this article:

Tahashildar MA, Rao MR, Hani U, Shivappa SG, Mahale RP, Kumar A, Kumar R. Distribution of HCV genotypes among patients attending south Indian tertiary care centre. *J Krishna Inst Med Sci Univ* 2023; 12(2):3-10

Submitted: *10-Jan-2023* ***Accepted:*** *20-Mar-2023* ***Published:*** *01-Apr-2023*