IJPSR (2023), Volume 14, Issue 10 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 21 February 2023; received in revised form, 16 May 2023; accepted 31 May 2023; published 01 October 2023

MOLECULAR CHARACTERIZATION OF HEPATITIS C VIRUS IN A TERTIARY CARE HOSPITAL IN RURAL INDIA OF WESTERN UTTAR PRADESH

R. K. Verma [1], Radhika [2], D. P. Singh [1], Khilika Sethi [* 1] and Sweta Singh [1]

Department of Microbiology [1], Uttar Pradesh University of Medical Sciences, Saifai - 206130, Uttar Pradesh, India.
Department of Microbiology [2], Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow - 226014, Uttar Pradesh, India.

Keywords:

Anti-HCV ELISA, HCV genotypes, Hepatitis C virus, Real-time PCR

Correspondence to Author:
Dr. Khilika Sethi

Junior Resident,
Department of Microbiology,
Uttar Pradesh University of Medical Sciences, Saifai - 206130, Uttar Pradesh, India.

E-mail: sethikhilika@gmail.com

ABSTRACT: Background: Hepatitis C virus (HCV) has been recognized as one of the main causes of chronic liver disease worldwide. It causes chronic hepatitis, which can progress to liver cirrhosis and hepatocellular carcinoma. HCV has different genotypes that can vary in pathogenicity and impact treatment outcomes. Identifying HCV genotypes is very important in selecting antiviral therapy, dose adjustment of antiviral drugs, determination of duration of treatment, and follow-up of the treatment response. **Objectives:** To determine the genotype of HCV in HCV-positive patients. **Materials and Methods:** We included 100 anti-HCV-positive patients. HCV RNA (Ribo Nucleic Acid) identification and HCV genotyping assays of the positive specimens were performed by Real-Time Polymerase Chain Reaction (PCR). **Results:** Of the 52 HCV RNA-positive cases, Males were more preponderant than females, and the were years. Genotype 3 (n = 40; 76.9%) accounted for the highest number of cases, was only present one case (n = 1; 1.9%), while genotype 4 could not be detected at all was found to be genotypes in the Western part of Uttar Pradesh. Genotype 4 was not detected in any positive cases. Identifying HCV genotypes is important for the optimum management of chronically infected HCV patients.

INTRODUCTION: Hepatitis C virus is one of the primary factors that result in chronic liver disorders Amongst people had acute hepatitis after a blood transfusion were the first to be diagnosed with non-A along with non-B hepatitis [1, 2]. Post-transfusion hepatitis is caused most often by Hepatitis C Virus. All across the globe, hepatitis is a major concern for medical professionals. It is estimated that 300 million individuals throughout the globe have HCV infection.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.14(10).4924-28

This article can be accessed online on www.ijpsr.com

DOI link: https://doi.org/10.13040/IJPSR.0975-8232.14(10).4924-28

Amongst the primary reason for hepatocellular carcinoma, HCV infection accounts for 20% of all instances of acute hepatitis as well as 85% of all cases of chronic hepatitis [3]. Approximately 0.5–2% of the global population is infected, with IV drug users and hemophiliacs being the most vulnerable [4].

The number of people suffering from chronic hepatitis C infection is approximately 58 million, with an additional 1.5 million being infected yearly. Hepatocellular carcinoma and Cirrhosis accounted for most of the anticipated 290,000 hepatitis C-related deaths in 2019 [1]. It is believed that between 10 and 15 million individuals in India are living with HCV, and 350,000 people in the country die yearly from liver disorders caused by HCV [5, 6]. On World Hepatitis Day in 2021, the

WHO (World Health Organization) highlighted the urgency of hepatitis eradication with the subject "Hepatitis Can't wait," with the goal of eradicating the disease by 2030 [1]. HCV is a 50nm, single-stranded, positive Ribonucleic Acid (RNA) virus that is part of genus Hepacivirus (derived from the Greek word hepatos, hepar, liver) [7]. The RNA genome contains around 9600 nucleotides and encodes a polypeptide precursor with 3000 amino acids. The genome's 5' and 3' ends are flanked by non-coding regions. Both structural (envelope E1, along with E2, core) and non-structural proteins are generated from the polyprotein precursor by co-translational processing by host signal peptidases (NS5B, NS5A, NS4B, NS4A, NS3, NS2, and NS1). Similarly, the protein p7 seems to have a not structural role [8].

Genome sequencing has shown remarkable diversity in the HCV virus. To test for HCV, most labs focus on the virus's 5' Untranslated Region (5'UTR), a highly conserved area across different HCV isolates. However, this area also includes genotypically variable sequences which formerly allowed the virus to be divided into 6 classical genotypes; these classifications have now been modified to account for seven primary genotypes that vary from one another by more than 30% in nucleotide sequence. These genotypes (1 to 7) Show different concern and their worldwide distribution, transmission, and disease progression [9, 10]. Several molecular biology tests are available for detecting and analyzing HCV-RNA (Hepatitis C virus ribonucleic acid). Almost all available commercial techniques use PCR (Polymerase Chain Reaction) and aim for the same 5'UTR region. Both detection and analysis of viral loads benefited from the development of real-time PCR tests [11-13]. Millions of individuals likely live with hepatitis C in India, but the nation lacks reliable statistics on the disease's prevalence. In the absence of a comprehensive HCV monitoring system, the true prevalence of HCV-related liver disorders in India remains unidentified. This research aimed to determine how HCV genotypes were distributed among HCV-infected patients treated in rural Indian tertiary care hospital.

MATERIALS AND METHODS:

Study Design: This prospective cross-sectional study involves purposive and convenient sampling. The study was conducted between January 2017 and June 2018. The Institutional Ethics Committee approved the study with Clearance Code: 148/2017.

Selection of Patient: The patients admitted to the clinical departments or attending Outpatient Departments at the study site and undergoing laboratory tests for Hepatitis C as part of routine preoperative screening or for diagnostic purposes were included in the study after prior consent. However, the patients with Hepatitis B Virus or HIV infections, and those on antiviral treatment for HCV infection, were excluded from the study.

Methodology: 5ml of blood sample was collected in a plain vial with proper aseptic. The serum was separated by centrifugation for serological analysis, and the remaining serum was stored at -20^{o}C for Real-Time PCR and genotyping analysis.

All samples were tested for anti-HCV antibodies by immunochromatography-based rapid diagnostic test (RDT) using ASPEN HCV® (Aspen Diagnostics Pvt. Ltd., Delhi, India) cards as per manufacturer's instructions. All the blood samples reporting positive by HCV RDT were further subjected to Anti-HCV ELISA Qualisa HCV™ (Qualpro Diagnostics, Goa, India) as per the manufacturer's instructions for further confirmation. The samples reporting positive by both tests were further subjected to HCV RNA detection and HCV genotyping. For HCV RNA detection and genotyping, 100 samples were selected randomly from the ELISA-positive cases. These samples were subjected to genotype determination by real-time PCR by Geno-Sen's HCV Genotyping 1/2/3/4 Real-Time PCR Kit (Genome Diagnostics Pvt. Ltd. Himachal Pradesh, India). The HCV RNA was extracted from the serum sample using PurelinkTM Viral RNA Mini Kit per the manufacturer's instructions (Invitrogen by Thermo Fisher Scientific India Pvt. Ltd., Mumbai, India). HCV RNA was extracted from 200µl serum and eluted with 60µl sterile RNAase-free water. In HCV RNA positive samples, genotyping of HCV was done by performing the real-time PCR by using Geno-Sen's HCV genotyping 1/2/3/4 Real-Time Polymerase Chain Reaction kit (Genome Diagnostics Pvt. Ltd. Himachal Pradesh) as per manufacturer's protocol.

The Geno-Sen's HCV Genotyping PCR Reagents constitute a ready-to-use system for the detection of HCV genotypes 1/2/3/4 using PCR instrument (7500 Fast Dx PCR Instrument, Applied Biosystem™, Thermo Fisher Scientific India Pvt. Ltd., Mumbai, India). The software analyzed data generated in the PCR according to the manufacturer's instructions.

RESULTS: 100 Anti-HCV ELISA positive samples were subjected to genotype determination by Real-Time PCR. Out of 100 seropositive samples, HCV RNA was detected in 52 (52%) samples. Among the HCV-positive patients (52), in whom HCV RNA was detected, 29 (55.76%) were males while 23 (44.23%) were females, with male-female ratio of 1.26:1.

HCV-infected patients were categorized into four age groups. Analysis of the association between patients' age group and HCV positivity revealed that the maximum number of cases belonged to the age group of 31-50 years, illustrated in **Table 1.**

TABLE 1: DISTRIBUTION OF AGE AMONG THE HCV RNA POSITIVE AND NEGATIVE CASES

Age group (years)	Positive (n=52)	Negative (n=48)	Total (n=100)
0-18	3 (5.8%)	3 (5.8%)	6
19-30	13 (25%)	18 (37.5%)	31
31-50	19 (36.53%)	18 (37.5%)	37
≥50	17 (32.7%)	9 (18.75%)	26

HCV RNA-positive samples were subjected to genotype determination by Real-Time PCR. Genotype 3 was the commonest type observed in 40 (76.92%) cases followed by genotype 1 in 11 (21.15%) cases, as seen in **Table 2.**

TABLE 2: DISTRIBUTION OF DIFFERENT HCV GENOTYPES

Genotype	Distribution (n=52)
Genotype 1	11 (21.15%)
Genotype 2	1 (1.92%)
Genotype 3	40 (76.92%)
Genotype 4	00

Distribution of genotypes among the genders reveals that Genotype 3, being the commonest in both genders, was identified in 24 (82.75%) males and 16 (69.5%) females. Please refer to **Table 3** for the gender-wise distribution of all 3 genotypes.

TABLE 3: DISTRIBUTION OF HCV GENOTYPES AMONG HCV-POSITIVE PATIENTS

Gender	Genotype 1	Genotype 2	Genotype 3
Male (n=29)	4 (13.8%)	1(3.44%)	24 (82.75%)
Female (n=23)	7 (30.43%)	00	16 (69.5%)
Total	11(21.1%)	1(1.9%)	40 (76.9%)

We also studied HCV genotype distribution in different age groups– 0-18 years, 19-30 years, 31-50 years, and ≥50 years.

In all these age groups, Genotype 3 was the most common genotype. In the age group of 31-50 years, which were commonly affected by HCV, genotype 3 was detected in 30.8% of cases, followed by genotype 1 (5.8%) and genotype 2 (1.52%) **Table 4.**

TABLE 4: AGE-WISE DISTRIBUTION OF HCV GENOTYPES AMONG THE HCV RNA-POSITIVE CASES

Age-group (in years)	Genotype 1	Genotype 2	Genotype 3
0-18	1 (1.92%)	0	2 (3.84%)
19-30	3 (5.8%)	0	10 (19.23%)
31-50	3 (5.8%)	1 (1.52%)	16 (30.8%)
≥50	4(7.7%)	0	12 (23.07%)
Total	11	1	40

DISCUSSION: The distribution of HCV genotypes varies substantially in different parts globally. HCV genotype should be correctly determined before initiation of treatment, as it determines the appropriate selection of antiviral drugs, the duration of treatment, the dose of the drug, and the viral monitoring procedure and prediction of prognosis [14]. A study in Serbia showed that genotype 1 (A and B) was the most prevalent genotype with a distribution of over 60%, followed by genotype 3 [15]. Genotype 4 is mainly seen in Egypt and the Middle East and genotype 6, earlier restricted to the Southeast Asian countries, is being increasingly reported in India, [16] with genotype 3 being predominant in the northern, eastern, and western regions. Genotype 3 has also been reported as the commonest genotype from the neighbouring countries of Nepal and Pakistan. Increased prevalence of genotypes 4 and 6 have

been noted in certain regions of India [17]. Genotype 4 is found in patients from Southern India, especially those in Andhra Pradesh and Tamil Nadu. Genotype 6 is prevalent exclusively in patients from North-Eastern parts of India [16]. Another study showed that genotype 1 was the most dominant HCV genotype, followed by genotype 3, genotype 6, genotype 2, genotype 4, and genotype 5 [18]. A study from North India showed genotype 3 as the commonest type (61.2%), followed by genotype 1 (29%) and genotype 2 (9.6%), respectively [17]. This data corresponds to our results. In our study, the most common genotype detected was genotype 3 (76.9%), followed by genotype 1 (21.1%) and genotype 2 (1.92%) referenced in **Table 4**. Christdas J *et al.*, stated HCV genotype 3 as the predominant genotype (63.85%), followed by genotype 1 (25.72%) in India. Genotypes 4 (7.5%) and 6 (2.7%) appear to be somewhat geographically restricted in their distribution within India [19].

A recent study from Mumbai revealed that genotype 3 is the most frequent genotype, followed by genotype 1b in HCV-infected patients [20]. Another study showed that genotype 3a (68.07%) was the most common genotype, followed by genotype 1a (25%) and 1b (2.9%) [21]. It has been reported that the distribution of HCV genotype varies with age. A study from Pakistan showed genotype 1 seen more commonly in younger patients, while genotypes 2 and 3 were more prevalent in older patients [22].

Another study from North India, Delhi showed a higher percentage of genotype 3(38.7%) in the age group 18-40 years, followed by genotype 1(19.3%) and genotype 2(6.5%). In the age group of 41-60 years, genotype 3 and genotype 1 were 19.4% and 6.4%, respectively. There is a piece of evidence suggesting that different types of HCV genotypes may be associated with different transmission routes [17]. Our study showed the highest distribution of genotype 3 (30.8%) in the age group of 31-50 years, favored by the exposure of this population to the environment, workplace, and other factors likely to transmit infectious diseases and conditions. Genotype 2, being the rarest, was detected only in one case in this age group. Genotype 1 was common in the elderly population of the age group ≥50 years (7.7%), followed by the age group 19-50 years (5.8%), and only one case (1.92%) in 0-18 years **Table 4**. We also found that genotype 3 was predominant in both males and females among all genotypes. The information provided by the present study and other studies from different regions provides relevant information to physicians in clinical decision-making to determine the correct treatment, the duration of treatment, the dose of the drug, and the viral load monitoring and prediction of prognosis. Infection with genotype 1 is reportedly associated with more severe liver disease and has a more aggressive course than other HCV genotypes. Improved treatment response rates have been observed with genotypes 2 and 3, compared to genotypes 1 and 4 [23]. Such epidemiological studies and trend analysis are also important to document in the wake of the licensure of the newer generation of directly acting antiviral drugs for HCV. In addition, this epidemiological information may also help in vaccine development strategies [19].

CONCLUSION: In the present study, we detected the most common genotype as genotype 3 followed by genotype 1 and genotype 2. Genotype 2 could be detected only in one case, while genotype 4 was not. This study showed a higher percentage of genotype 3 in the age group 31-50 years followed by ≥50 years, 19-30 years, and age group 0-18 years. Genotyping of HCV has become important in treating infection, monitoring the disease progression, and epidemiology studies.

The World Health Organisation aims to eradicate HCV by 2030 (World Hepatitis Summit 2017). Our study provides comprehensive data for HCV epidemiological research. It is very important to Educate and create awareness amongst the population about the virus and its modes of transmission, disease progression, and methods of prevention of the acquisition of viruses. This could be prevented by education, screening of blood products, greater availability of disposable needles and needle exchange programs for drug abusers, proper counseling, and treatment of the affected person, and thus may result in the reduction of transmission of the virus.

Limitations: This study's impact and weightage are restricted due to the small sample size.

ACKNOWLEDGMENT: Nil

Ethical Approval: Informed and written consent were taken from study participants. The Institutional Ethics Committee approved the study with Clearance Code: 148/2017.

Funding Information: This work received no specific grant from any funding agency.

CONFLICTS OF INTEREST: Nil

REFERENCES:

1. World Health Organisation (WHO). Diseases; Hepatitis C. https://www.who.int/news-room/fact-sheets/detail/hepatitis-c [Last accessed on 30 July 2021].
2. Alter MJ, Margolis HS and Krawczynski K: The natural history of community-acquired hepatitis C in the United States. The Sentinel Counties Chronic non-A, non-B Hepatitis Study Team. N Engl J Med 1992; 327(27): 1899-1905. doi:10.1056/NEJM199212313272702
3. El-Serag HB: Epidemiology of viral hepatitis and hepatocellular carcinoma. Gastroenterology 2012; 142(6): 1264-1273.e1. doi: 10.1053/j.gastro.2011.12.061. PMID: 22537432; PMCID: PMC3338949.
4. Mehta P and Reddivari AKR: Hepatitis. [Updated 2021 Jan 16]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK554549
5. Hoofnagle JH: Course and outcome of hepatitis C. Hepatology 2002; 36(51): 21-29.
6. Lee MH, Yang HI, Yuan Y, L'Italien G and Chen CJ: Epidemiology and natural history of hepatitis C virus infection. World Journal of Gastroenterology WJG 2014; 20(28): 9270.
7. Shi G and Suzuki T: Molecular Basis of Encapsidation of Hepatitis C Virus Genome. Front Microbiol 2018; 9: 396. doi: 10.3389/fmicb.2018.00396
8. Konan KV, Giddings TH, Ikeda M, Li K, Lemon SM and Kirkegaard K: Nonstructural protein precursor NS4A/B from hepatitis C virus alters function and ultrastructure of host secretory apparatus. J Virol 2003; 77(14): 7843-7855. doi:10.1128/jvi.77.14.7843-7855.2003
9. Casanova YS, Boeira Tda R and Sisti E: A complete molecular biology assay for hepatitis C virus detection, quantification and genotyping. Rev Soc Bras Med Trop 2014; 47(3): 287-294. doi:10.1590/0037-8682-0040-2014
10. Chen M, Zheng F, Yuan G, Duan X, Rong L, Liu J, Feng S, Wang Z, Wang M, Feng Y, Zhou Q, Li J, Deng K, Li C, Xia J, Rao G, Zhou Y, Fu Y and Li YP: Development of an Infectious Cell Culture System for Hepatitis C Virus Genotype 6a Clinical Isolate Using a Novel Strategy and Its Sensitivity to Direct-Acting Antivirals. Front. Microbiol 2018; 9: 2950. doi: 10.3389/fmicb.2018.02950
11. Li HC and Lo SY: Hepatitis C virus: Virology, diagnosis and treatment. World J of Hepatology 2015; 7(10): 1377.
12. Halfon P, Bourlière M, Pénaranda G, Khiri H and Ouzan D: Real-time PCR assays for hepatitis C virus (HCV) RNA quantitation are adequate for clinical management of patients with chronic HCV infection. J Clin Microbiol 2006; 44(7): 2507-2511. doi:10.1128/JCM.00163-06
13. Sizmann D, Boeck C and Boelter J: Fully automated quantification of hepatitis C virus (HCV) RNA in human plasma and human serum by the COBAS AmpliPrep/COBAS TaqMan system. J Clin Virol 2007; 38(4): 326-333. doi:10.1016/j.jcv.2006.12.021
14. Chevaliez S and Pawlotsky JM: Hepatitis C virus: virology, diagnosis and management of antiviral therapy. World J Gastroenterol 2007; 13(17): 2461-2466. doi:10.3748/wjg.v13.i17.2461
15. Đorđević V, Stanković Đorđević D, Kocić B, Dinić M, Sokolović D and Pešić Stanković J: The Impact of Hepatitis C Virus Genotypes on Oxidative Stress Markers and Catalase Activity. Oxid Med Cell Longev 2021; 2021: 6676057. Published 2021 Feb 25. doi:10.1155/2021/6676057
16. Irekeola and Ahmad Adebayo: "Prevalence of HCV genotypes and subtypes in Southeast Asia: A systematic review and meta-analysis." PloS One 2021; 16(5): 0251673. doi:10.1371/journal.pone.0251673
17. Chakravarti A, Ashraf A and Malik S: A study of changing trends of prevalence and genotypic distribution of hepatitis C virus among high risk groups in North India. Indian J Med Microbiol 2013; 31(4): 354-359. doi:10.4103/0255-0857.118877.
18. Irekeola AA, Malek NA, Wada Y, Mustaffa N, Muhamad NI and Shueb RH: Prevalence of HCV genotypes and subtypes in Southeast Asia: A systematic review and meta-analysis. PLoS One 2021; 16(5): 0251673. Published 2021 May 20. doi:10.1371/journal.pone.0251673.
19. Christdas J, Sivakumar J, David J, Daniel HD, Raghuraman S and Abraham P: Genotypes of hepatitis C virus in the Indian sub-continent: a decade-long experience from a tertiary care hospital in South India. Indian J Med Microbiol 2013; 31(4): 349-353. doi:10.4103/0255-0857.118875.
20. Yabaji PM, Shankarkumar A, Shukla A and 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): 352-356. doi:10.4103/ijmm.IJMM_18_221.
21. Prakash S, Shukla S, Ramakrishna V and Jain A: Distribution of hepatitis C genotypes in Uttar Pradesh, India; rare genotype 4 detected. J Med Virol 2018; 90(12): 1875-1881. doi:10.1002/jmv.25277.
22. Idrees M and Riazuddin S: Frequency distribution of hepatitis C virus genotypes in different geographical regions of Pakistan and their possible routes of transmission. BMC Infect Dis 2008; 8: 69. Published 2008 May 23. doi:10.1186/1471-2334-8-69.
23. Okanoue T, Minami M, Makiyama A, Sumida Y, Yasui K and Itoh Y: Natural course of asymptomatic hepatitis C virus-infected patients and hepatocellular carcinoma after interferon therapy. Clin Gastroenterol Hepatol 2005; 3(102): 89-91. doi:10.1016/s1542-3565(05)00701-9.

How to cite this article:
Verma RK, Radhika, Singh DP, Sethi K and Singh S: Molecular characterization of hepatitis C virus in a Tertiary Care Hospital in Rural India of Western Uttar Pradesh. Int J Pharm Sci & Res 2023; 14(10): 4924-28. doi: 10.13040/IJPSR.0975-8232.14(10).4924-28.