

Distribution of Genotypes of Hepatitis C Virus Studied in a Tertiary Care Hospital in Uttarakhand, India: A Retrospective Study

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ABSTRACT

Introduction: With more than one million cases of Hepatitis C viral infection reported annually, it has become a major global problem. Hepatitis C Virus (HCV) is classified into 7 genotypes and 67 subtypes. Various genotypes show a wide geographical distribution worldwide. Genotypes 1 and 3 are the most common genotypes reported from India. There is minimal data available on the distribution and frequency of HCV genotypes in Uttarakhand.

Aim: This three years retrospective study was undertaken to find out the distribution pattern of HCV genotypes among HCV-infected patients attending a tertiary care hospital in this Sub-Himalayan state of Uttarakhand.

Materials and Methods: A total of 306 samples were tested for HCV genotyping in 3 years duration i.e., January 2018 to December 2020 at the Molecular Laboratory of Shri Guru Ram Rai Institute of Medical and Health sciences and associated

Shri Mahant Indiresh Hospital, Dehradun. Genotyping was performed using HCV genotype specific Real-Time Polymerase Chain Reaction (RT-PCR) assay on Rotor-Gene Q using TRUPCR HCV Genotyping Kit (3B BlackBio Biotech India Ltd.) as per manufacturer's instructions. This test kit detects HCV genotypes 1, 1a, 2 (2a/ 2b), 3, 4, 5a, and 6.

Results: Genotype 3 (72.7%) was the commonest genotype detected, followed by genotype 1 (13%), genotype 1a (6.25%) and genotype 4 (2.8%). Genotype 3 was the most dominant genotype in all the 3 years of the study. Two cases of genotype 5a (1.13%) and three cases of genotype 6 (1.7%) were also detected in our study. We also detected a case of mixed infection by genotype 1 and genotype 6.

Conclusion: The most common genotype detected was three during the study period. Detection of genotype 5a and 6 ascertained the impact of interstate migration of people.

Keywords: Genotyping, Hepatocellular carcinoma, Real-time PCR, Viral infection

INTRODUCTION

Viral hepatitis has become a big global challenge. Annually, more than one million new cases of infection are reported globally. It is believed that HCV is more prevalent than HBV infection [1]. Up to 80% global burden of HCV infection is borne by low resources area like India, East Asia, North Africa and the Middle East [2]. Nine percent of the world's HCV infected persons reside in India (~12-18 million). But due to a big population size, the overall prevalence remains low to moderate (1-1.5%) [3,4].

The HCV genome consists of 10 kb long, positive-sense, single-stranded Ribonucleic Acid (RNA). Nucleotide sequence variability in the viral genome of different isolates of HCV around the world is substantial [5]. Rapid globalisation has led to genetic diversity of HCV, which impacts the response to various therapies in different geographical areas. Based on genetic variability HCV is divided into 7 genotypes (designated as genotype 1 to 7) and 67 subtypes [6]. In some types, the closely related strains of HCV are assigned as subtypes. Genetic complex variants found in individuals isolate is given the terminology quasi species. These quasi species of HCV results from mutations that have accumulated during viral replication [7-11].

The variation of genomic sequences of various HCV isolates is up to 35% [12]. Two important parameters influencing the treatment outcomes are HCV genotype and viral load. HCV remains one of the major aetiology of chronic liver diseases like liver fibrosis, liver cirrhosis and Hepatocellular Carcinoma (HCC), having high morbidity and mortality rate [13].

In India, with approximately 63.85%, genotype 3 predominates while genotype 1 with approximately 25.72% follows. Genotype 1 is more prevalent in Southern India while genotype 3 is distributed

more in Northern, Eastern and Western parts of India. Genotype 2 is rarely reported while genotype 5 has yet to be reported from India [14].

In spite of broader prevalence of genotypes 3 and 1, in some parts of India, genotype 4 and 6 are showing an increase in prevalence. States of Southern India (Andhra Pradesh and Tamil Nadu) have reported genotype 4 while North-Eastern regions of India have higher prevalence of genotype 6 [15]. This observation has been linked to the political international boundaries shared between North-Eastern states of India and Myanmar where genotype 6 is prevalent [16].

Various serological and molecular methods are available for detecting HCV infection [17]. Accurate diagnosis of HCV is highly recommended before starting the treatment. Stage of infection is also crucial because the infection get spontaneously cleared in about 15-45% persons within 6-12 months. Thereafter, anti HCV antibodies may remain positive while viraemia is not detectable. So both serological and molecular tests for HCV infection significantly help in differentiating infected from recovered persons [18]. Previously, anti HCV antibody positive individuals were confirmed for their infectivity by Recombinant Immune Blot Assay (RIBA). Currently, Nucleic Acid Testing (NAT) methods have replaced RIBA as confirmatory test for the diagnosis of infectivity status of HCV and are considered the gold standard for confirming active HCV infection [19].

HCV genotyping along with HCV RNA quantitative viral load assessment may be helpful in selecting the treatment modalities like, interferons in chronic HCV infections. Currently, the relationship between HCV genotype and selection of candidates for treatment

is not yet explored. However, the duration of treatment has been found to have some role in treating HCV genotype 1, where 48 weeks course of interferon and ribavirin treatment has led to sustained long term remission. Sequencing of specific PCR amplified product of HCV genome from the patient followed by phylogenetic analysis is considered the reference standard for HCV genotyping [20].

In a study by Amoroso P et al., the role of HCV genotypes in persistence of HCV infection after an acute exposure was assessed. They found that 92% patients infected with HCV genotype 1b developed chronic infection after an acute illness, as compared to other genotypes. This suggests that apart from host factor, certain viral factors, like HCV genotype may play an important role in progressing to chronicity after acute exposure to HCV infection [21].

There is a paucity of data for the circulating HCV genotypes in the young state of Uttarakhand [22]. This retrospective study was designed with the aim to determine the different HCV genotypes present among HCV-infected patients attending a tertiary care hospital in this Sub-Himalayan state of Uttarakhand.

MATERIALS AND METHODS

This retrospective study was performed in the Molecular Testing Laboratory under the Department of Microbiology, of a tertiary care hospital in Dehradun, Uttarakhand after the approval from Institutional Ethical Committee (Registration no. ECR/710/Inst/UK/2015/RR-18) vide letter no. SGRR/IEC/34/21 dated 18.02.2021. Data for the study was extracted from laboratory information system and laboratory registers from January 2018 to December 2020. Data collection and analysis was done from March 2021 to July 2021. Case selection for HCV genotyping was done by the clinicians.

Procedure: A total of 306 blood specimens in Ethylenediaminetetraacetic Acid (EDTA) vial submitted to the Molecular Testing Laboratory for HCV Genotyping were subjected to purification of plasma followed by extraction of HCV RNA using QIAamp Viral RNA mini kit (Qiagen) as per manufacturer's instructions. Extracted RNA was subjected to HCV genotype specific real-time PCR assay on Rotor-Gene Q using TRUPCR HCV Genotyping Kit (3B BlackBio Biotech India Ltd) as per manufacturer's instructions. This test kit detects HCV genotypes 1, 1a, 2 (2a/ 2b), 3, 4, 5a, and 6. TRUPCR HCV Genotyping kit is a two-step real time reverse transcription PCR assay in which RNA templates are first reverse-transcribed to generate complementary cDNA followed by DNA PCR. After generation of cDNA, for each sample three separate reaction mixture have to be make (tube 1, tube 2 and tube 3). Tube 1 for HCV detection and HCV genotypes 1 and 5a detection, tube 2 for HCV genotypes 1a, 4 and Internal Control (IC) detection and tube 3 for detection of HCV genotypes 2a/2b, 3 and 6. The limit of detection of the TRUPCR HCV Genotyping kit is 500 IU/mL. All instruments used were calibrated as per protocol. Result analysis was done according to the following [Table/Fig-1] (as per kit insert).

Tube no.	Channels	Signal	HCV genotype
1	FAM/Green	Present	HCV detection
	HEX/VIC/Yellow	Present	5a
	Tex Red/ROX/Orange	Present	1
2	FAM/Green	Present	1a
	HEX/VIC/Yellow	Present	4
	Tex Red/ROX/Orange	Present	Internal control
3	FAM/Green	Present	3
	HEX/VIC/Yellow	Present	2a/ 2b
	Tex Red/ROX/Orange	Present	6

[Table/Fig-1]: Result interpretation as per kit instructions.

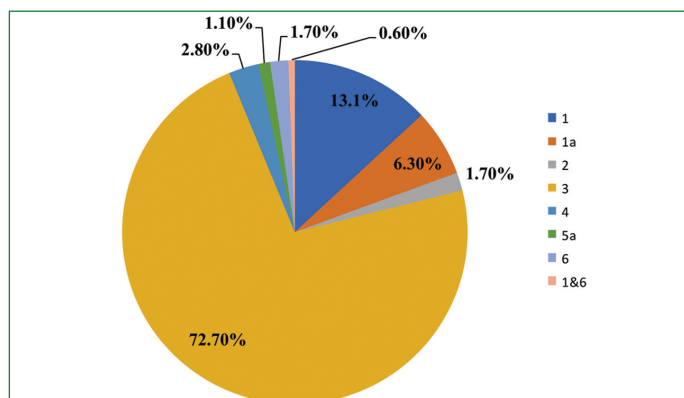
STATISTICAL ANALYSIS

Lab Information System of the hospital was the source of demographic data of study population. The collected data was imported into Microsoft Excel spreadsheet. The important patient identifiers were properly and safely discarded to maintain patient confidentiality. The information regarding the patient age group, sex, genotype was studied and analysed.

RESULTS

During the study period of three years i.e., January 2018 to December 2020, a total of 306 samples were received for HCV genotyping. A total of 116 samples were received annually in both 2018 and 2019. A total of 74 samples were received in the year 2020. Out of 306 samples, 153 belonged to male patients and 153 belonged to female patients coincidentally. The most dominant age group was 21-40 years (129 samples) followed by 41-60 years (111 samples), >60 years (56 samples) and <20 years (10 samples). Risk factors and social data were unavailable at the time of study.

Out of these 306 samples, genotypes were detected in 176 (57.5%) samples. In 130 samples, no target was detected. One sample was detected with two genotypes, namely genotype 1 and 6. In the span of three years, the annual distribution of positive samples was as follows: 55/176 (31.25%) in 2018, 78/176 (44.31%) in 2019 and 43/176 (24.43%) in 2020. When gender distribution of the positive cases was analysed it was found that 52.8% of positive samples belonged to female patients while 47.15% belonged to male patients. [Table/Fig-2] depicts the distribution of various genotypes detected during the study period in which Genotype 3 (72.7%) was the most common genotype detected, while the least detected was genotype 5a (1.1%). Genotype 3 was the commonest genotype in all three years [Table/Fig-3].



[Table/Fig-2]: Distribution of various HCV genotypes in the study.

Genotype → Year ↓	3	1	1a	4	2	5a	6	Others	Total
2018	37	2	11	1	2	1	1	0	55
2019	60	12	0	2	1	0	2	1 (1 and 6)	78
2020	31	9	0	2	0	1	0	0	43
Total	128	23	11	5	3	2	3	1	176

[Table/Fig-3]: Year wise distribution of most common genotypes detected.

The distribution of various genotypes among both genders when analysed shows that in males, genotype 3 (69.8%) was the commonest followed by genotype 1 (16.8%) and genotype 1a (7.2%) and in females, genotype 3, genotype 1 and genotype 1a were detected in 75.2%, 9.6% and 5.3%, respectively. [Table/Fig-4] shows age-group wise genotype distribution. In our study, 21-40 years was the predominant age-group affected accounting for 42.6% of the positive samples. This was followed by 41-60 years age-group (38.63%). It was also observed that genotype 3 was the most common genotype in all the age groups. On using one-way Anova, it was observed that the mean age of positive cases for

Genotype → Age group ↓ (years)	1	1a	2	3	4	5a	6	Others	Total	Cumulative percentage (total sample=176)
≤20	1 (20)	1 (20)	0	2 (40)	0	0	1 (20)	0	5 (100%)	2.8%
21-40	8 (10.66)	3 (4)	1 (1.3)	58 (77.3)	3 (4)	1 (1.3)	1 (1.3)	0	75 (100%)	42.6%
41-60	12 (17.6)	3 (4.4)	2 (2.9)	49 (72.0)	1 (1.4)	1 (1.4)	0	0	68 (100%)	38.6%
>60	2 (7.14)	4 (14.28)	0	19 (67.8)	1 (3.5)	0	1 (3.5)	1 (3.5)	28 (100%)	15.9%

[Table/Fig-4]: Age-group wise genotype distribution (n=176).
Figures in parentheses indicate percentage

Year	Mean age±SD (years)	p-value
2018	45.09±15.58	0.13
2019	44.52±15.92	
2020	39.51±12.26	

[Table/Fig-5]: Year wise distribution of mean age of positive cases.

2018, 2019 and 2020 was 45.09±15.58 years, 44.52±15.92 years and 39.51±12.26 years, respectively [Table/Fig-5].

DISCUSSION

The HCV infection is a major health problem with 71 million people infected worldwide. India has 6-12 million people who are anti-HCV positive [23]. HCV is the commonest cause of HCC, cirrhosis and chronic hepatitis [23]. Thus, it poses a chronic and continuous disease burden as the age advances. HCV is classified into 6 major genotypes and many subtypes. It shows marked geographical diversity [24]. Genotypes 3 and 1 are the most prevalent genotypes worldwide. In South-East Asia, genotype 1 is the most dominant followed by genotype 3, genotype 6, genotype 2, genotype 4 and genotype 6 [25]; whereas, in Europe, the predominant genotype is 1 followed by 3, 2 and 4 [26]. Determination of the type of genotype is important to ascertain as different genotypes show varied responses to the available treatment. The knowledge of genotype helps in deciding the treatment regime for the patient. India has very insufficient data of the genotype distribution. Although, some data is available for the major metropolitan cities [27-29], but small states such as Uttarakhand continue to face paucity of information [22].

Our study showed genotype 3 (72.7%) to be the most dominant followed by genotype 1 (13%), genotype 1a (6.25%) and genotype 4 (2.8%). Similar results were obtained by Chatterjee K *et al.*, from Uttarakhand: genotype 3 (77%), genotype 1a (21%) and genotype 4 (2%) [22]; whereas, a study from Hyderabad reported genotype 1 (45.5%) as the most common genotype followed by genotype 3 (39.9%) [27]. Similar results were reported from Karnataka which showed genotype 1 (78.38%) as the predominant genotype followed by genotype 3 (18.9%) [28]. All these results ascertained genotype 3 to be predominant in Northern India and genotype 1 in Southern India.

In our study, females (52.8%) were more than males (47.15%). The most common age group to be affected was 21-40 years (42.6%) followed by 41-60 years (38.63%). Our findings were similar to study by Chatterjee K *et al.*, which also had major proportion belonging to the age group of 21-40 years (37.2%) and 40-60 years (39.7%) [22]. Similar results were also shown in the study by Malhotra P *et al.*, in Haryana. About one fourth (26.02%) of the patients were in the age group 35 to 45 years. A 3.32% patients belonged to age group 11-20 years and 1.03% of the patients were over 60 years of age [29]. These results highlight the importance of creating awareness against the risk factors amongst targeted population of young adults. Heightened screening of HCV should be done in the high-risk groups such as intravenous drug abusers and blood transfusion recipients in the age group of 20-40 years. We observed the mean age of positive patients showed a decreasing trend. 45.09±15.58 years (2018), 44.52±15.92 years (2019) and 39.51±12.26 years (2020). Although this association was not found to be statistically significant (p-value 0.13) but it highlighted the need for increased screening of HCV infections in younger age groups.

In our study, we found two cases of genotype 5 (1.13%) and three cases of genotype 6 (1.7%). There are very few documented data for these genotypes in India. Malhotra P *et al.*, had reported 0.2% cases of genotype 5 and 0.1% cases of genotype 6 cases in their study from Haryana [29]. Mishra BK *et al.*, in their study from Nepal reported 3.1% cases of genotype 5 [30]. Barman B *et al.*, from Meghalaya had reported genotype 6 in 30.8% patients in their study [31]. A study from Hyderabad reported 8 (0.9%) cases of genotype 6 [27]. Genotype 6 is reported from many South East Asian countries such as Thailand and Indonesia [25]. Uttarakhand receives a lot of migrant population from Nepal and other regions of India where these genotypes are commonly found. Due to which such rare genotypes which are foreign to Uttarakhand are being reported. The increase in movement of individuals for employment and tourism from one area to another and across international borders may blur the clearcut distribution of genotypes in coming years.

We also detected a case of mixed infection by genotype 1 and genotype 6 in a 64-year-old man who gave a history of multiple sex partners in the past. Nguyen T *et al.*, reported three cases of mixed genotype infections in men who have sex with men [32]. Repeated intravenous drug abuse and having multiple sexual partners can lead to concurrent HCV infection with different genotypes.

Limitation(s)

Due to resource limitations, we could not perform sequencing for confirmation of the mixed infection (Genotype 1 and 6). The impact of mixed infection on direct-acting antivirals (DAAs) is not known and should be investigated.

CONCLUSION(S)

This study was undertaken to determine the recent trends in the HCV genotype distribution in the region of Uttarakhand. This three years retrospective study ascertained the fact that genotype 3 is the most common genotype in this region. Detection of cases of uncommon genotypes like 5 and 6 in this study was an eye-opener. In this era of good connectivity between different states and countries, genotypes distribution is no longer restricted to a geographical region. Detection of mixed genotypes paves the way for further research using sequencing techniques for their confirmation.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]

- Plagiarism X-checker: Nov 14, 2023
- Manual Googling: Jan 03, 2023
- iThenticate Software: Jan 06, 2023 (9%)

ETYMOLOGY: Author Origin**AUTHOR DECLARATION:**

- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? No
- For any images presented appropriate consent has been obtained from the subjects. No

Date of Submission: **Nov 10, 2022**Date of Peer Review: **Dec 06, 2022**Date of Acceptance: **Jan 07, 2023**Date of Publishing: **Jul 01, 2023**