Prevalence of Hepatitis C Virus Genotypes in Jammu: A Hospital Based Observational Study

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Abstract
Background and Objectives: Hepatitis C Virus (HCV) is an emerging infection in India and one of the major global health issues with an overall prevalence of 1%, among which 71 million people are living with chronic HCV infection. Every year 1.7 million of hepatic burden is added with an estimated 399,000 deaths. In India, North eastern tribal populations, and areas of Punjab are the major hotspots of HCV infection. HCV has been divided into 8 genotypes, each showing wide variation in their distribution. The present study was designed to estimate the prevalence of various HCV genotypes in Jammu region of J&K and study the correlation between detected genotypes and clinical presentation.

Materials and Methods: Blood samples of patients attending the Department of Medicine, Government Medical College, Jammu referred to VRDL section, Department Of Microbiology, GMC Jammu for serological testing by anti-HCV ELISA wherein the positive samples were subjected to genotyping.

Results: This study showed that, out of 50 HCV positive patients, 37 (74%) were positive for genotype 3, 8 (16 %) for genotype 1, 1 (2%) for genotype 4 and in 4 (8 %) genotype couldn’t be detected.

Conclusion: Jammu region of J&K has very few reports on the prevalence of HCV infection and hence knowledge of the predominant HCV genotypes is important for better management of chronic HCV infection and also helps clinicians in guiding appropriate antiviral therapy.

Introduction
Hepatitis C Virus (HCV) infection, one of the major global health issues and an emerging infection in India, is one of the important risk factor for Liver cirrhosis (LC), Hepatocellular carcinoma (HCC), and chronic hepatitis (Patel PH et al., 2017)¹. According to World Health Organisation (WHO), overall prevalence of HCV infection is estimated to be 1% of which 71 million people worldwide are living with chronic HCV infection, with 1.7 million new cases and 399,000 deaths occurring each year (WHO, 2017)². Most common transmission route for HCV is estimated to be parenteral like Intravenous...
drug abuse, reuse or inadequate sterilization of medical equipment, transfusion of unscreened blood and blood products (Sy T and Jamal MM, 2006)\textsuperscript{3}, sexual transmission and mother to child transmission being the less common modes of transmission.

HCV, a small enveloped virus belongs to genus Hepacivirus, family Flaviviridae with a positive sense single stranded ribonucleic acid (ss-RNA) genome. Viral genome has a total length of 9.6 kb with one open reading frame (ORF) and 5’ and 3’ untranslated regions (UTRs) at both edges and shows high degree of genetic variability and widespread mutations because of imperfect proof reading ability of viral RNA-dependent RNA polymerase (Ashfaq UA et al., 2011)\textsuperscript{4}.

On basis of phylogenetic and sequence analysis of whole viral genome, HCV strains have been classified into 7 major genotypes and a newly detected Genotype 8 (Borgia SM., et al 2018)\textsuperscript{5} with 67 confirmed and 20 provisional subtypes. Strains belonging to same subtypes differ at <15\% of nucleotide sites (Smith DB et al., 2014)\textsuperscript{6}. The distribution of HCV genotypes is highly variable with Genotype1 being most prevalent in Europe, Genotype 2 in Central Africa and Genotype 3 in India, Pakistan, Bangladesh, Myanmar, Nepal, Thailand and Northern European countries. Genotype 4 is most frequently found in Central Africa and the Middle East, genotype 5 is most common in Southern Africa while as Genotype 6 is the dominant genotype in Laos and Vietnam and is present at the highest frequencies in East and Southeast Asia. Increased prevalence of genotypes 4 and 5 is due to emigration from the Middle East and Africa.

Genotypic distribution of HCV in India suggests genotype 3 to be commonest (61.8\%), followed by genotype 1 (31.2\%). Genotypes 2, 4, 5, 6 have been detected in 0.05\% to 4.5\% cases (Satsangi S and Chawla YK, 2016)\textsuperscript{7}. Genotype 3 is most common in Northern, Western and Eastern India whereas genotype 1 is common in Southern India. Genotype 4 is recognised as an emerging threat and a cause of chronic liver disease in South India especially from states of Andhra Pradesh, Tamil Nadu and Kerala (Raghuraman S et al., 2004)\textsuperscript{8}. In north eastern part of India genotype 6 is exclusively prevalent (Christdas J et al., 2013)\textsuperscript{9}. Genotype 2 has rarely been reported from India whereas genotype 5 is yet to be reported. Such epidemiological and trend analysis can help in revolutionizing management of HCV by introduction of Directly-acting antivirals (DAAs).

India, second most populated country after China is a major contributor to global HCV burden, harbouring about 12-18 million HCV infected people, with an estimated prevalence of infection to be 0.5\% -1.5\% (Barman B et al., 2018)\textsuperscript{10}. The virus genotype does not influence the presentation of the disease, though it is a major predictor of course and severity of disease process. Study of genotypes has clinical significance as it is seen that serum HCV RNA levels are higher in patients infected with HCV genotype 1 than in those with genotype 2 (Kohara M et al., 1995)\textsuperscript{11}. Also, serum HCV RNA levels were found to be reduced to 4 times more in genotype 2 infected patients than in patients infected with genotype 1, thereby determining the type of antiviral drugs to be administered along with the duration, efficacy and response to the treatment (Kumar JP and Puttamaregowda H, 2016)\textsuperscript{12}.

Jammu and Kashmir has very few reports about the surveillance of HCV and being in the neighbourhood of Punjab which is a hot spot of HCV infection it becomes more important to study the prevalent genotypes of HCV in this state. Hence, this study was planned to detect prevalent genotypes of HCV in patients of Jammu region and their association with disease progression which eventually affect the selection of appropriate antiviral therapy.

Materials and Methods
This research and observational study was conducted over a period of one year i.e. November 2018 to October 2019 at Govt. Medical College Jammu, J&K, India, a tertiary care teaching
hospital, after clearance from the Institutional Ethical Committee. Blood samples were received from patients attending medical outpatient department and various wards of the hospital and processed in the Department of Microbiology. HCV infected patients above 15 years with jaundice, hepatic disease, and deranged liver function tests were included in the study, while those infected with hepatitis B virus, human immunodeficiency virus, and other hepatitis viruses were excluded from the study. Blood samples collected from medicine OPD were referred to VRDL section, Department of Microbiology, GMC Jammu. Serum was separated and samples were subjected to anti-HCV testing by ELISA (Merilisa HCV) as per manufacturer’s instructions. Anti-HCV positive samples were included for HCV RNA detection and genotyping studies.

**HCV RNA Extraction and Detection**

RNA extraction of anti-HCV positive samples was done by using Lysis buffer extraction method (Tan SC and Yiap BC, 2009)\(^1\). Extracted RNA was further subjected to agarose gel electrophoresis.

**HCV Genotyping**

Extracted RNA was further subjected to HCV genotyping using TRUPCR HCV Genotyping kit. Genotyping method consisted of a two step real time reverse transcription PCR assay; RNA templates were first reverse – transcribed to generate complementary c DNA strands followed by DNA polymerase- mediated c DNA amplification.

Results

![Microtitre plate for anti-HCV ELISA](image)

**Figure 1:** Microtitre plate for anti-HCV ELISA
Figure 2: Agarose gel electrophoresis showing RNA extraction

Figure 3: HCV Genotyping Agarose Gel Electrophoresis Results Lanes 1, 2, 4 & 6 show approximately 260 bp band corresponding to genotype 3 while in wells 3, 5 & 7 show ≈230 bp bands of genotype 1.

Among 50 HCV positive patients, 27 (54%) had high viral load values (> 8 lac IU/ml), while 23 (46%) had low viral load (< 8 lac IU/ml) (Fig: 4).
Figure 4: Figure depicting viral load of the infected patients

50 anti-HCV positive samples were subjected to genotyping studies. Most prevalent genotype was Genotype 3, reported in 37(74%) cases followed by Genotype 1 in 8(16%). In 1(2%) cases, genotype 4 was seen and in 4 (8%) cases Genotype was undetected (Fig 5).

Figure 5: Figure depicting the distribution of genotypes among the infected patients

Among 50 HCV positive patients, 40 (80%) patients were found to be asymptomatic i.e. they had no sign and symptoms of disease and were diagnosed during routine investigation for operation, blood donation or routine investigation for antenatal checkups etc. While 10 (20%) patients presented with common symptoms of hepatitis for example pain in right upper quadrant abdomen, vomiting, jaundice etc (Fig 6).

Figure 6: Clinical Profile of the patients

Discussion
Hepatitis C continues to be a major cause of concern with its hepatic wrath contributing towards a huge burden on public health systems. It is estimated that a minimum of 3% of the World’s population is chronically infected with HCV.
A knowledge of HCV genotyping has gained importance over the years as it has a role in elucidating the therapeutic, prognostic & clinical status of the HCV infection (A'iz H et al., 2013). Therefore, the present study was planned to determine the prevalence of various genotypes in Jammu region which would further help the clinicians in guiding appropriate therapy to control HCV infection.

In the present study, samples were collected from November 2018 to October 2019 from Medicine OPD, GMC Jammu. 5ml of venous blood was collected from each patient and sent to VRDL, Department of Microbiology, GMC Jammu for further processing. Serum was separated by centrifugation, aliquoted and stored at -20°C. Anti-HCV ELISA confirmed 50 HCV positive patients.

This study showed that, out of 50 HCV positive patients, 37 (74%) were positive for genotype 3, 8 (16 %) for genotype 1, 1 (2%) for genotype 4 and in 4 (8 %) genotype couldn’t be detected. Non-detection of Genotype in four patients would be due to low viral load, technical error or the fact that patients were on treatment since longer duration. In a similar study from North India by Sharma M et al., 2017 genotypes 3 and 1 were most commonly found, Chakravarti A et al., 2011 also reported genotypes 3 (63 %) and 1 (30.98 %) to be predominant in Delhi. Kumar S et al., 2014 reported that HCV genotype 3 is the most common genotype found in North India. Shah NA et al., 2017 in a study from Kashmir reported genotype 3 in 90.8% and genotype 1 in 8.1% cases. Studies by Al Mahtab M, 2016, Khan AW et al., 2017, Chaudhary S 2018, conducted in neighbouring countries of Nepal, Pakistan and Bangladesh have documented genotype 3 to be most prevalent. Contrary to this, Chen Y et al., 2017 reported genotype 1 (52.18%) to be most prevalent in China followed by genotype 2 (28.69%). Findings of our study also correlate with study from South India by Christdas J et al., 2013 where genotypes 3 and 1 were documented in 63.85% and 25.72 % cases respectively. Messina JP et al., 2015 from their study concluded that genotype 1 was most prevalent (46.2%) followed by genotype 3 (30.1%). Another study in Maharashatra by Mohanraj U et al., 2015 reported a rare genotype 5 in 1.25 % cases. Genotype 5 is predominantly found in South Africa. Occurrence of this rare genotype may be because of sporadic cases of transmission of genotypes from other geographical regions, where endemicity of this genotype is higher. Our study has reported a rare genotype- 4 in 2 % of the cases which is in accordance with study by Das BR et al., 2002 where genotype 4 was seen in 4% of the cases. Prakash S et al., 2018 in a study from Uttar Pradesh reported genotypes 3 and 1 to be most prevalent while genotype 4 was seen in 0.24% cases. Study by Raghuraman S et al., 2004 found genotype 4 in 7.2% cases. Genotype 4 is recognised as an emerging threat and a cause of chronic liver disease in South India especially from states of Andhra Pradesh, Tamil Nadu and Kerala (Raghuraman S et al., 2004). It is hypothesized that mass migration of individuals from South India to oil rich Middle East countries was followed by return of immigrants to India and HCV genotype 4 may have entered India because of transmission from these returning émigrés (Zachariah KC et al, 2001, 2002). In our study one case of genotype 4 was reported. In this region part of a population go to Middle East for better job opportunities and a large number of emigrants come to Jammu from UP and Bihar. Middle East & UP, Bihar are the hotspots of genotype 4 and this might be attributed for genotype 4 in our case. Study by Barman B et al., 2018 in North Eastern region of Meghalaya documented Genotype 3 (48.7%) to be most prevalent followed by genotype 6 (30.8%). Christdas J et al., 2013 reported genotype 6 to
be exclusively prevalent in North Eastern India. Also, study from Punjab by Borgia SM et al., 2018 documented presence of a novel HCV Genotype 8, thus expanding classification of HCV into 8 genotypes.

The present study showed that 87% cases of HCV genotype 1 had high viral load values while in HCV genotype 3, 46% cases had high viral load values. This is in accordance with study by Chakravarti A et al., 2011 which showed that genotype 1 had significantly higher viral load than genotype 3 which is attributed to more efficient viral replication of genotype 1 as compared to the others. Rong X et al., 2012 reported that genotype 3 is associated with lowest viral loads while another study by Nabi SG et al., 2013 and Rastin M et al., 2014 also reported that genotype 1 had high viral load values.

In our study genotypes 3 and 1 were most prevalent whereas similar studies from neighbouring states of Punjab, Haryana, and Delhi have also reported preponderance of HCV genotypes 3 and 1. In the neighbouring countries of our region like Nepal, Bangladesh, Pakistan genotypes 3 and 1 were found to be most common. Punjab is an important hotspot of HCV infection and a growing epidemic of IV drug abuse is an important risk factor for HCV infection in this region. Therefore, because of geographical niches and travelling to neighbouring countries could be the reason for detection of genotypes 3 and 1 in Jammu region.

**Conclusion**

HCV is a major cause of morbidity globally, with estimated 495,000 deaths; in India HCV infection was estimated to be responsible for 37,000 deaths in 2015 (National Guidelines for Diagnosis and Management of Viral Hepatitis, 2018). HCV infection can be a cause of substantial economic burden if patients are not provided with accurate and timely treatment. Considering the alarming increase in the trend of HCV infection, National Viral Hepatitis Control Programme (NVHCP) was launched by the Ministry of Health and Family Welfare, Government of India with the goal of ending viral hepatitis as a public health threat by 2030 in the country. Over the last decade HCV infection was predominantly treated with a combination of pegylated (PEG) interferon (IFN)-α combination with ribavirin (RBV), a nucleotide analogue. However, with the availability of new direct acting antiviral agents (DAAs), there has been a paradigm shift in management of HCV infected patients, with hope of eliminating HCV infection as a public health threat by 2030. Recently there has been an approval and adoption of pan genotypic DAAs. These pan genotypic drugs provide new opportunities for the public health response to HCV infection, with simplified procurement and no frequent laboratory monitoring. First pan genotypic regimen under NVHCP was combination of Sofosbuvir and Velpatasvir for a duration of 12 weeks.

Control of this infection requires a comprehensive approach including access to primary prevention of infection, enhanced screening against HCV in healthcare settings, and increased public awareness about the various modes of transmission and risk factors involved in spreading of infection. Therefore this study was planned to detect prevalent genotypes of HCV in patients of Jammu region and their association with disease progression which eventually affect the selection of appropriate antiviral therapy.

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