

Epidemiology and Genotypes of Hepatitis C Virus: A First Study from Jammu (J&K), India

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ABSTRACT

Background and objectives: Globally, around 200 million people are infected with hepatitis C virus (HCV). India contributes a big proportion of HCV burden with the prevalence estimated between 0.5 and 1.5%. Northeastern tribal populations and areas of Punjab represent the HCV infection hotspots, while in Western and Southern parts of the country, the prevalence is lower. The distribution of HCV genotypes is highly variable. This study was particularly planned to attain knowledge of the prevalent HCV genotypes in Jammu province of Jammu and Kashmir (J&K) state.

Materials and methods: Blood samples of patients attending the Department of Medicine, Government Medical College (GMC), Jammu and Kashmir, India, for HCV testing were subjected to serological test at Department of Microbiology, GMC, Jammu. The serum samples were tested for anti-HCV antibodies by enzyme-linked immunosorbent assay (ELISA) and positive samples were subjected to genotyping.

Conclusion: Of the 396 samples tested, 33 (8.33%) were found to be HCV positive and 23 of these were included for genotyping. Genotypes 3 and 1 were detected in this region and this was in accordance with other national studies. There is a need for larger field studies to better understand the HCV epidemiology and identify higher prevalence areas and also the distribution of genotypes of HCV. This, being a maiden study from this region, will shed light to allow apposite choice and target efforts for better management of the disease and reduce the burden of chronic liver disease due to HCV.

Keywords: Chronic liver disease, Genotyping, Hepatitis C virus, Seroprevalence.

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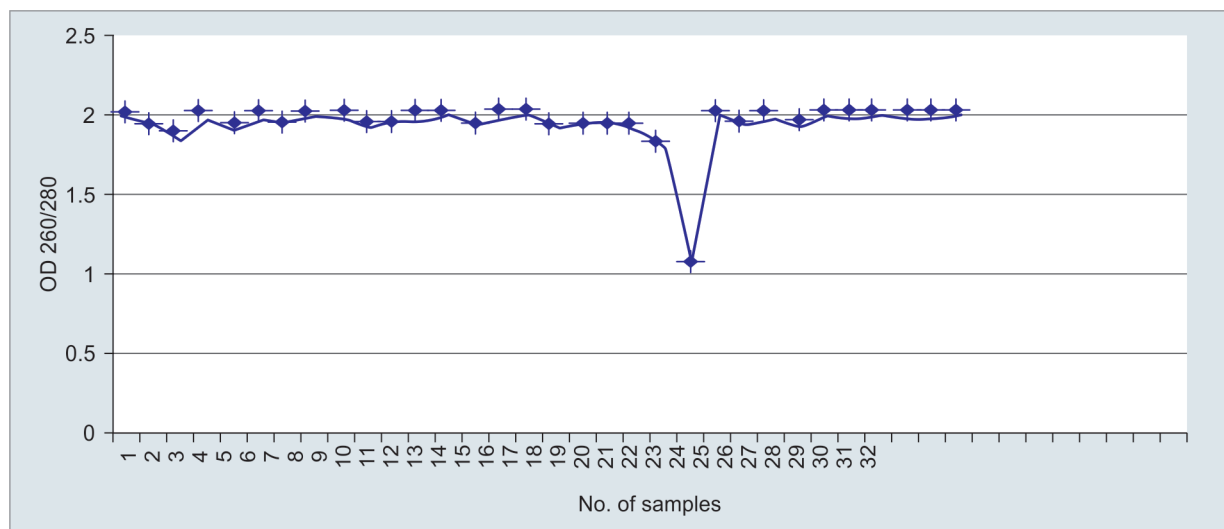
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INTRODUCTION

Hepatitis C virus infection is one of the major global health issues. The World Health Organization (WHO) estimates that HCV infects about 3% of the world's population, with 1.7 million new cases and 399,000 deaths occurring each year.¹ Hepatitis C virus is one of the significant causes of chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC). About 70 to 80% of infected individuals develop chronic hepatitis C (CHC) infection,² of which 3 to 11% develop liver cirrhosis within 20 years, along with the risk of liver failure and HCC,³ and the burden of the disease is expected to increase further as the infection persists lifelong.⁴ The WHO reports that about 71 million people have CHC infection globally and majority of infected people belong to the Asian subcontinent. It has been reported that HCV is transmitted primarily through the parenteral route in 20 to 80% due to iatrogenic exposures (transfusion/needlestick accidents/transplantation/dialysis of infected blood/blood products or organs/tissues) and use and sharing of contaminated injections/equipment. Sexual and maternal–infant transmissions of hepatitis C are less common.⁵

Hepatitis C virus is an enveloped icosahedral virus of 55 to 65 nm size and belongs to the family *Flaviviridae* with about 9.6 kb single-stranded, positive sense ribonucleic acid (RNA) genome.⁶ The poor fidelity of RNA-dependent-RNA-polymerase (NS5B protein) contributes to a high level of nucleotide sequence heterogeneity in the viral genome.⁷ Based on the sequence homology, six major HCV genotypes (1–6) and numerous distinct subtypes exist, e.g., 1b, 3a, etc.⁸ The distribution of HCV genotypes is highly variable. Genotypes 1 to 3 are distributed globally, whereas genotypes 4 and 5 are restricted to the Middle East and Africa, and genotype 6 occurs predominantly in South-East Asian countries.⁹ The mode of HCV transmission has an association with genotype distribution as spread of HCV subtype 1a via blood transfusion and unsafe medical practices has been documented.¹⁰

The genotypic distribution of HCV in India suggests genotype 3 to be the commonest (61.8%), followed by genotype 1 (31.2%). Genotypes 2, 4, 5, and 6 have been detected in 0.05 to 4.5% of cases.¹¹ The seroprevalence of



Graph 1: Quantification of RNA by nanodrop method

HCV in general population of India has been reported between 0.22 and 1.8%.^{12,13} Although there is availability of various antiviral drug regimens, determination of HCV genotypes remains an important determinant to establish the course and severity of the disease process and duration and efficacy of the antiviral therapy.¹⁴ Recent studies have shown an influence on the configuration of diagnostic assays and vaccine design and recognized a key role of genotyping in orthotopic liver transplantation. Moreover, the mode of transmission of HCV in 39% infected individuals remains unknown. Thus, genotyping may also attest to be a useful tool for tracing the source of infection.¹⁵

Jammu province of Jammu and Kashmir state has 10 districts, and this tertiary care center caters to the population from all the districts. The number of HCV infection is not well acknowledged in Jammu (J&K), India. Hence, we planned this hospital-based study to determine epidemiological aspects, district-wise distribution, and HCV genotypes prevalence in the patients attending the tertiary care center.

MATERIALS AND METHODS

This prospective study was conducted over a period of 1 year, i.e., March 2016 to Feb 2017 at Government 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. The HCV infected patients with jaundice, hepatic disease, and deranged liver function tests were included in the study, while those infected with hepatitis B virus (HBV), human immunodeficiency virus, and other hepatitis viruses were excluded from the study. Serum

was separated from 5 mL of blood collected aseptically from each patient. The serum samples were tested for anti-HCV antibodies by ELISA using SD HCV ELISA 3.0 (SD Biostandard Diagnostics Pvt. Ltd) as per manufacturer's instruction. Anti-HCV antibody-positive sera were included for HCV RNA detection and genotyping studies.

HCV-RNA Extraction and Detection

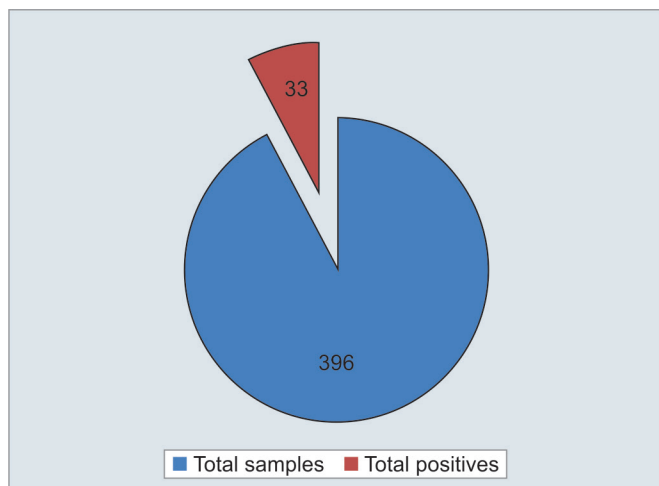
Extraction of RNA from sera was carried out manually using the protocol given by Okamoto et al¹⁶ with slight modifications. The enzyme inactivation was carried out by incubating the samples at 92°C for 10 minutes. Quantification of RNA was carried out by Nanodrop (Thermo Fisher) which functions by combining fiber optic technology and natural surface tension properties to capture and retain minute amounts of sample independent of traditional containment apparatus, such as cuvettes or capillaries. The RNA was successfully isolated and analyzed from only 32 sera (Graph 1).

HCV Genotyping

Samples from HCV patients were subjected to genotyping based on HCV core region-specific primers, using the method by Okamoto et al¹⁷ with slight modifications. The RNA was reverse-transcribed into complementary deoxyribonucleic acid using an antisense primer of the core region. For genotyping, HCV-specific amplicons were amplified using first- and second-round polymerase chain reaction, as reported by Sandres-Sauné et al¹⁸ using the genotype-specific primers. Amplicons were run and resolved on 2.5% agarose.

RESULTS

During the study period, 396 samples were tested for HCV and 33 tested positive (8.33%) (Graph 2). The



Graph 2: Total number of HCV samples tested from March 2016 to February 2017

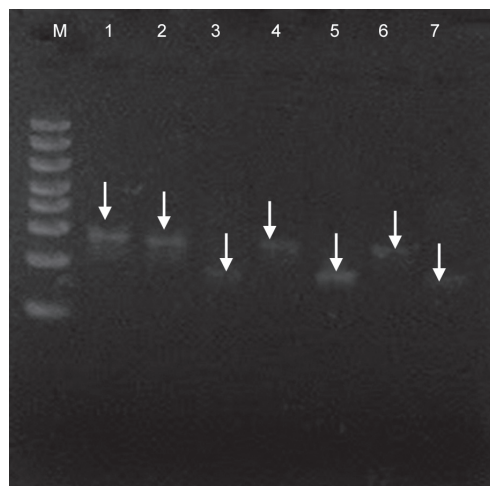
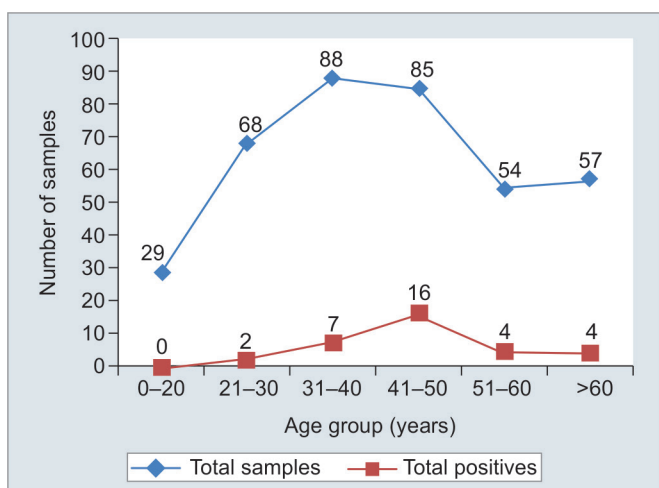
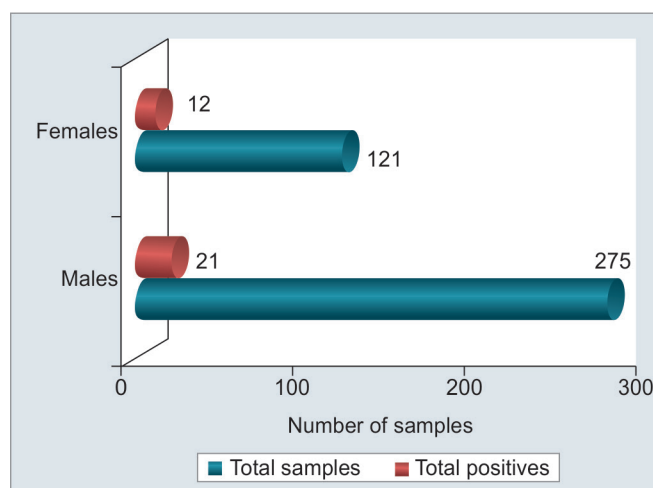


Fig. 1: Lanes 1, 2, 4, and 6 show approximately 260 bp band corresponding to genotype 3, while wells 3, 5, and 7 show ≈230 bp bands of genotype 1

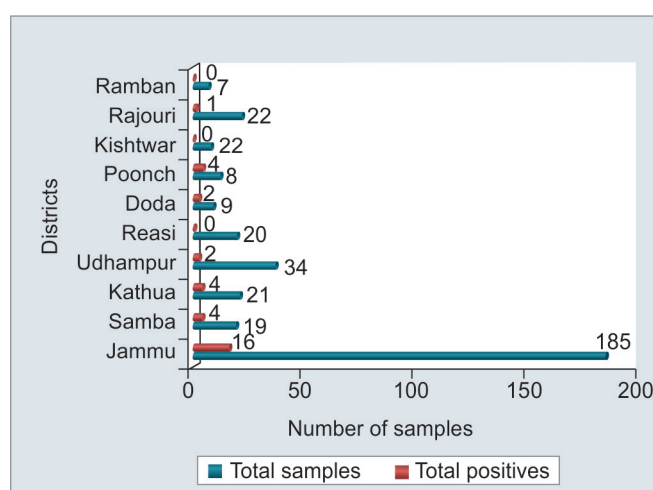


Graph 3: Age distribution of the total and positive HCV samples



Graph 4: Gender-wise distribution of the HCV samples

genotyping of 23 samples was performed (14 genotype 3; 9 genotype 1). Genotyping results of the seven samples in Figure 1 illustrate that sample number 1, 2, 4, and 6 showed approximately 260 bp band corresponding to genotype 3, while samples 3, 5, and 7 had an amplification at 230 bp, which concluded them to be of genotype 1. Majority of cases were reported between 41 and 50 years of age, followed by 31 to 40 years with 48.48% and 21.21% seropositivity respectively. Age groups of 51 to 60 years had 12.12% seropositivity, while 21 to 30 years and >60 years had an equal seropositivity of 6.06% each (Graph 3). Male preponderance was observed in gender distribution, wherein 63.63% (21/33) males and 36.36% (12/33) females were observed (Graph 4). Jammu district reported the maximum number of cases 48.48%, followed by Samba, Kathua, and Poonch with 12.12% seropositivity each. Udhampur and Doda reported seropositivity of 6.06% each and the district of Rajouri had



Graph 5: District-wise distribution of HCV-positive samples

seropositivity of 3.03% (Graph 5). All the samples from Kishtwar, Ramban, and Reasi districts were reported to be negative.

DISCUSSION

Over the last two decades, epidemiological studies have revealed the intricate patterns of infection affecting the global prevalence of this virus as well as its clinical presentation and treatment response.¹⁹ Hepatitis C virus infection is a preventable disease and the burden of the disease could be reduced to a significant extent if its seroprevalence is better known in the population. Therefore, this maiden study was carried out to achieve and document the baseline data of both the seroprevalence and also to determine various genotypes of HCV in patients attending the tertiary care center.

The current study reported a seropositivity of 8.33% in our hospital-based population, which is in concordance with the study from Delhi reporting seroprevalence of 5.5%.²⁰ In India, the seroprevalence of HCV varies among hospital-based populations with 1.57% from Cuttack,²¹ 1.7% from Jaipur,²² and 4.8% reported from Puducherry.²³

International studies from Mauritius,²⁴ Ethiopia²⁵ and Pakistan²⁶ have reflected a seroprevalence of 5.9, 6, and 9% respectively. On the contrary, a study from Iraq documented comparatively low seroprevalence of 0.81% for the year 2013.²⁷

Genotypes have a significant role in guiding the therapeutic, prognostic, and clinical outcome of the HCV infection. Chronic hepatitis C can be treated with antiviral therapy. Patients infected with HCV genotype 1 are treated with pegylated interferon and Ribavirin, while those infected with HCV genotype 2 or 3 have better response to interferon alpha.²⁸ Several clinical trials advocate that pretreatment determination of HCV genotype helps in deciding the appropriate treatment regimen and duration of therapy.²⁹ Our study has reported the predominance of HCV genotype 3 followed by genotype 1 in HCV infected patients, which is in accordance with the study from Delhi.³⁰ They further concluded that HCV genotypes 3 and 1 accounted for approximately 95% of the HCV infection in Delhi and surrounding areas. The epidemiological patterns of HCV genotypes have variegated geographical distribution among different regions of the same country. Several studies from India have reported all the genotypes, with genotype 3 being the most common in Northern, Eastern, and Western India,³¹⁻³³ whereas data from South India depicts high occurrence of genotype 1.³⁴

Several international studies from Pakistan and Southern Asia have also documented the predominance of genotype 3.³⁵ On the contrary, Southern and Eastern Europe, Japan, and North Africa show high prevalence of genotype 1, while genotype 2 is common in Northern

Italy. Genotypes 4 and 5 are highly common in Arabian countries and South Africa respectively. Genotype 6 prevails in Hong Kong and Vietnam.³⁶

In our study, male dominance was observed with seropositivity of 63.63% and is in agreement with the studies from Uttarakhand, Delhi, and Haryana.^{37,38} Another study from Delhi revealed the male preponderance with overall male to female ratio of 1.5:1.²⁰ A study from Pakistan revealed 46.29% of the males affected in comparison to females.³⁹ The male gender is most likely to indulge in risky behavior and practices, such as IV drug abuse, unprotected sex, etc., explaining the male predominance. However, Ramarokoto et al⁴⁰ in their study on the seroprevalence of hepatitis C in urban areas of Madagascar reported that the prevalence did not differ significantly according to gender but increased with age.

In the present study, the age group of 41 to 50 years showed the highest seropositivity of 3.28%, which is in concordance with other North Indian studies.^{41,42} The reason behind the dominance of HCV in a particular age group may be due to the long asymptomatic period of hepatitis C infection with symptoms appearing after a long latent period.⁴³ Adults are at higher exposure risk due to unsafe practices.⁴⁴ Hepatitis C virus is usually transmitted by sharing infected needles, unsafe transfusion practices, and accidental exposures.

The limitation of this study is that it is an institution-based study, so the patients going to private sector could not be included in the study. Hence, there is an expected underreporting. Also, the serological assay fails to detect the patients in the window period.

Although the sample size is small in this hospital-based study, but the findings throw light on the prevalent genotypes. However, more meticulous characterization of HCV subtypes needs to be done further on a greater sample size. Also, this familiarity of region and age-specific distribution of HCV infection is imperative for national disease prevention and control efforts, treatment, evaluating vaccination program, and also in liver transplantation. In addition, implementation of research focusing on region-specific endemicity levels is mandatory in developing countries where the burden of viral hepatitis is greater, ensuring greater prominence on innovative, safe, and cost-effective solutions.

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