

# Detection and distribution of genotypes of Hepatitis C in a tertiary care hospital

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## ABSTRACT

Hepatitis C virus (HCV) has emerged as a leading cause of chronic hepatitis and hepatocellular carcinoma. Thus, this study was undertaken for detection and to observe genotypic distribution of the virus in this geographical region. **Aims and Objectives:** The aim of the study was to quantitatively detect HCV RNA in Hepatitis C patients and to determine the distribution pattern of its genotypes by real time polymerase chain reaction. **Materials and Methods:** This is a retrospective study conducted in a tertiary care hospital. In seropositive Hepatitis C patients, quantification of HCV-RNA was done by real time PCR and in HCV-RNA positive samples, genotyping for HCV was conducted. **Result:** A total of 300 patients were recruited in the study, of which 165 were anti HCV positive and among them, 127 were HCV-RNA positive. These positive samples were further subjected to genotype determination using real time PCR. 98 samples were positive for genotype, where genotype 3 was seen in 90 patients, genotype 1, in 3 patients and genotype 4, in 5 patients. **Conclusion:** Knowledge of genotype is crucial for management of HCV infection and prediction of prognosis. Patients infected with HCV genotype 1 and 4 will have to receive IFN and ribavirin for a period of 48 weeks and show a poor sustained viral response. On the contrary, patients infected with HCV genotype 2 and 3 are reported to have better response to therapy.

**Keywords:** Anti HCV, HCV genotype, HCV-RNA, hepatitis C virus

## Introduction

Chronic infection with hepatitis C virus (HCV) is one of the major causes of liver cirrhosis and hepatocellular carcinoma. According to the World Health Organization, there are 180 million people affected with HCV worldwide. and about 12.5 million carriers in India.<sup>[1]</sup> The high rate of chronicity combined with the lack of a successful vaccine makes HCV infection a serious public health challenge. Early stages of the infection are missed because the antibodies develop only after one and half months of infection and the tests for anti

HCV antibody may be negative in the initial period before the seroconversion phase. HCV RNA detection by polymerase chain reaction (real time PCR) is highly sensitive and is a reliable test in the early diagnosis of HCV infection.<sup>[2]</sup>

HCV is an enveloped ribonucleic acid (RNA) virus belonging to family Flaviviridae. It was discovered in 1989 and was the first virus to be detected by employing molecular techniques.<sup>[3]</sup> HCV has been classified into six major genotypes and into more than 90 subtypes distributed across the world.<sup>[4]</sup> Hepatitis C virus (HCV) has population-specific genotypes and provides valuable epidemiological and therapeutic information. Hence, the importance of genotype knowledge is high for clinicians in devising therapeutic strategies.<sup>[5]</sup>

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Received: 18-04-2020

Revised: 10-06-2020

Accepted: 30-07-2020

Published: 30-10-2020

### Access this article online

#### Quick Response Code:



Website:  
www.jfmpc.com

DOI:  
10.4103/jfmpc.jfmpc\_651\_20

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**How to cite this article:** Bhumbra U, Shekhawat L, Kothari A, Rao J. Detection and distribution of genotypes of Hepatitis C in a tertiary care hospital. J Family Med Prim Care 2020;9:5249-51.

## Objective

The main objective of the study was, quantitative detection of HCV-RNA in Hepatitis C patients and to determine the distribution pattern of its genotypes by real time polymerase chain reaction.

## Materials and Methods

### Patients and study design

This is a retrospective study conducted in the Department of Microbiology in a 800 bedded tertiary care hospital. All the patients with known chronic liver disease attending the Department of Gastroenterology were referred to the Department of Clinical Microbiology and Serology of the Institution.

The study protocol was approved by the Institutional Ethics Committee. Written informed consent was obtained from all study subjects.

Five ml of blood samples were aseptically collected by venipuncture and stored in the sterile tubes containing the anticoagulant ethylene diamine tetra acetic acid. Plasma was separated and was subjected to Anti HCV serology, HCV-RNA quantification, and Genotyping by PCR.

### Inclusion criteria

Patients with established chronic liver disease (CLD).

### Exclusion criteria

Patients positive for Hepatitis B antigen (HBsAg), Human immunodeficiency virus (HIV), or those having history of alcohol intake were excluded from the study.

### Serological studies

Screening for HBsAg and anti-HCV antibody was conducted.

HBsAg: using commercially available ELISA Kit- (Hepalisa, J. Mitra and Co., India), as per manufacturer instructions.

Anti-HCV antibodies—using commercially available third generation ELISA Kits which comprised of Core, E1, E2, NS3, NS4, and NS5 antigens of HCV (Microlisa, J. Mitra and Co., India; Sensitivity: 100%, Specificity: 99.73%) as per manufacturer instructions.

HBsAg positive samples were excluded while anti-HCV antibodies positive samples were further processed for next step evaluation, that is quantitative detection of HCV-RNA was carried out by real time polymerase chain reaction and further in positive samples and genotyping was done.

HCV RNA:

1) Viral RNA extraction was performed by Geno Sen's Viral RNA Extraction Mini Kit (Genome Diagnostics Pvt. Ltd.).

2) HCV-RNA real time amplification was done by Geno Sen's HCV (Rotor Gene) Real Time PCR Kit (Genome Diagnostics Pvt. Ltd.).

3) HCV-RNA genotyping was conducted by Geno Sen's HCV Genotyping Real Time PCR Kit – Qualitative (Genome Diagnostics Pvt. Ltd.).

### Statistical analysis

Descriptive and inferential statistics were computed with SPSS for Windows version 17.0 software. Quantitative variables were expressed as median with range. The qualitative variables were expressed with numbers and percentage. A 95% confidence interval was calculated. Pearson's Chi square was used to compare categorical variables as applicable. *P* values less than 0.05 were considered significant.

## Results

A total of 320 patients were recruited in the study. Twenty patients, whose samples were found positive for HBsAg, were excluded from the study. Thus, 300 patients were included in the final analysis. Majority of these patients belonged were from, both rural and urban regions. These patients were divided into five major risk groups (blood transfusion recipients, IV drug users, unsafe medical procedures (including injections and minor surgeries not requiring blood transfusion), dental procedures, and tattooing.

Of the 300 patients screened, 165 (55%) were found positive for anti HCV antibody. 127/300 (42.3%) patients were found to have HCV RNA positive.

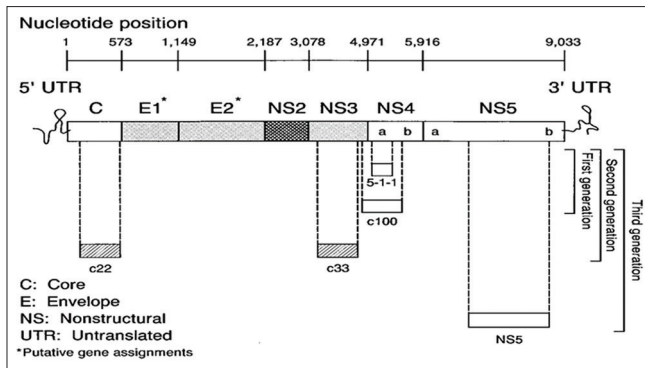
Total samples	Anti HCV positive	HCV-RNA positive	HCV-Genotype positive
300	165	127	98

A total of 98 (77.1%) samples were positive for genotype out of 127 HCV-RNA positive samples. Genotype 3 was the commonest type observed in 90 (91.8%) patients, followed by genotype 1 (3.06%) and 4 (5.1%). Other genotypes such as Type 2, 5, 6, and 7 were not detected.

## Discussion

A quantitative PCR helps in early diagnosis and also ascertains the baseline viral load before initiation of therapy on these patients. Earlier clinical and therapeutic trials have shown that patients with baseline HCV RNA level of more than  $8 \times 10^5$  IU/ml had 9% lower sustained virological response rate as compared to those with a viral load of less than  $8 \times 10^5$  IU/ml. It is also important to note that patients with low HCV RNA levels have 15–39% better response to therapy than those with high RNA levels.<sup>[6]</sup> Moreover, early source tracing can also prevent further transmission.

For physicians, knowing the genotype of Hepatitis C is helpful in deciding type and duration of therapy.<sup>[7]</sup> Several clinical



**Figure 1:** Genomic organization of HCV. First generation, second generation, and third generation refer to serologic assays for detection of HCV antibodies

trials of Pegylated interferon/ribavirin therapy have revealed significant differences in response rates for the various HCV genotypes. Individuals with genotypes 2 and 3 are more likely than individuals with genotype 1 to respond to therapy with alpha interferon or the combination of alpha interferon and ribavirin.<sup>[8]</sup> One probable reason for more treatment failures with HCV genotype 1 could be its efficient replication ability enabling it to establish higher viral RNA compared to other genotypes.<sup>[9]</sup> In the present study, patients with HCV genotype 1 had significantly higher viral load as compared to genotype 3 and 4. Patients with high viral load present a poor response to interferon therapy than those with lower levels [Figure 1].

In the United States, about 70% of cases are caused by genotype 1, 20% by genotype 2, and about 1% by each of the other genotypes. Genotype 1 is also the most common in South America and Europe. But in this study genotype 3 was the most common (90%) which was similar to other studies from North India. This was followed by genotype 1 (7%). Genotype 4 which was not observed by Chakravarti *et al*, was present in 3 (3%) of our patients. It has also been reported in Punjabi population of Lahore in Pakistan. Amarapurkar *et al* stated that 13/61 (21%) were Genotype 1, 15/61 were Genotype 2 (25%), and 33/61 (54%) were Genotype 3.<sup>[10]</sup>

## Conclusion

In conclusion, our data highlights that the rampant use of injections (unsafe), unscreened blood transfusion, and dental procedures are playing a significant role in increasing the reservoir of HCV infection in our country. This technique has proved its advancement and needs over conventional serological methods. Therefore, the implementation of RT PCR will be of great benefit to low-resource countries due to high prevalence of HCV. This underscores the need of strict

implementation of infection control practices in healthcare settings and creating awareness among public by mass media, public health education and proper counseling of persons with high-risk practices. Our study also shows that although genotype 3 is the most common genotypes in our region, other genotypes 1 and 4 are also prevalent. This is important since this could influence configuration of diagnostic assays as well as vaccine designs for our population. With the advancement in medical research and clinical trials of new drugs, the treatment of HCV infection has been shifted from pegylated interferon/ribavirin to direct-acting antiviral (DAA) combination therapies.

## Financial support and sponsorship

Nil.

## Conflicts of interest

There are no conflicts of interest.

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