

DETECTION AND DISTRIBUTION PATTERN OF PREVALENT GENOTYPES OF HEPATITIS C VIRUS IN A TERTIARY CARE HOSPITAL IN NORTH INDIA

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ABSTRACT

BACKGROUND

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.

The aim of the study is 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 study was conducted in a tertiary care hospital in north India from January 1, 2013 to June 30, 2015. Quantification of HCV-RNA was done by real time PCR and in HCV-RNA positive samples, genotyping for HCV was conducted.

RESULTS

A total of 465 patients were recruited in the study, of which 217 were anti-HCV positive and 420 were HCV-RNA positive. These positive samples were further subjected to genotype determination using real time PCR. 163 samples were subjected to genotyping, where genotype 3 was the commonest as seen in 102 patients, followed by genotype 1 seen in 25 patients and genotype 4 in 8 patients, and in 27 patients genotype was undetected.

CONCLUSION

Without reliable assays for antigenemia and the inability of antibody tests to define viraemia in all cases, the detection of viral nucleic acid is necessary for diagnosis of active HCV infection. Genotype is clinically important in determining potential response and duration to interferon-based therapy. Genotype 1 and 4 are less responsive to interferon based treatment than are the other genotypes (2, 3, 5 and 6). Duration of standard interferon based therapy for genotypes 1 and 4 is 48 weeks, whereas treatment for genotypes 2 and 3 is completed in 24 weeks.

KEYWORDS

Hepatitis C Virus, Anti-HCV, HCV-RNA, HCV Genotype.

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BACKGROUND

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. Approximately, 50%-70% patients infected with HCV develop chronic liver disease.¹

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.²

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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.³

Hepatitis C virus has been classified into seven major genotypes and into more than 90 subtypes distributed across the world.⁴

Hepatitis C virus (HCV) has population-specific genotypes, which provides valuable epidemiological and therapeutic information. Hence, the importance of genotype knowledge is high for clinicians in devising therapeutic strategies.⁵

Till date, no scientific data is available on the confirmation of genotypic distribution of HCV in the state of Punjab in northern India.

Aims and Objectives

The aim of the study is 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

Patients and Study Design

This is a study conducted in the Department of Microbiology in a 700 bedded tertiary care hospital in Punjab, North India from January 1, 2013 to June 30, 2015.

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 venepuncture and stored in the sterile tubes containing the anticoagulant ethylenediaminetetraacetic acid (EDTA). Plasma was separated and the investigation work-up included anti-HCV antibody and PCR based HCV-RNA quantitative assay.

Serological Studies

Screening for anti-HCV was conducted.

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. Positive and negative controls provided with the kit were used to validate the test and calculate the cut-off values (COVs) as per the manufacturer's guidelines.

HCV-RNA was quantified using standardised RNA extraction kits and amplification using TaqMan principle which states that during PCR, forward and reverse primers hybridise to a specific sequence product. A TaqMan probe, which is contained in the same reaction mixture and consists of an oligonucleotide labelled with a 5'- reporter dye and a downstream, 3'- quencher dye, hybridises to a target sequence within the PCR product. A Taq polymerase which possesses 5' to 3' exonuclease activity cleaves the probe. The reporter dye and quencher dye are separated upon cleavage, resulting in an increase in fluorescence for the reporter. Thus, the increase in fluorescence is directly proportional to the target amplification during PCR.

Quantitative detection of HCV-RNA was carried out by real time polymerase chain reaction and further in positive samples, genotyping was done by PCR.

HCV-RNA

1. Viral RNA extraction was performed.
2. HCV-RNA real time amplification was done.
3. HCV-RNA genotyping was conducted.

Estimation of ALT and AST

In all cases these biochemical parameters were estimated at the time of sample collection.

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 465 patients were recruited in the study. Majority of these patients belonged to Punjab, from both rural and urban regions. These patients could be 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 465 patients screened, 217 (46.66%) were found positive for anti-HCV antibody. 420/465 (90.32%) patients were found to have HCV-RNA positive.

Total Samples	Anti-HCV Positive	HCV-RNA Positive
465	217	420

Table 1

A total of 163 samples were subjected for genotyping. Genotype 3 was the commonest type observed in 102 (62.57%) patients, followed by genotype 1 in 25 samples (15.33%) and genotype 4 in 8 samples (4.90%). Out of these 163 samples, in 27 (16.56%) samples genotype was not detected due to low viral count. Other genotypes such as Type 2, 5, 6 and 7 were not detected.

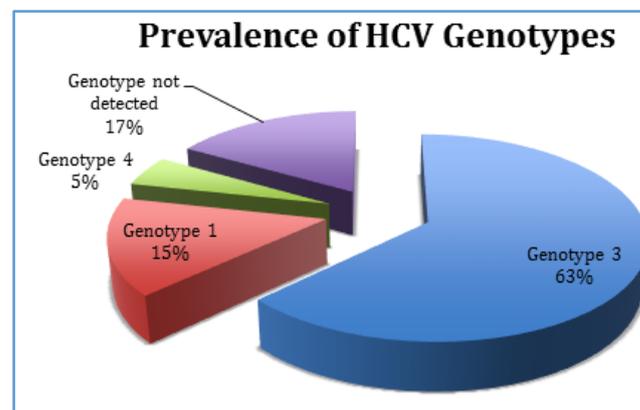


Figure 1

DISCUSSION

HCV-RNA has been found in the blood long before other markers and often within days of infection. All the reported studies from India on HCV infection are based on the detection of HCV infection using anti-HCV, which is relatively insensitive as compared to HCV-RNA which is more sensitive. Since the antibody test does not define viraemia in all cases, the detection of HCV-RNA is necessary for diagnosis of active HCV infection. A quantitative PCR helps in early diagnosis and also ascertains the baseline viral load before initiation of therapy on these patients. 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.⁶ Moreover, early source tracing can also prevent further transmission.

In the present study, HCV-RNA was used as the gold standard to document HCV infection. A correlation with anti-HCV antibody detection was included.

For physicians, knowing the genotype of Hepatitis C is helpful in deciding type and duration of therapy.⁷ Several clinical 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.⁸ 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.⁹ 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.

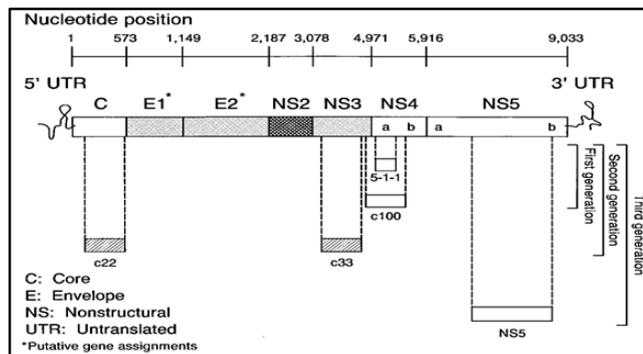


Figure 2. Genomic organisation of HCV. First generation, second generation, and third generation refer to serologic assays for detection of HCV antibodies.

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 (62.57%) which was similar to other studies from North India. This was followed by genotype 1 (15.33%). Genotype 4 which was not observed by Chakravarti et al was present in 8 (4.90%) of our patients. It has also been reported in Punjabi population of Lahore in Pakistan.¹⁰

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 Punjab. 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 counselling of persons with high-risk practices. Our study also shows that although genotype 3 is the most common genotype 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.

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