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Original Research Article

Hepatitis C Virus (HCV) RNA Quantification and Typing for the Identification of HCV Genotypes in clinical isolates

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E-mail: sharmanarotam5@gmail.com**Abstract**

The frequency of infection with the major genotypes of hepatitis C virus (HCV) was investigated in the current study. 30 serum specimens were quantified for HCV RNA viral load and their titre values were observed with the usage of Real Time PCR in-between 1.05×10^1 - 9.58×10^7 IU/ml. HCV genotype 3a was found in 17(56.6%) cases with highest prevalence. Genotypes 1a, 1b, 2a, 2b, 3b, 4, and 5a was found in 2(6.6%), 15(50%), 5(16.6%), 15(50%), 2(6.6%), 2(6.6%) and 2(6.6%) respectively. The findings also included that HCV viral RNA titre was elevated in HCV genotype 3a, assuming that HCV genotype 3a is the most replicated virus and need to be monitored thoroughly during diagnosis. Although type 1a, 1b, 2a, 2b, 3b, 4, 5a were also present but in lower proportion.

Keywords: HCV genotypes, Nested PCR, Viral load, Real time PCR, Amplicon, Quantitation Standard

1.Introduction

Hepatitis C virus (HCV) infection has reached epidemic proportions. Worldwide, more than one million new cases of infection are reported annually, and HCV is believed to be more prevalent than hepatitis B virus infection (HBV). Hepatitis C virus (HCV) infection is one of the most important Flaviviridae infections with significant clinical problems throughout the world in humans and it is responsible for the second most common cause of viral hepatitis [1]. To date at least six major genotypes of HCV, each having multiple subtypes, have been identified worldwide [2]. The different genotypes are relevant to epidemiology, vaccine development and clinical management of chronic HCV infection [3]. Also, the HCV genotype is the strongest prognostic parameter for continued virological response [4]. This clinical relevance of HCV genotyping attracted attention from studies that reported an influence of HCV genotypes on the

clinical course of disease and response to interferon therapy, as patients with different HCV genotypes respond differently to alpha interferon [5]. Firm evidence has been established that patients with type 2 and type 3 HCV infections are more likely to have a sustained response to therapy than patients with type 1 HCV infections [6]. Rates of sustained virological response to combination therapy in patients infected with HCV-2/3 and HCV-1 genotypes are 65% and 30%, respectively [7,8]. Therefore the patient genotype should be taken into consideration when prescribing interferon standard therapy. HCV genotypes 1, 2, and 3 appear to have a worldwide distribution and their relative prevalence varies from one geographic area to another. HCV subtypes 1a and 1b are the most common genotypes in the United States. These subtypes also are predominant in Europe [9-11]. The predominant subtype reported from Japan is subtype 1b that is

responsible for up to 73% of cases of HCV infection[12]. HCV subtypes 2a and 2b are relatively common in North America, Europe, and Japan and subtype 2c is found commonly in northern Italy. HCV genotype 4 appears to be prevalent in North Africa and the Middle East, and genotypes 5 and 6 seem to be confined to South Africa and Hong Kong, respectively. HCV genotypes 7, 8, and 9 have been identified only in Vietnamese patients, and genotypes 10 and 11 were identified in patients from Indonesia. There has been disagreement about the number of genotypes into which HCV isolates should be classified. Investigators have proposed that genotypes 7 through 11 should be regarded as variants of the same group and classified as a single genotype, type 6. Thus the current study was done to evaluate the Hepatitis C Virus (HCV) RNA Quantification and Typing for the Identification of HCV Genotypes 1a, 1b, 2a, 2b, 3a, 3b, 4, 5a, and 6a in the clinical isolates.

2. Materials and Methods

2.1 Source of Clinical Samples

30 serum samples were received along with all the clinical details of the subjects collected from different Departments of Shri Mahant Indresh Hospital, Dehradun which includes the wards and outdoor patients. The study was approved by institutional ethical clearance body and written consent was taken from the individual patients where ever required for the detection of HCV during the course of this study. The quantitation of HCV viral RNA is performed using the HCV Quantitation Standard. The HCV Quantitation Standard is non-infectious armored RNA construct that contains the HCV sequences with identical primer binding sites as the HCV RNA target and a unique probe binding region that allows HCV Quantitation Standard amplicon to be distinguished from HCV target amplicon. The HCV Quantitation Standard is incorporated into each individual specimen and control at known copy number and is carried through the specimen preparation, reverse transcription, PCR amplification and detection steps along with the HCV target. The COBAS Taqman 48 Real time PCR Analyzer calculates the HCV RNA titre in the test specimen and control. The HCV Quantitation Standard compensates for effects of inhibition and controls for the preparation and amplification processes to allow the accurate quantitation of HCV RNA in each specimen. RNA was isolated by high pure viral nucleic acid extraction system and then converted into cDNA which was utilized as template for HCV quantification as well for HCV genotyping.

2.2 cDNA Synthesis

For cDNA synthesis from HCV RNA, accuscript kit from agilent biotech was used as per the manufacturer's protocol.

2.3 HCV Genotyping

HCV genotyping was done with the nested PCR. The first round includes, 5µl PCR buffer, 5µl dNTPS, 5µl MgCl₂, 0.5µl each forward and reverse primers, 0.25µl of Taq DNA polymerase, 8µl of nuclease free water with final addition of 5µl of extracted RNA. Second round of amplification also includes almost the same constituents with only difference in primers difference. The PCR was set in ABI veritti 9600 therocycler. The first round cycling conditions includes; Initial Denaturation at 95°C for 5 min. Then 40 repetitive cycles of denaturation, annealing and extension was given at 94°C, 59°C and 72°C respectively for 1 minute. Final extension was given at 72°C for 5 minutes. Second round of amplification was given as 95°C for 5 minutes as initial denaturation followed by 30 repetitive cycles of denaturation, annealing and extension at 1 minute, 45 seconds and 1 minute respectively. Final extension of the amplicons was given at 72°C for 7 minutes. The amplicons were subjected for gel electrophoresis on 1.6% gel. The expected sizes of the genotype-specific bands amplified by PCR typing are as follows: genotype 1a, 208 bp in size; genotype 1b, 234 bp; genotype 2a, 139 bp and 190 bp, genotype 2b, 337 bp; genotype 3a, 232, bp; genotype 3b, 176 bp; genotype 4, 99 bp; genotype 5a, 320 bp; and genotype 6a, 336 bp (Fig. 1).

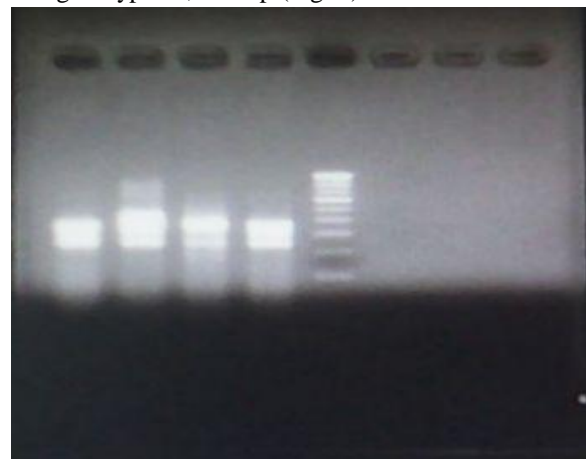


Figure 1: Gel picture showing the positive result of HCV and its genotyping.

3. Results

The current study includes collection of 30 blood specimen from the different Departments of Shri Mahant Indresh Hospital, Dehradun (U.K.). Serum/Plasma was collected from the entire blood sample and further subjected for different molecular parameters. Out of 30 specimens HCV RNA was

quantified in all samples by the utilization of TaqMan probes in COBAS TaqMan48 Real Time PCR and their titer values were observed and ranges in-between from 1.05×10^1 - 9.58×10^7 IU/ml. The COBAS TaqMan48 Analyzer automatically determines the HCV RNA titre of the specimen or control based upon the Cycle Threshold values for the HCV RNA and HCV Quantitation Standard cDNA and the lot-specific calibration coefficients. The HCV RNA titre is expressed in International Units (IU)/ml. The conversion factor between HCV copies/ml and HCV

International Units/ml is 5.82 copies/ IU using the WHO HCV International Standard for NAAT (Nucleic Acid Amplification Test) testing. The viral load was estimated in International Unit (IU)/ml (Table 1). The HCV genotypes were characterized through 30 specimens, subjected for HCV genotyping out of which genotype 3a was most prevalent and was found in 17(56.6%) cases. Genotypes 1a, 1b, 2a, 2b, 3b, 4, and 5a was found 2(6.6%), 15(50%), 5(16.6%), 15(50%), 2(6.6%), 2(6.6%), 2(6.6%) respectively (table 2).

Table 1: No of cases of HCV Genotypes

HCV RNA Titer (IU/ml)	No. of cases	HCV genotypes	1a	1b	2a	2b	3a	3b	4	5a
$1.00 \times 10^1 - 1.00 \times 10^3$	9	1a, 1b, 2a, 2b, 3a	2	6	1	5	6	-	-	-
$1.00 \times 10^4 - 1.00 \times 10^7$	20	1b, 3a, 2b, 2a, 5a, 4, 3b	-	9	4	10	11	2	2	2
$>1.00 \times 10^8$	1	2b, 3a	-	-	-	-	-	-	-	-
Total cases =30			02	15	05	15	17	02	02	02

Table 2: Percentage wise distribution of HCV genotypes

Sr. No	HCV Genotype detected	Number of cases N=30	Percentage
1.	1a	2	6.6%
2.	1b	15	50%
3.	2a	5	16.6%
4.	2b	15	50%
5.	3a	17	56.6%
6.	3b	2	6.6%
7.	4	2	6.6%
8.	5a	2	6.6%
9.	6a	0	0%

4. Discussion and Conclusion

The amount of HCV RNA in the blood (viral load) is believed to represent the steady state of viral replication and clearance. Therefore, it is important to utilize a highly specific and sensitive assay to quantify precisely HCV RNA in the blood of patients with HCV. This is particularly helpful for monitoring effect of anti-HCV drug therapy [15,16]. It is well established that in the patient with chronic HCV infection the response to alpha interferon therapy is correlated with serum HCV RNA. Although the viral load has been suggested to correlate with HCV activity, degree of liver damage, the studies from different investigators have generated controversial data. In this study we found that HCV genotype 3a is the most prevalent genotype associated in HCV infected individuals and in Patients with high HCV RNA viral load. The genotypes are divided into several subtypes with the number of subtypes depending on the genotype. In conclusion, 30 serum specimens were quantified for HCV RNA viral load and their titre values were observed in-between 1.05×10^1 - 9.58×10^7 IU/ml. HCV genotype 3a was found in 17(56.6%) cases with highest prevalence. Genotypes 1a, 1b, 2a, 2b, 3b, 4,

and 5a was found 2(6.6%), 15(50%), 5(16.6%), 15(50%), 2(6.6%), 2(6.6%), 2(6.6%) respectively. The findings also included that HCV viral RNA titre was elevated in HCV genotype 3a, assuming that HCV genotype 3a is the most replicated virus and need to be monitored thoroughly during diagnosis. Although type 1a, 1b, 2a, 2b, 3b, 4, 5a were also present but in lower proportion. The utility of nested PCR is that it consists of multiple primer sets within a single PCR reaction to produce amplicons of varying sizes that are specific to different DNA sequences. This assay system is very useful for rapid and sensitive genotyping of the HCV genomes when their epidemiological and transmission studies of this agent are carried out in large scale [17-20].

Conflict of interest: None

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