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Hepatitis C Virus (HCV) Genotyping and its Clinical Utility for Disease Monitoring

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Abstract: The hepatotropic viruses are a major public health problem representing the most common cause of liver disease worldwide. The distribution of HCV genotypes vary according to the geographical region. Genotypes 1-3 are widely distributed throughout the world. A virus's genotype usually stays the same. Genetic changes, or mutations, can occur at random or in response to the environment. Current study focuses on the Hepatitis C Virus (HCV) Genotyping and its Clinical Utility for Disease Monitoring.
Keywords: HCV Genotyping, RT-PCR, hepatocellular carcinoma, Copies, liver cirrhosis.

INTRODUCTION

Hepatitis C virus (HCV) is a parenterally transmitted hepatotropic RNA virus that causes acute and chronic hepatitis and hepatocellular carcinoma. HCV has been classified into the genus hepacivirus of the family Flaviviridae [1]. This virus is responsible for causing infection in three percent of world's population with approximately 170 million persons at risk of developing chronic hepatitis [2]. HCV is a single-stranded linear RNA virus with a high mutation rate; an estimated frequency of 10^{-2} mutations per nucleotide per year [3]. HCV has six main genotypes with many subtypes, which have variable sequence homology with each other. Symptoms can appear anytime from 2 weeks to 6 months, which include jaundice, fatigue, gray - colored stool, joint pain, belly pain, weakness, anorexia, itchy skin and dark urine.

Genotypes 1, 2, and 3 are most prevalent globally, while other genotypes are limited to specific regions [4]. HCV genotyping provides information about variability in the viral genome, likely disease progression and possible treatment strategies [5]. In contrast, most patients with chronic hepatitis C show elevations in liver enzyme levels as a consequence of hepatic inflammation, and many steadily progress to liver cirrhosis [6-10]. Genotyping is more significant for planning of HCV treatment period and helps to cure HCV infections [11]. For the quantification and identification of hepatitis C virus - ribonucleic acid, many molecular techniques are performed; the most significant are HCV ELISA, quantitative HCV - RNA PCR and recombinant immunoblot assay. HCV infection has reached epidemic proportions [12]. In the current research work we will be working on HCV RNA genotypes.

METHOD AND METHODOLOGY

5 ml EDTA blood were collected from Shri Mahant Indiresh Hospital Dehradun (U.K) and were utilized further for different parameters. RNA was isolated from the separated serum from the whole blood. Pathogen detection by the polymerase chain reaction (PCR) is based on the amplification of specific regions of the pathogen genome. In real-time PCR the amplified product is detected via fluorescent dyes. The quantity of under-test specimen, negative control and positive control, and pipette appropriate quantity of these four PCR mixes and RT-PCR enhancer in four centrifuge tubes, according to the ratio of PCR mix was considered. Hepatitis C virus genotype distribution was studied in a spectrum of HCV RNA viral load.

RESULTS

A total of 94 EDTA blood, 5 ml were collected from HCV reactive patients from different departments

of Shri Mahant Indiresh Hospital, Dehradun which includes OPDs and IPDs of Gastroenterology, Medicine, Gynecology, Pediatrics, Tuberculosis and Chest, Surgery etc. The serum was separated from all of the 94 samples and RNA was extracted using QIAamp® Viral RNA mini kit cat. No. 52904 which was quantified by the utilization of Rotor Gene Q Real Time PCR. All the 94 cases were considered for the study. Firstly all the RNA from all the 94 cases was quantified for HCV RNA quantitation by Rotor gene Q Real Time PCR. Only the cases with more than 2000 copies/ml of HCV RNA were further considered for the HCV Genotyping. 32 cases were with HCV RNA titer more than 2000 copies/ml. The diagnostic kit uses magnetic bead technology to extract HCV-RNA from serum. By applying one-step RT-PCR technology, the kit uses several specific pairs of HCV primers to target conserved regions of different HCV genotypes, including genotypes 1b, 1, 2, 3, 4, 5 and 6, as well as Taqman fluorescence probes to achieve genotyping detection of HCV RNA through fluorescent signal changes**.** Out of 32 cases, HCV genotype 3 was most prevalent with total of 21 (65.62%) cases followed by HCV genotype 1a with 4 (12.5%) cases followed by HCV genotype 6 with 3(9.37%) cases followed by HCV genotype 1b with 2 (6.25%) and thus followed by HCV genotype 2 and 4 with 1(3.12%), as depicted in figure 1.

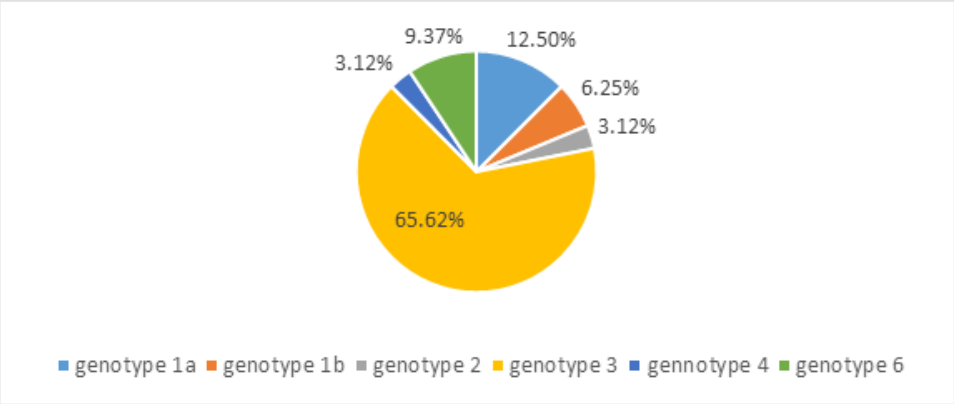

Fig-1: HCV genotypic distribution

DISCUSSION

This study was carried out at tertiary care hospital thus the clinical relevance is of utmost priority. This assay enabled us to detect 6 HCV genotype (1a, 1b, 2, 3, 4, 5, 6). However, HCV genotype 3 was most frequently detected. HCV genotype 3 is also the most common genotype in India and Pakistan. HCV genotype 3 contributes to the development of steatosis (fatty liver disease) and insulin resistance, both of which can directly influence HCV disease progression including cirrhosis and liver cancer. There is evidence to suggest that people with this genotype experience a faster rate of fibrosis progression. HCV genotype 1a is the other genotype common. Study revealed that the prevalence of genotype 1a in HCV patients was significantly higher than in chronic hepatitis and liver cirrhosis patients. Multiple logistic regression analysis revealed that, after adjusting for age and serum HCV RNA levels, HCV genotype 1b infection was still a significant risk factor. The level of SGOT, SGPT, Alkaline phosphatase, Bilirubin and Globulin were also examined and found maximum in high viral load cases, which were further used for genotyping.

CONCLUSION

Genotypes 1-3 are widely distributed throughout the world. From the study, we concluded that the HCV genotype 3 followed by 1b & 1a, 6, 2 and 4 were the most prevalent genotypes detected. The biochemical findings also included that the parameters SGOT, SGPT, Alkaline phosphatase, Bilirubin and Globulin were found higher in the HCV RNA high viral load cases. The outcome of HCV genotyping is of almost clinical value as there are various regimens were available to treat different types of HCV genotypes like Simeprevir, Sofosbuvir etc for genotype 1. Sofosbuvir/R for genotype 2 & Sofosbuvir/R for genotype 3. However, as we conclude the most common regimen to treat the HCV infection for all genotypes was Sofosbuvir, interferons, Ribavirin, Viramidine etc. As the genotype 3 was, prevalent in this work so there is a regimen available to treat the infection & these are Sofosbuvir/ Ribavirin & the duration time for the treatment is 24 weeks. The HCV genotype 3 is one of the most replicating viruses known to damage hepatic cells & thus requires proper line of treatment thoroughly during the diagnosis.

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