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Cellular and Molecular Profiling of Hepatitis C Virus (HCV) and to Study its Genotypic Heterogeneity in Clinical Isolates

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Abstract

The distribution of HCV genotypes vary according to the geographical region. Genotypes 1-3 are widely distributed throughout the world. The outcome of HCV genotyping is of almost clinical value. Current research work was carried out to study the Cellular and Molecular profiling of Hepatitis C Virus (HCV) and its clinical importance that includes the correlation of the different laboratory parameters with respect to HCV RNA Viral load and its genotypes. 94 EDTA blood samples were collected from HCV reactive patients and further processed for cellular and molecular profiling. Out of 94 HCV RNA quantified, 42 (44.6%) were TND, 52(55.4%) with HCV RNA viral load >34 IU/ml. HCV genotype 3 came in 21 (65.62%) cases followed by HCV genotype 1a with 4 (12.5%) and 6 with 3(9.37%) and 1b with 2 with 6.25%. SGOT, SGPT, Alkaline phosphatase, Bilirubin and Globulin was elevated in 28 (62%), 22(48.88%), 23 (51.11%), 12(26.66%) and 20(44.44%) respectively. Maximum number of cases with HCV RNA Viral load was found in the 1.00 X 104 -1.00 X 107 IU/ml range with 27 cases. The HCV genotype 3 is one of the most replicating virus known to damage hepatic cells & thus requires proper line of treatment thoroughly during the diagnosis. Thus, the area of molecular testing for the diagnosis and management of HCV infection has shown steady improvement in technology and standardization. Further to say, development of proper algorithm by combining the profiling of cellular, molecular and Biochemical studies can be a new era for the proper management of this virus.

Keywords: Hepatocellular carcinoma, Real Time PCR, Viral load, Genotyping, Reverse transcription

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Introduction

Viral hepatitis has developed as a major public health problem throughout the world affecting several hundreds of millions of people [1-7]. Viral hepatitis is a cause of substantial morbidity and mortality in the human population, both from acute infection and chronic sequel that include, in the case of hepatitis B, C and D, chronic active hepatitis and cirrhosis [8,9]. Hepatocellular carcinoma, which is one of the ten most common cancers worldwide, is closely associated with hepatitis B, and at least in some regions of the world with hepatitis C virus [10-13]. There are very few studies on the clinical co-relation of HCV RNA viral load and its implications on other affected organs of the body, as well as the impact of different HCV Genotype/s for the proper management of the patients from the northern parts of India [14-17]. Thus, the current research work was carried out to study the Cellular and Molecular profiling of Hepatitis C Virus (HCV) and its clinical importance that includes the correlation of the different laboratory parameters with respect to HCV RNA Viral load and its genotypes.

Materials and Methods

94 EDTA blood samples were collected from HCV reactive patients from different departments of Shri Mahant Indiresh Hospital, Dehradun, Uttarakhand, India, which includes OPDs and IPDs of Gastroenterology, Medicine, Gynecology, Pediatrics, Tuberculosis and Chest and Surgery. The serum was separated from all of the 94

samples and RNA was extracted using Qiagen QIAamp Viral RNA mini kit (cat. No. 52904), Germany. Extracted RNA was further utilized as template for HCV RNA quantification, which was quantified by the utilization of Rotor Gene Q 5 Plex Real Time PCR machine. For all the 94 clinical samples, the viral load was estimated. Further, for the same master mix was prepared for the quantification of HCV RNA by the usage of Artus Amplification Kit from Qiagen (catalog no. 4518263). The Hepatitis C Virus RG Master A and B reagents and enzymes were utilized for the reverse transcription and specific amplification of a 240 bp region of 5'-3' untranslated region [UTR] of HCV genome. HCV RNA was quantified within the range of 34 IU/ml to 1.00×108IU/ml (as depicted in Table 2). HCV genotyping characterization was done with the usage of Real Time PCR technology utilizing Hepatitis C Virus Genotype Diagnostic Kit (PCR-Fluorescence Probing) from Sansure, Korea, Biotech kit (Reference Number S3034E). This diagnostic protocol 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.

Results

Out of 94 HCV RNA quantified, 42 (44.6%) cases came with target not detected and 52(55.4%) were with HCV RNA viral load >34 IU/ml. Further the cases greater than or equal to 34 IU/ml (Table 3) with HCV RNA viral load greater than 500 IU/ml were considered for the HCV genotyping. 32 cases were considered for HCV genotyping because the HCV RNA viral load was above 500 IU/ml. Out of 32 cases, subjected for HCV genotyping, HCV genotype 3 was the most prevalent which came in 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%). In 3.12% cases HCV genotype 2 and 4 came (table 2). Biochemical investigation was done for 86 cases including SGOT, SGPT, Alkaline Phosphatase, albumin and globulin with high HCV RNA viral load (>34 IU/ml) and with cases in which target was not detected.

It was observed that out of 45 cases where the HCV RNA viral load was >34 IU/ml, parameters SGOT, SGPT, Alkaline phosphatase, Bilirubin and Globulin was elevated in 28 (62%), 22(48.88%), 23 (51.11%), 12(26.66%) and 20(44.44%) respectively. When studied gender wise prevalence of HCV viral infections both the gender came with nearly equal proportion with 47.3% for male and 66.6% for female (Table 1)

Age group (In years)	Total cases	Cases with HCV RNA viral load detected	Target not detected
0-20	**03** (3.2%)	**03**(100%)	**00** (0%)
21-40	**32**(34%)	**18** (56.25%)	**14** (43.75%)
41-60	**42**(44.7%)	**22**(52.4%)	**20** (47.6%)
Above 60	**17**(18.1%)	**9**(52.94%)	**8** (47.06)
Total	**94** (100%)	**52**(55.4%)	**42** (44.6%)

Table 1: Age wise distribution of Hepatitis C Viral infection.

It was also analyzed that the age group 21-40 and 41-60 years of age were with high HCV RNA viral load with 56.25% and 52.4% cases respectively. HCV genotype and subtype were studied in different spectrum of HCV RNA viral titer ranging from 1.00x103 10/ml to 1.00x108 10/ml. It was seen that maximum number of cases with HCV RNA Viral load in different ranges was found in the 1.00 X 104 -1.00 X 107 IU/ml range (table 2).

Range of HCV RNA titre (IU/ml)	No. of cases	Total no. of cases with HCV RNA viral load ≥ 500 IU/ml	HCV Genotype/s detected	HCV Genotype/s distribution						
				1a	1b	2	3	4	5	6
1.00×10^{1}-1.00×10^{3}	22	3	1a,3	1	-	-	2	-	-	-
1.00×10^{4}-1.00×10^{7}	28	27	3,1a,1b,2,4,6	3	2	1	17	1	-	3
$>1.00\times10^{8}$	02	2	3	-	-	-	2	-	-	-
Total	94	32		**04 (12.5%)**	**02 (6.25%)**	**01 (3.12%)**	**21 (65.62%)**	**01 (3.12%)**	**00 (0%)**	**03 (9.37%)**

Table 2: Spectrum of HCV RNA viral load w.r.t. its genotype/s detected.

In this range a total of 27 cases were there where in the same range HCV genotype 3 was the most prevalent which was found in 17 cases out of 27 cases considered for the same, followed by HCV genotype 1a and 6 with 3 cases each (tabulated in table 12). Present study reveals about the co- relation of different HCV RNA viral load w.r.t to biochemical profiling. Out of 86 cases considered for comparative evaluation of Molecular and Biochemical, profiling it was seen that in 45 cases, HCV RNA viral load was ≥ 34 IU/ml, whereas 41 cases came with target not detected. It was analyzed that out of 45 cases where HCV RNA was detected, SGOT, SGPT, AP, Bilirubin and Globulin was raised in 28 (62.22%), 22 (48.88%), 23 (51.11%),12 (26.66%) and 20(44.44%) respectively. In 41 cases, target was not detected, but SGOT, SGPT, AP, Bilirubin and Globulin was elevated in 12 (29.26%), 7 (17.07%), 11(24.44%), 10(24.39%) and 7(17.07%) cases respectively (table 3).

	Total no of cases	Range of [1]SGOT (**14-36)U/L**	Range of [2]SGPT **(9-12)**U/L	Range of [3]AP **(38-126)U/L**	Range of Bilirubin **(0.2-1.3)mg/dl**	Range of globulin **(2.3-3.5)g/dl**
Cases greater than upper value of normal range	86	40 (46.51%)	29 (33.7%)	34 (39.53%)	22 (25.58%)	27 (31.39%)
Total cases lower than lower value of normal range	86	14 (16.27%)	11 (12.79%)	7 (8.13%)	13 (15.11%)	16 (18.60%)
Total cases within normal range	86	30 (34.8%)	42 (48.83%)	47 (54.65%)	46 (53.48%)	43 (50%)
HCV RNA viral load (Above ≥34 IU/ml)s	45	28 (62.22%)	22 (48.88%)	23 (51.11%)	12 (26.66%)	20 (44.44%)
Cases with target not detected.	41	12 (29.26%) in high range	7(17.07%)	11(24.44%)	10 (24.39%)	7 (17.07%)

Table 3: Comparative results interpretation for biochemical investigations and HCV RNA viral load.

1SGOT= Serum glutamic oxaloacetic transaminase
2SGPT= Serum glutamic pyruvic transaminase
3AP = Alkaline phosphatase

Discussion

According to the WHO, there are 180 million people affected with HCV worldwide and about 12.5 million carriers in India [18, 19]. Hepatocellular carcinoma accounts for 85 to 90% of the cases of primary liver cancer. Chronic hepatitis and cirrhosis constitute the major preneoplastic conditions in the majority of HCC. The risk of developing HCC for a patient with HCV-related cirrhosis is approximately 2-6% per year [20, 21]. HCC risk increases to 17-fold in HCV-infected patients compared to HCV-negative subjects. In general, HCC develops only after two or more decades of HCV infection and the increased risk is restricted largely to patients with cirrhosis or advanced fibrosis. HCV Quantification & Genotypes is known to have a distinct pattern of geographic distribution. The genotype/s determination depends based on target-conserved region in the genome of HCV. This study used a quantitative assay to measure virus load in individuals infected with different HCV genotypes. Real time PCR remains to date the most reproducible and sensitive technique to track the putative presence of virions, passively absorbed or replicating, in cells. Present study reveals that out of total 94 clinical samples, processed 52(55.4%) cases were with HCV RNA high viral load and 42(44.6) cases were with target not detected. The study is very relevant for the proper management of the patients infected with HCV and undergoing treatment. As the HCV RNA, viral loads will be responsible for the monitoring of the therapy provided by the clinicians. Increase, decrease as well as no change in HCV RNA viral load is also essential for studying further the drug resistance, susceptibility pattern in HCV infected patients.

The high viral load cases must be further processed for genotyping. Knowing the viral load before starting treatment is useful because patients with "high" viral loads can have a difficult time getting the virus to become completely undetectable on treatment. Target not detected are the cases were the hepatitis C virus is present in the bloodstream, but at a very low level, too low to be measured by a quantitative test. Unlike the flu virus, which has an incubation period of less than a week, incubation for chronic HCV can take between 14 to 180 days. The incubation for acute hepatitis C is typically about six to 10 weeks. The incubation period of HCV differs from that of other types of hepatitis. Viral infection may also be depending on the age of an individual and its immunity. Cases with 41-60 years of age group in the present study were mostly affected with HCV infection, having maximum number of HCV RNA high viral load with 42 cases. This might be due to repeatedly being exposed to infected blood. The age group of 0-20 years were least susceptible with 3 cases positive for HCV infection. This may be due to protective antibody titer against HCV due to vaccination in early age. Accretion of nucleotide switch in the HCV genome results in diversification and evolution into different genotypes. Differences among HCV genotypes in geographic distributions have provided investigators with epidemiologic markers that can be used to find the source of HCV infection in a given population & for further prognosis.

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. This may also contribute to the risk of liver failure. There is evidence to suggest that people with this genotype experience a faster rate of fibrosis progression. This means that the liver tissue may thicken and scar faster than that of someone with a different genotype. Our study were also with maximum number of cases with HCV RNA viral load >500 IU/ml with HCV genotype 3, which was found in 65.62% patients. Thus, the genomic composition of the HCV and its difference in genetic expression can play an important role for the management of the patients affected by this lethal virus. HCV genotype 1a is the other genotype common. Study revealed that the prevalence of genotype 1a in HCC 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. However, there are conflicting reports on the relationship between the biochemical markers of inflammation alanine transaminase (ALT), histological degree of inflammation, and serum HCV-RNA levels by reverse transcription (RT)-PCR. In individuals with chronic hepatitis C, viral load and elevated serum ALT levels may have clinical relevance.

ALT is most concentrated in liver and released into the bloodstream as the result of liver injury. It, therefore, serves as a fairly specific indicator of liver status. SGOT is normally found in a diversity of tissues including liver, heart, muscle, kidney, and brain. It is released into serum when any one of these tissues is damaged. It is, therefore, not a highly specific indicator of liver injury. The present study revealed that SGOT levels varied significantly among the three groups of HCV genotypes. All other biochemical parameters were deranged but changes remained non-significant as also reported earlier. A low globulin level in patients with hepatitis C can be a sign of cirrhosis (advanced liver disease). Globulin levels can go up and down slightly. Very low globulin levels can cause symptoms of edema, or fluid accumulation, in the abdomen (called ascites) or in the leg. Low levels of total protein in the blood can occur because of impaired function of the liver.

A high alkaline phosphatase level does not reflect liver damage or inflammation. A high alkaline phosphatase level occurs when there is a blockage of flow in the biliary tract or a buildup of pressure in the liver--often caused by a gallstone or scarring in the bile ducts. The level of SGOT, SGPT, Alkaline phosphatase, Bilirubin and Globulin are found maximum in high HCV RNA viral load cases in comparison to target not detected in the current study.

Conclusion

The distribution of HCV genotypes vary according to the geographical region. Genotypes 1-3 are widely distributed throughout the world. 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. But as we conclude the most common regimen to treat the HCV infection for all genotypes was Sofosbuvir, interferons, Ribavirin, Viramidine etc. These are various alternative therapies also available to treat Hepatitis C infection like Milk Thistle, Green tea extract, Glycyrrhizin but currently there is no vaccine available to prevent the Hepatitis C infection. The HCV genotype 3 is one of the most replicating virus known to damage hepatic cells & thus requires proper line of treatment thoroughly during the diagnosis. Thus, the area of molecular testing for the diagnosis and management of HCV infection has shown steady improvement in technology and standardization. Further to say, development of proper algorithm by combining the profiling of cellular, molecular and Biochemical studies can be a new era for the proper management of this virus.

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Conflict of interest: None

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