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Hepatitis C Virus and its Genotypes detection in Serum Specimens: Clinical Relevance for the Management

Sakshi Kumari[1], Usha Chand[2], Rashmi Bisht[3], Sonali Sundriyal[4], Narotam Sharma[5], Satish Chandra Nautiyal[6]

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Abstract

In India, the cases of Hepatitis C Virus are increasing at a very rapid rate. Hepatitis C virus (HCV) often causes persistent infection, and is important factor in the cirrhosis and hepatocellular carcinoma (HCC). Current study focuses on the various factors associated with the HCV infection. Study reveals about the different HCV Genotypic distribution and its RNA viral load in different spectrum. The study also correlates about the evaluation of various other biochemical and serological factors.

Keywords: Hepatitis; Chronic; Real Time PCR; Hepatocellular Carcinoma; Serology.

Introduction

Hepatitis is a member of Hepacivirus C species, is a small (55-65 nm in size), enveloped, positive sense single stranded RNA virus. HCV comes under the flaviviridae family and genus Hepacivirus [1]. Hepatitis C virus particle consists of a lipid membrane envelope that is 55 to 60 nm in diameter. Two viral envelope glycoproteins E1 and E2 are embedded in the lipid envelope [1]. They take part in viral attachment and entry into the cell. Hepatitis C Virus (HCV) is a major cause of liver disease including liver cirrhosis and scarring And some cancers such as liver cancers (hepatocellular carcinoma, abbreviated HCC) and lymphomas in humans [2,16]. It causes infection in two phases, first involve acute attack that last for few weeks and if untreated then it may persist for long time that is termed as chronic hepatitis C. This chronic infection may often lead to chronic liver disease (CLD) that may ultimately lead to even hepatic failure. Regarding the role of cellular immune response to HCV infection it has been thought to be crucial in the inhibition of hepatitis C viral replication. Several studies have reported that $CD8^+$ lymphocytes play a key role as effectors of liver damage during chronic HCV infections [3]. On the other hand, total serum cholesterol, triglycerides and glucose have been linked to liver transaminase

Author's Affiliation: [1,4]Research Scholar, Department of Microbiology, D.B.S. College, Dehradun, Uttarakhand 248001, India. [2]Department of Microbiology, Sai institute of Paramedical and Allied Sciences, Dehradun, Uttarakhand 248001, India. [3]Research Scholar, Department of Microbiology, Uttaranchal (P.G.) College of Bio-Medical Sciences & Hospital, Dehradun, Uttarakhand 248001, India. [5]Scientist [6]Senior Technician, Central Molecular Research Laboratory, Biochemistry Department, SGRR Institute of Medical and Health Sciences, Dehradun, Uttarakhand 248001, India.

Corresponding Author: Narotam Sharma, Scientist, Central Molecular Research Laboratory, Biochemistry Department, SGRR Institute of Medical and Health Sciences, Dehradun, Uttarakhand 248001, India

E-mail: sharmanarotam5@gmail.com

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levels in healthy populations. Alterations of lipid metabolism, particularly in apolipoprotein B and C have been described in hepatitis C with genotype 1b or 3a and hypocholesterolemia has been associated with HCV infection and HCV/HIV co-infection. There are seven genotype (1-7) and numerous subtypes of HCV. Genotypes differ by 30-35% of the nucleotides sites over the complete genome. The difference in genomic composition of subtypes of genotype is usually 20-25%. Subtypes 1a and 1b are found worldwide and cause 60% of all cases [4-6]. The aim of this study is to distinguish molecular characterization of Hepatitis C Virus and its genotype detection in serum specimen.

Material and Methodology

A total of 62 caseswho were infected with HCV and who had not previously been treated with interferon and Ribavirin are then collected in lavender colored vacutainer tube which ensure mixing anticoagulant (EDTA) with blood to prevent clotting. All samples were taken from the infected cases of Hepatitis c virus and. Further samples were transported to Central Molecular Research Laboratory (CMRL) and transported in the 4*C temperature. Samples which were collected were subjected serological and pathological parameters such TLC, DLC RBC, LFT Samples with high infection were further processed for molecular diagnostic by latest version of technologies like conventional thermal cyclers, Real Time PCR, Comprehensive Biological Analytical System (Roche TaqMan 48) semi-automated thermal cycler.

Results

Viral load of HCV was detected by Roche TaqMan 48 Real Time PCR. Among the 62 cases for Hepatitis C, only 26 (41.93%) cases were positive for HCV among which 25 cases were having high viral load and 1 was having low viral load whereas 36 (58.07%) cases were negative for the same. The detection limit is 25IU/ml – 1.10 $\times 10^8$ IU/ml where high viral load range is $\geq 10^3$ IU/ml of HCV RNA whereas low viral load is below 10^3 IU/ml and TND is below 25 IU/ml. Most of the positive cases were in the age range of 41-60 years, which is the average active age group. Of the total positive cases, 44.4% were males and 55.6% were females.High positive cases of HCV were further diagnosed through genotyping in which genotype 3 was prevalent. Out of 18 cases of genotyping 14 (77.77%) cases found to be prevalent for genotype 3 and 3 (16.66%)cases for genotype 1 whereas only 1 (5.55%) case for genotype 6. The HCV Genotyping was done by manufacturer protocol which detects HCV genotype 1, 1b, 2, 3, 4, 5, 6, gene target was conserved region by Qiagen RT PCR open channel. Then biochemical profiling was done by the cases of HCV in which GGT, SGOT, SGPT, albumin, TSH etc. are included. Bilirubin normal range is of 0.2-1.3 mg/dl, Gamma glutamyl transferase (GGT) normal range is of 12-43 U/L, and total protein normal range is 6.30-8.20 g/dl and uric acid content range is of 6.30-8.20 g/dl. Total bilirubin was in normal range of 0.2-1.3 mg/dl among all the cases except 1 case which was having high bilirubin. Gamma glutamyl transferase (GGT) was depicted low in 4 cases and high in 3 cases from normal range of 12-43 U/L. The total protein was average in all the cases between the rangesof 6.30-8.20 g/dl whereas 02 cases were below this range of total protein. The 01 case was having low uric acid content whereas rest of the cases was in normal range of 3.5-8.5 mg/dl.

Table 1: Age wise Distribution of Hepatitis C Virus cases.

Age (years)	Number of cases		Positive cases with high viral Load	Target not Detected
	Male	Female		
0-20	1	0	1	0
21-40	10	18	10	18
41-60	13	14	13	14
Above 60	3	3	3	3

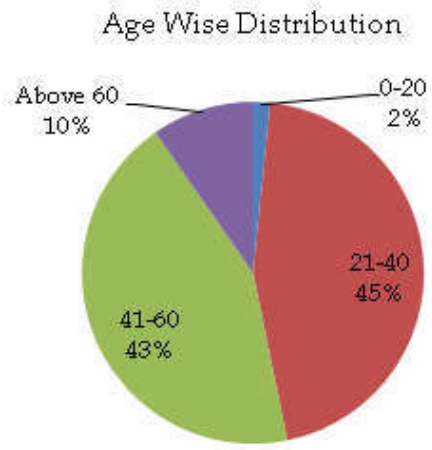

Fig 1: Age wise distribution of HCV

Table 2: Gender wise distribution

	Male	Female
HCV RNA Viral load	26	36
TND (Target not detected)	15	21
High viral load	11	15

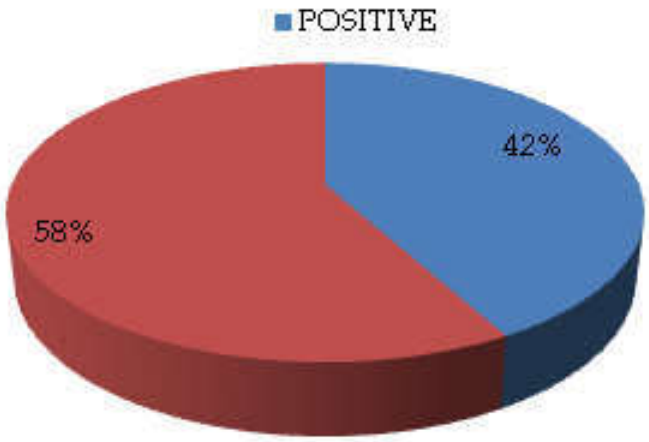

Fig. 2*:* Positive and TND cases of hepatitis C Virus

Table 3: Genotype distribution in HCV

Genotype	Total cases
1	3(16.6%)
1b	-
2	-
3	14(77.77%)
4	-
5	-
6	1(5.5%)

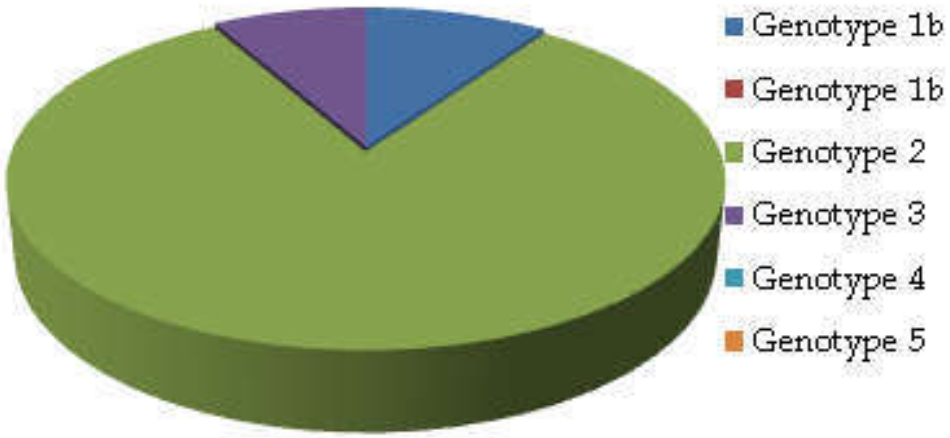

Fig. 3: Hepatitis C Virus Genotype distribution

Discussion

The intent of this study was to assess different type of genotyping, their serological and biochemical analysis of their viral load. The hepatitis C virus is a blood-borne virus and following initial infection, approximately 80% of people do not exhibit any symptoms [7,14]. Those who are acutely symptomatic may exhibit fever, fatigue, decreased appetite, nausea, vomiting, abdominal pain, dark urine, grey-coloredfaces, joint pain and jaundice (yellowing of skin and the whites of the eyes) Hepatitis C is Chronic manifestation of liver diseases. It is difficult to diagnose due to a lack of rapid, sensitive, and specific tests. Newer methods which are easy and reliable are required to diagnose Hepatitis C at a very early stage [8,18]. HCV infection is diagnosed in 2 steps: Screening for anti-HCV antibodies with a serological test identifies people who have been infected with the virus. If the test is positive for anti-HCV antibodies, a nucleic acid test for HCV ribonucleic acid (RNA) is needed

to confirm chronic infection because about 15–45% of people infected with HCV spontaneously clear the infection by a strong immune response without the need for treatment. Although no longer infected, they will still test positive for anti-HCV antibodies [9,10]. From the above result discussed, it is evident that for positive cases in which viral load detected was high as where viral load was very high i.e 1.64×10^8 whereas in viral load was low i.e. 9.84×10^3 IU/ ml. Out of 62 samples considered for study out of which 35 were females and 27 were males 24 (38.7%) were positive for HCV and 38 (61.2%) were negative. A majority of patients 27 (43.5%) in our study were between age group of 41-60 yrs out of which 11 were positive for Hepatitis C. High positive cases of HCV were further diagnosed through genotyping in which genotype 3 was prevalent. Out of 18 cases of genotyping 14 cases found to be prevalent for genotype 3 and 3 cases for genotype 1 whereas only 1 case for genotype 6. Total bilirubin was in normal range of 0.2-1.3 mg/dl among all the cases except 2 cases which was having high bilirubin. Gamma glutamyl transferase (GGT) was depicted low in 5 cases and high in 8 cases from normal range of 12-43 U/L. The total protein was average in all the cases between the range of 6.30-8.20 g/dl whereas 4 cases were below this range of total protein. The 2 cases was having low uric acid content whereas rest of the cases was in normal range of 3.5-8.5 mg/dl.

Conclusion

HCV remains a major cause cirrhosis, liver failure and liver cancer despite recent dramatic advances in anti viral treatment [11,15]. Some patients may experience progression of liver disease or HCC despite viral clearance. Trace amount of HCV RNA from successfully treated patients can be infectious. We do not know the long term efficacy of treatment with the new generation of DAAs, particularly with interferon free regimens; and generation of potential resistant virus. Our study made it evident that Roche Cobas TaqMan 84 PCR and Realtime PCR is a rapid and cost effective diagnostic test for Hepatitis C that shows good sensitivity and specificity [12,16]. PCR has been a great advance technology used in the diagnosis of HCV worldwide. This PCR has a sensitivity of about 95- 100% accuracy. Thus results should always be viewed parallel with clinical findings. Recent advances in our understanding of HCV structure, genome and its lifecycle have revealed numerous target sites for potential pharmacological invention. These should help in further improving HCV treatment [14,17].

Conflict of interest: None

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