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# Hepatitis C Virus (HCV) and its Genetic Diversity in clinical Isolates from Uttarakhand Population

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## ABSTRACT

Hepatitis C is major cause of chronic liver disease. It has been recognised as a global health problem because of the progression to cirrhosis and hepatocellular cancer. Quantization and genotyping of HCV RNAs are important to determine the optimal duration of anti-viral therapy and predict likelihood of response. Total 77 samples were tested biochemically, serologically and molecular assay (Roche COBAS TaqMan 48 Real Time PCR). Out of 77 cases 33(42.85%) were with high viral load (>$10^3$IU/ ml of HCV RNA) and low viral load (below $10^3$IU/ml) 2 (2.59%) and 42 (54.54%) were target not detected (below 25 IU/ml). Genotype 3 was prevailed with 68.42% out of 35 cases followed by HCV genotype 15.78% in 1, 5.26% in 2 and 6, 2.63% in 1b and 4. In addition, our studies showed that genotype 1, 2, 4 and 6 (mixed genotype was detected in 1 cases with viral load 6.62 × $10^8$IU/ml). Total protein content in serum in all the cases was average except 04 cases that was having low protein content. 02 cases were having low uric acid content that was having high viral load. From all high positive (high viral load) cases which were further diagnosed for their genotyping in which genotype 3 was prevalent following by genotype1, 1b, 2, 4 and 6. Study signifies the gene based diagnosis and its clinical relevance for the proper management of the patients.

**Keywords:** Hepatitis, Chronic, Real Time PCR, Hepatocellular Carcinoma, Serology

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## INTRODUCTION

According to the Centre for Disease Control and Prevention (CDC, 2009), hepatitis C is defined as a contagious liver disease resulting from infection with the hepatitis C virus (HCV) [1]. HCV is the main etiological agent for chronic hepatitis, liver cirrhosis and hepatocellular carcinoma. HCV is an enveloped, positive sense, single-stranded; RNA virus with a diameter of about 50 nm, its genome is of about 9.5 kb. It is classified as a separate genus (Hepacivirus) within the Flaviviridae family [2-4]. Like a blood-borne virus, HCV can be transmitted by blood and blood products. There are three forms of viruses present in the serum of infected individuals, including free virions, virions associated with immuneglobulins and virions associated with very low density and low density lipoproteins. There are very few studies on the clinical correlation of the HCV RNA viral load and its implications on other affected body organs, as well as on the impact of different HCV genotypes for the proper management of patients in the northern regions of India [5-12]. This study is focus on Hepatitis C Virus and its Genetic Diversity in clinical Isolates from Uttarakhand population

## MATERIALS AND METHODS

Total number of cases with serologically HCV reacted were considered for the proposed study. The whole blood sample collected in lavender coloured vacutainer tube which ensure mixing anticoagulant (EDTA) with blood to prevent clotting and samples is transported in properly sealed insulted thermocol box with ice gel pack maintaining 4°C temperature[13,14]. Central Molecular Research Laboratory day to day and the same day serum was separated for further processing. The cases in which we have targeted the *Untranslated region* (UTR) for HCV RNA viral load monitoring from the symptomatic patients and RNA was

isolated by Roche high pure system (cat P/N: 03 531 376-001) solid phase nucleic acid extraction method.

## RESULT

Viral load for HCV was done by Roche COBAS TaqMan 48 RT PCR with detection limit 25IU/ml-1.10×$10^8$IU/ml in which HCV RNA viral load was detected for all the 77 cases it was found that out of 77 cases were high viral load (>$10^3$IU/ ml of HCV RNA) 33(42.85%) and low viral load (below $10^3$IU/ml) 2(2.59%) and TND (below 25 IU/ml) 42 (54.54%).The cases with high viral load were further subjected to HCV genotyping by Real time PCR in which we have targeted type and subtypes of the HCV gene *Conserved regions* of different HCV genotypes including 1b, 1, 2, 3, 4, 5, 6 as well as TaqMan florescence probes to achieved genotyping detection of HCV RNA though fluorescent signal changes. Further all the case with HCV RNA viral load ≥ $10^3$ IU/ml were further compared for HCV genotyping. It was observed that out of 77 cases, 35 were HCV RNA viral load ≥ $10^3$, which was further considered for HCV genotyping. Genotyping was done by Sansure Biotech Inc. (cat number 655654654) The HCV genotyping was done as per manufacturer protocol which detects HCV genotype 1, 1b, 2, 3, 4, 5, 6 gene target was *Conserved region* by Qiagen RT PCR open channel. Genotype 3 was prevailed with 68.42% out of 35 cases followed by HCV genotype 15.78% in 1, 5.26% in 2 and 6, 2.63% in 1b and 4.Also our studies showed that genotype 1, 2, 4 and 6 (mixed genotype was detected in 1 cases with viral load 6.62 × $10^8$ IU/ml).

Further, Biochemical profiling was done by the cases. Biochemical examination (bilirubin, SGOT, SGPT, albumin, alkaline phosphatase, TSH etc.). Total bilirubin was in normal range of 0.2-1.3mg/dl among all the cases except 4 cases which was having high bilirubin. Gamma glutamyl transferase (GGT) was depicted low in 3 cases and high in 12 cases from normal range of 12-43 U/L. The total protein was average in all the cases between the ranges of 6.30-8.20g/dl whereas 7 cases were below this range of total protein. The 3 cases was having low uric acid content whereas rest of the cases was in normal range of 3.5-8.5mg/dl. The 11 cases was having low and 3 cases was high Creatinine serum content whereas rest of the cases was in normal range of 0.8-1.5mg/dl.

**Table 1: Gender wise Distribution of high and low viral load**

| | Female | Male |
|---|---|---|
| HCV RNA viral load status | 43 (55.84%) | 34(44.15%) |
| TND (Target not detected )below 25 IU/ml | 25 (59.52%) | 17(40.47%) |
| High viral load (above$10^3$ IU/ml) | 18 (54.54%) | 15 (45.45%) |
| Low viral load(below $10^3$ IU/ml) | 2(100%) | 0 (0%) |

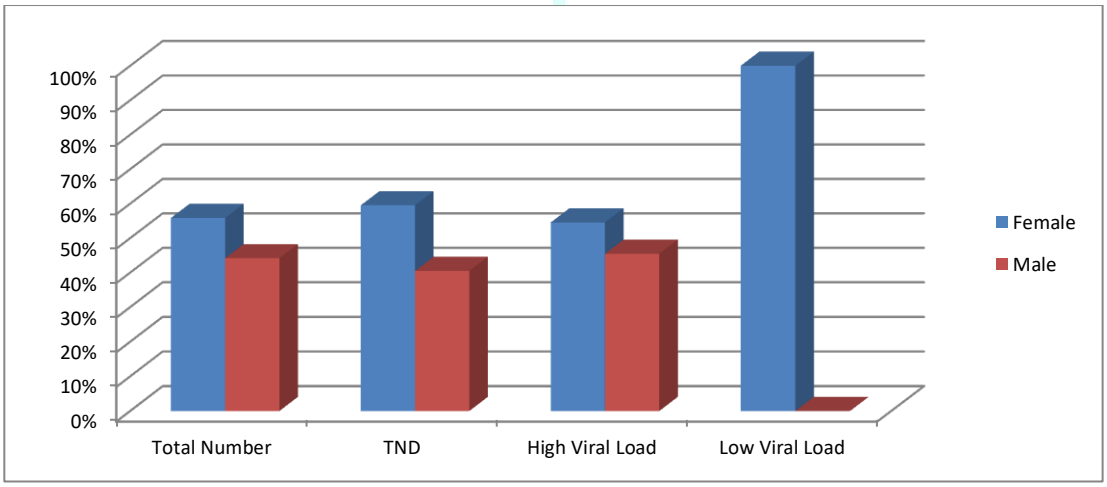


**Figure 1: Gender wise Distribution of high and low viral load for Hepatitis C Virus**

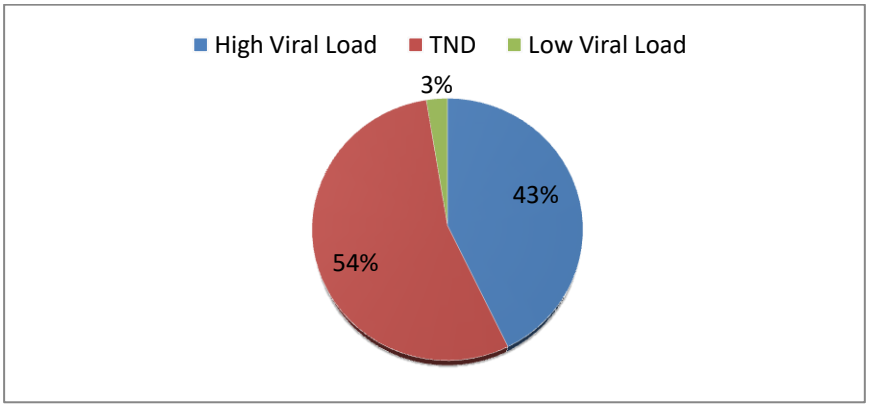


**Figure 2: Pie chart of Positive and Negative cases of Hepatitis C Virus**

**Table 2: Age wise Distribution of Hepatitis C Cases**

| Age (years) | Total Number of cases | High viral load(>$10^3$IU/ml) | Target not detected (Below 25IU/ml) | Low viral load (below $10^3$) |
|---|---|---|---|---|
| **0-20** | 0 | - | - | - |
| **21-40** | 28 | 13(46.42%) | 16(57.14%) | 2 |
| **41-60** | 38 | 16(42.10%) | 20(52.63%) | - |
| **Above60** | 11 | 4(36.36) | 6(54.54%) | - |

**Table 3: Genotype distribution of HCV Cases**

| HCV Genotype | Total cases |
|---|---|
| 1 | 6(15.78%) |
| 1b | 1(2.63%) |
| 2 | 2(5.26%) |
| 3 | 26(68.42%) |
| 4 | 1(2.63%) |
| 5 | - |
| 6 | 2(5.26%) |
| 7 | - |

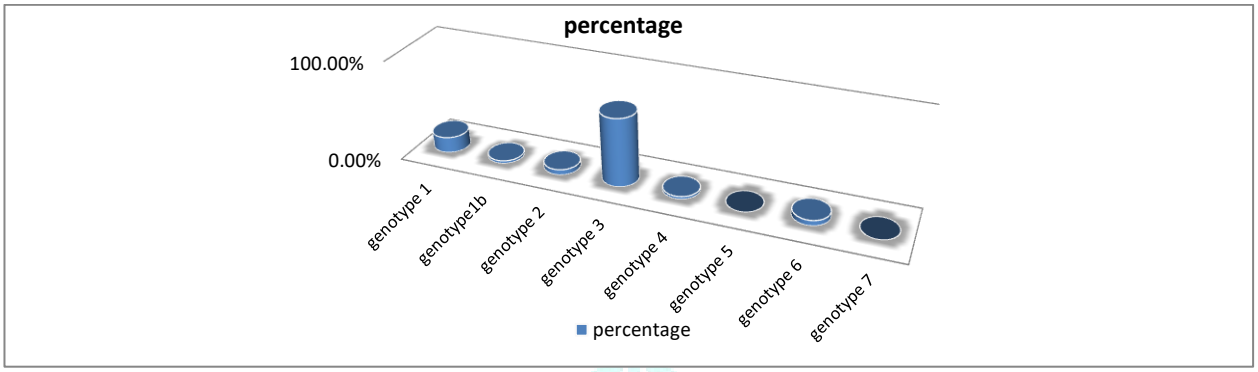


**Figure 3: Genotypic prevalence among suspected cases of HCV**

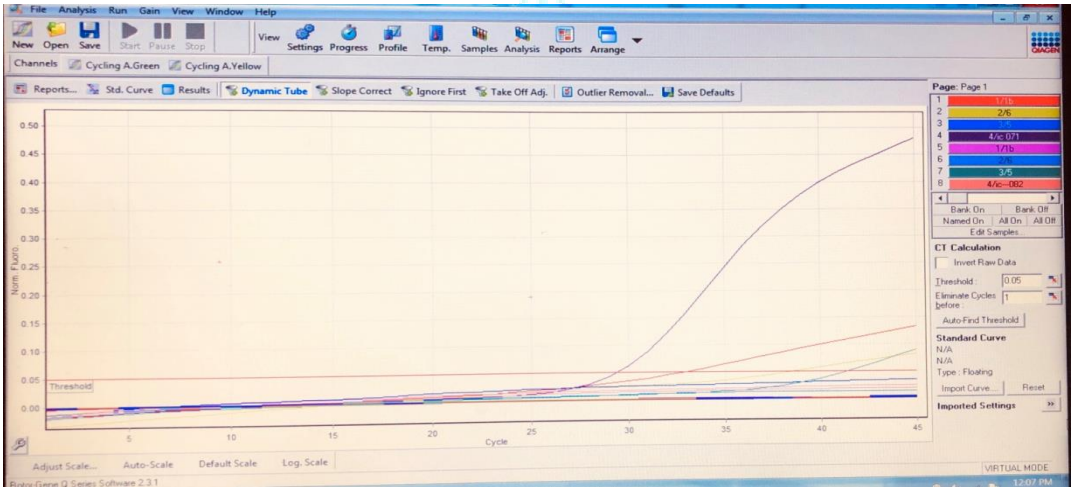

**Figure 4: Multiple Genotype in Hepatitis C Virus**

**Table 4: Spectrum of HCV viral load**

| Range of HCV RNA titer (IU/ml) | No. of cases | Total no. of Cases with HCV RNA viral≥500 IU/ml | HCV Genotypes detected | HCV Genotype/s distribution | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 1 | 1b | 2 | 3 | 4 | 5 | 6 |
| TND | 42 | None | None | None | None | None | None | None | None | None |
| $1.10\times10^1$ $1.10\times10^3$ | 2 | No | No | No | No | No | No | No | No | No |
| $1.00\times10^4$ $1.00\times10^7$ | 19 | 19 | 1, 2, 3, 4, 6 | 3 | No | 2 | 15 | 1 | - | 2 |
| $>1.00\times10^8$ | 14 | 14 | 1b,1,3 | 3 | 1 | - | 14 | - | - | - |
| Total | 77 | 33 | | 06 (15.78%) | 01 (2.63%) | 02 (5.26) | 29 (68.42) | 01 (2.63%) | - | 02 (5.26) |

**Table 5:** Comparative result interpretation for biochemical investigations LFT

| | Total number 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 | 77 | 15 (19.48%) | 12 (15.58%) | 7 (9.09%) | 4 (5.19%) | 7 (9.09%) |
| Total Cases lower than lower value of normal range | 77 | 2 (2.59%) | 1 (1.29%) | 1(1.29%) | 1(1.29%) | 00 |
| Total Cases within normal range | 77 | 20 (25.97%) | 24 (31.16%) | 28 (36.36%) | 32 (41.55%) | 29 (37.66%) |

[1]SGOT = Serum glutamic oxaloacetic transaminase

[2]SGPT = Serum glutamic pyruvic transaminase

[3]AP = Alkaline phosphatase

**Table 6:** Comparative result interpretation for biochemical investigations RFT

| | | | | | | |
|---|---|---|---|---|---|---|
| Cases greater than upper value of normal range | 77 | 3 (3.89%) | 3 (3.89%) | 00 (0%) | 00 (0) % | 2 (2.59%) |
| Total Cases lower than lower value of normal range | 77 | 00 (0%) | 11 (14.28%) | 4 (5.19%) | 6 (7.79%) | 00 (0%) |
| Total cases within normal range | 77 | 16 (20.77%) | 9 (11.68%) | 11 (14.28%) | 9 (11.68%) | 13 (16.88%) |

## DISCUSSION

In this study, we have taken a total no. of 77 symptomatic specimens for HCV tri dot examination. Only 1(1.29%) case was non-reactive for tri dot out of 77 cases. The sensitivity considered for HCV tri dot in this study approximately 95%-99%. 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[15,16,17]. 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 build-up 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. 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 load scan have a difficult time getting the virus to become completely undetectable on treatment [18,19,20]. Target was 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 16 (48.18%), 20(52.63%), TND and 2(5.01) cases. This might be due to repeatedly being exposed to infected blood. The age group of 0-20 no viral load was detected in this age group. 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[21]. This assay enabled us to detect 6HCV genotype (1, 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. Our study were also with maximum number of cases with HCV RNA viral load >500 IU/ml with HCV genotype 3, which was found 68.42% in patients and other genotypes and subtypes were 1 (15.78%), 1b (2.63%), 2 (5.26%), 4(2.63%), 6 (5.26%) respectively. 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. Study revealed that the prevalence of genotype 1 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 1 and 1b infection was still a significant risk factor. From the above result discussed, it is evident that for positive cases in which viral load detected was very high (> 1.10 X $10^8$) in 14 cases whereas in only 1 case was found very low viral load <25IU/ml. Total prevalence of HCV viral load was calculated was 45.45% of total suspected cases. In some positive cases like case no.19 levels of bilirubin, and globulin were found to be high among all the cases whereas Gamma glutamyl transferase(GGT) was depicted low in 5 cases and high in 8 cases. Total protein content in serum in all the cases was average except 4cases which was having low protein content.2 cases was having low uric acid content which was having high viral load. From all high positive(high viral load) cases which were further diagnosed for their genotyping in which genotype 3 was prevalent following by genotype1, 1b, 2, 4 and genotype 6.

## CONCLUSION

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. 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. Efforts by investigators across the globe have led to detailed molecular characterization of the virus but despite these advancements in our standing, many fundamental questions will remain unanswered. The membrane associated RNA replication complex presumably involves various host proteins. However, the precise host components and mechanisms for replication are not understood yet.

**Conflict of interest:** None

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