Prevalence of HCV and Its Correlation with HCV Genotypes

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ABSTRACT

Introduction: Hepatitis C virus is known to cause liver inflammation, which may progress to liver cirrhosis and hepatocellular carcinoma. The genomes of HCV display sequence heterogeneity, thus, are classified in different genotypes and subtypes. Genotyping aids in understanding the epidemiology, biological features of the virus and inspecting the outbreak of the viral infection.

Materials and Methods: In the present study, HCV viral RNA was isolated and quantified by RT-PCR technique followed by determining its genotype by Sanger sequencing method from the blood samples of infected patients.

Results: Out of 40 samples, 25 showed positive results and 15 samples showed copy number lower than detection limit (< 35 IU/ml). Of 25 patients, the predominant genotype was 3 (a, b, g, i, k) followed by genotype 1 (a, b, g) and 4(a, c).

Conclusion: The results revealed that genotype 3b accounted for the highest number of cases with positive HCV viral load as compared to genotype 1 and 4. The higher prevalence of genotype 3 shows that the needful measure and timely treatment should be conducted.

Keywords: HCV, heterogeneity, genotype, RT-PCR.

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I. INTRODUCTION

Viral hepatitis is one of the major global health concerns which might be due to blood-borne pathogen that is, hepatitis C virus. This pathogen has been reported to cause the high prevalence of chronic type of infection in general populations [1], [2].

According to reports from the World Health Organization (WHO), countries with high prevalence rates of Hepatitis C infection found in Egypt approximately $>10\%$, Pakistan $>2\%$, China $>2\%$, and most of the other African countries with $>3\%$. However, total 3% of world's population were infected with Hepatitis of which approximately 0.8-1.5% patients found having HCV infection in India [3], [4].

About 25-30% of infected cases attributed liver cirrhosis [5], which further increases their risk of developing hepatocellular carcinoma [3]. Also, it is major cause of liver transplantation [6]. The main route of HCV transmission in developing countries is through transfusion of infected blood and reuse of needle, syringe (particularly among drug users) [3].

On the basis of the phylogenetic and sequence analysis of the HCV viral genome which are recovered from different geographical areas, they are classified into 6 genotypes and these genotypes are further subdivided into different subtypes [3]. The predominance of genotype 3 is seen in the northern,

western, and eastern regions of India, whereas genotype 1 is most commonly found in southern region of India [7]. The quantification of virus, clinical findings and determining the genotype act as a strong predictor for the antiviral therapy of HCV-infected individuals. The viral load is used to monitor the treatment response and relapse rates [8]. HCV genotyping also aids in understanding the epidemiology, biological features of the virus and inspecting the outbreak of the viral infection [3].

A major challenge is seen in developing pan-genotypic anti-viral drugs and vaccines due to the high genetic diversity of the virus. For this, a country-wide study on the prevalence of the HCV genotype is required [9]. Having millions of people affected by hepatitis C in India, there is still no accurate data about its prevalence and policies. Hence, a surveillance system is needed to know the actual number of HCV-infected individual [3].

The aim of the present study was to determine the prevalence of HCV and its correlation with genotypes.

II. MATERIALS AND METHODS

A. Clinical Samples

Total 40 blood samples were collected from suspected patients visited to Shree Nath Gene Laboratory and Research Centre (SNGLRC), Surat for analysis.

B. Viral RNA Extraction

From the suspected patients' sera (serum/plasma), RNA was extracted using Viral RNA extraction kit. The plasma/serum sample (150 µl) was subjected to lysis under the highly denaturing conditions provided by the lysis buffer. Further, the flocculating reagent was added to enhance the binding of viral RNA to the silica membrane. After binding, a series of washing steps were performed in presence of chaotropic salts. The pure RNA was eluted from the membrane in 50 µl of elution buffer and subsequently stored at -20 °C.

C. Quantification of HCV RNA

HCV RNA was amplified using artus HCV QS-RGQ kit as per manufacturer's recommendation and was quantified by using Rotor Gene Q Qiagen analyser. The lower detection limit for quantification is less than 35 IU/ml. The obtained value was multiplied by dilution factor to get the actual viral load in IU/ml.

D. Genotyping of HCV RNA

HCV RNA positive samples (>5000 IU/ml) were subjected to HCV genotyping by using specific HCV genotyping procedure.

E. Target Gene Amplification

Initially, 5 µl of HCV RNA was utilized for PCR amplification. The amplified 1st round for PCR product was subjected to 2nd round of nested PCR amplification, to obtain 232 bp of specific amplicon size. After amplification, the product was quantitatively analyzed by gel electrophoresis on a 2% agarose gel and documented under gel documentation system (Bio-Rad, USH) to visualize the desired 232 bp fragment.

F. Fluorescent Nucleotide Sequencing

The sequencing reaction was performed by using Brilliant Dye TM Terminator v 3.1 cycle sequencing kit as per manufacture protocol. The sequencing PCR product (amplicon) was then cleaned up and loaded into the 3500 Dx Genetic analyser.

G. Bioinformatic Analysis

The genotype was identified by using a web-based genotyping tool NCBI Viral Genotyping tool (<https://www.ncbi.nlm.nih.gov/projects/genotyping/formpage.cgi>).

III. RESULT

The distribution of different HCV genotype in HCV infected patients in studied population is shown in (Table I).

TABLE I: PREVALENCE OF HCV GENOTYPE IN STUDIED POPULATION

HCV Genotype	Number of cases
1a	3
1b	2
1g	1
3a	3
3b	6
3g	1
3i	1
3k	4
4a	1
4c	1

Results revealed that out of 40 patients, 25 (62.5%) patients were found positive with HCV infection and 15 (37.5%) patients were having HCV viral load less than 35 IU/ml (Fig. 1).

Fig. 1. Prevalence of HCV viral load.

Out of 25 patients, 2 patients were having viral load less than 5000 IU/ml, due to which the genotyping of this patients not conducted. Hence, total 23 patients were subjected for genotype determination.

Out of 23 patients, 15 showed the presence of Genotype 3 (65%), 6 showed Genotype 1 (26%) and 2 showed Genotype 4 (9%) (Fig. 2).

Fig. 2. Prevalence of HCV Genotypes.

The prevalence of Genotype 3b (26%) was found to be highest followed by genotype 3k (17.3%), 3a and 1a (13% each), 1b (8.6%), 1g, 3i, 3g, 4a, and 4c (4.3% each) (Fig. 3). Also, cases with mixed genotype infection were not found.

Fig. 3. Prevalence of HCV Genotypes and Subtypes.

IV. DISCUSSION

The prevalence and distribution of different genotypes of HCV varies significantly between countries and regions. In a retrospective study showing the global distribution and prevalence of HCV genotypes, conducted by Messina *et al*, [10], revealed that the most common HCV genotype was genotype 1 which mostly prevailed in Northern and Western Europe, Asia, North and South America, and Australia. While the second most common genotype was genotype 3 with the higher frequency of cases found in south Asia. These geographical differences may help in predicting the origin of HCV virus. Also, the above cited study signified the predominance of genotype 3 found in the present study.

Satsangi and Chawla [11], presented a study regarding the Indian scenario on Viral hepatitis which revealed that the hepatitis C virus genotype 3 was the most common reported genotype in India and predominantly found in northern, eastern, and western regions of India, while genotype 1 and 3 both were commonly found in Southern India. The present study showed the dominance of genotype 3 which was in consistent with the above cited study.

The present study shows the predominance of genotype 3, with highest frequency of genotype 3b, followed by 3k and 3a. Also, the prevalence of genotype 1 and 4 was seen. Most of the studies from India by Lole *et al* [12], Singh *et al* [13], Hissar *et al* [14], Narahari *et al* [15], revealed high prevalence of genotype 3 which was in accordance with the present study.

The HCV genotype 4 is known to be geographically restricted in most of the Arab countries, as suggested in a study by Shier *et al* [16], which characterized the HCV genotypes by direct sequencing method and found that out of 51 patients, 39 were having genotype 4 (76.4%) followed by 10 patients with genotype 1 (19.6%) and 2 patients with unidentified genotype. In India, the HCV genotype 4 was exclusively found in minority in mostly the southern regions of India as presented in a study by Raghuraman *et al* [17], which included 12 patients from north India, 73 from east India and 40 from south India, the HCV genotype 4 was found exclusively in 9 patients from south India only. However, in the present study, 2 cases of HCV genotype 4 was found which suggested that this genotype was no longer restricted to south India only.

The identification of HCV genotypes and subtypes in the patients from Maharashtra was carried out by Mohanraj *et al* [18], they revealed that out of 80 patients, 45 patients were found with genotype 3 (56.25%), 28 patients with genotype 1 (35%), 6 patients with genotype 4 (7.5%) and 1 patient with genotype 5 (1.25%). In the current study, the predominance of genotype 3 was found along with the presence of the genotype 1 and 4 which was in agreement with the above cited study. There was no case of genotype 5 found in the present study which differed from the above cited study. Also, the above study reported the presence of the following subtypes: 3a, 1a, 1b, 3i, 3g, 3k, 4c, 4d, 4l and 5a which showed similarity as the presence of subtype 3a, 3i, 3k, 3g, 4c, 1b, 1a were also seen in the current study. Genotype 2, 6 and 7 were not found in either of the above studies which indicated the overall low prevalence of these genotypes in India.

The Genotypes 5 and 6 are not detected in the present

study. But in a study conducted by Syed *et al* [19], had reported the presence of genotype 5a among the Indian population. And the prevalence of genotype 6 was found in the eastern and north-eastern parts of India as per the study conducted by Puri *et al* [20].

In a retrospective study conducted by Kanwal *et al* [21], regarding the association of HCV genotype 3 and increased risk of cirrhosis and hepatocellular cancer indicated that genotype 3 is associated with higher rates of liver cancer and fibrosis. The predominance of HCV genotype 3 in the current study and overall, in India may lead to an increasing number of chronic hepatitis cases in near future if the required treatment and preventive measures are not undertaken.

The study conducted by Raimondi *et al* [22], suggested that HCV genotype 1b played an important role in developing hepatocellular carcinoma. Since, HCV genotype 1 being the second most common genotype found in India, the infected individuals need to be treated to avoid the risk of hepatocellular carcinoma.

V. CONCLUSION

The present study showed highest prevalence of genotype 3, followed by genotype 1 and 4. The predominance of HCV genotype 3 in the current study showed the correlation with that found in overall India. However, no correlation was found between the HCV genotypes and viral load in the study. The limitation of the present study was that it included a small sample size which could not ascertain the proper correlation between HCV genotype and viral load. Whereas, considering the larger sample size would help to ascertain the correlation and prevalence of the genotypes better. The findings of this study can be helpful in planning preventive measures and treatment (antiviral therapy) against the most prevalent HCV genotypes in India.

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