

The predominance of Hepatitis C Virus Genotypes and Its Association with the Viral Load in Bangladeshi Population

BioResearch Communications
Volume 7, Issue 1, January 2021



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ABSTRACT: Determination of hepatitis C virus (HCV) genotype and viral load are two significant prognostic and assessment markers of treatment decisions. The study aimed to determine the predominant HCV types or subtypes and any association with the viral load in Bangladeshi chronic HCV infected patients. A total of 359 anti-HCV positive patients underwent investigation to estimate viral load and determination of genotype and subtype using real-time reverse transcriptase polymerase chain reaction (real-time RT-PCR). Among 306 detectable viral loads containing individuals, 278 (90.85%) genotyped successfully, and 28 (9.15%) had unknown genotypes. Among typable genotypes, 1a accounted for 14 (5.03%), 1b for 14 (5.03%), 3 for 247 (88.85%), 4 for 2 (0.72%) and genotype 6 for 1 (0.36%). Based on pre-treatment viral load levels, study subjects classified into three categories such as low (<50000 IU/mL), intermediate (50000-500000 IU/mL), and high (>500000 IU/mL). The majority of HCV other types (1a, 1b, 4, 6) infected patients (96.4%) had intermediate to high viral load compared to those infected with genotype 3 (77.7%) and unclassified types (55.0%) ($\chi^2 = 15.41$; $p = 0.004$). HCV type 3 was prevalent (68.4%) in the above 40 years of group compared to less than 40 years group (31.6%). HCV genotype 3 was the predominant genotype circulating in Bangladesh. Pre-treatment viral load demonstrated significant difference among individuals having HCV other types and type 3. However, sequencing the HCV genome analysis would determine the exact types and subtypes among all possible HCV strains available in Bangladesh.

KEYWORDS: Hepatitis C virus, Genotypes and Subtypes, Viral load; Real-time RT-PCR

RECEIVED: 17 September 2020, ACCEPTED: 14 December 2020

TYPE: Original Research

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Introduction

Hepatitis C Virus (HCV) infection is recognized as a well-known significant public health problem worldwide. Among about 1% of the world's population having chronic HCV infection, almost half are unacquainted as the disease is often asymptomatic. Around 1.75 million persons become newly infected each year. Chronic HCV infection perceives as one of the leading causes of chronic liver diseases, including liver fibrosis, liver cirrhosis, and hepatocellular carcinoma^{1, 2}. Successful health outcomes of HCV infection and preventing transmission require identifying chronic active infection as the primary step³⁻⁵. HCV is an enveloped flavivirus having positive single-stranded RNA as its genome (with approximately 9600 nucleotides), which encodes a polyprotein precursor of about 3000 amino acids⁶. Evaluation of the status of HCV infection has been identified as vital to predict the outcome of disease progression. Several factors, including age at infection, gender, genotype/subtype, viral load, and mode of infection, have been identified to determine the rate of disease progression. Hepatitis C virus genotypes vary in geographical distribution, and treatment response varies according to genotype⁷. Among diagnostic and prognostic assessment markers of chronic HCV infection, quantification of HCV-RNA is indicated to determine baseline viral load

prior to initiating antiviral therapy, to determine the treatment duration as well as to determine the therapeutic success by finding the viral load not detected^{7, 8}.

Based on the genetic differences, HCV has been classified into six genotypes with several subtypes. The predominance and distribution of HCV genotype vary globally. Of the six genotypes, three (genotype 1, 2, and 3) are prevalent throughout the world, and the remaining three are restricted to particular geographical areas. Higher prevalence of HCV genotype 1a and 1b was evident in the United States and Europe, respectively. Type 2 represents less in West Africa. Type 3 is predominant in South-East Asia and Australia as well as variable prevalence in different countries. Genotype 4 is mainly circulating in the Middle East, Egypt, and central Africa. Patients infected with different HCV genotypes exhibit differences in disease severity or outcome⁹⁻¹³.

HCV genotypes' determination is the prognostic indicator of response to antiviral therapy associated with the pre-treatment viral load. Detection of HCV genotypes is also indicated to determine the duration and dose of antiviral therapy. Individuals with genotype 1 and 4 demonstrate an inadequate response to interferon alone, whereas genotypes 2 and 3 more favorable responses. It was evident from other studies that

infection with 1b genotype exhibits a worst prognosis than other types and subtypes. Hence, when a combination of interferon and ribavirin therapy is used, patients with genotypes 2 or 3 are recommended to receive treatment for 24 weeks, whereas patients infected with genotype 1 for 48 weeks^{7,14}.

Increased viral load before antiviral therapy has been associated with low response rates to standard antiviral therapy by several studies¹⁴⁻¹⁶. Several studies have demonstrated that patients with lower pre-treatment viral load are more likely to positively respond to currently available antiviral therapy as compared to high pre-treatment viral load¹⁷⁻¹⁹. Several studies have found significant association of the pre-treat viral load with the particular HCV genotype and subtype²⁰⁻²².

Although some sporadic studies on the prevalence of HCV genotype were carried in Bangladesh, no studies addressed the association of pre-treatment viral load with the particular HCV genotype. Therefore, this study aimed to investigate the distribution of different HCV genotypes and subtypes in Bangladesh and determine any correlation between particular genotype with the HCV viral load.

Materials and Methods

Patients and study design

The study comprised three hundred fifty-nine individuals who were anti-HCV antibodies positive and referred by the specialist physician to determine HCV genotypes. Informed consent obtained from the individual, and the institutional ethical review committee approved the study protocol. Individuals, who were positive for anti-HCV antibodies, however negative for HCV RNA or were under treatment, were excluded. Out of 359 anti-HCV antibody-positive patients, 306 had the detectable level of HCV RNA. All HCV RNA positive patients were selected for the study and subjected to HCV genotype and HCV viral load determination.

Samples

Three milliliters (3 ml) venous blood samples were collected from each patient in K₃EDTA vacutte tubes. After centrifugation, plasma was separated immediately after collection and stored at -80°C before analysis.

HCV RNA viral load

HCV RNA was extracted using the INSTANT Virus RNA kit (AJ Roboscreen GmbH, Germany), and quantification was performed using the Smart Cycler II Real-time PCR platform (Cepheid Inc., USA) with RoboGene HCV RNA Quantification Kit (AJ Roboscreen GmbH, Germany). The lower and upper limits of detection the used assay was 250 to 5.0×10^8 IU/mL, respectively. Specimens yielding values above the upper limit were diluted 100-fold, retested, and the obtained values were multiplied by this dilution factor to get

the actual HCV RNA concentration in international units (IU) per ml.

HCV genotyping

Samples with a detectable HCV RNA level were subjected to HCV genotyping using HCV Genotype Plus Real-TM (Sacace Biotechnologies Srl, Italy). This kit detected genotypes 1a, 1b, 2, 3, 5a, and 6. 5' untranslated region (5' UTR) of HCV was used as the target region. The determination of HCV genotyping was done using Smart Cycler II Real-time PCR (Cepheid Inc, USA). All procedures were directed according to the manufacturer's recommendations.

Statistical Analysis

Statistical analysis was carried out using Statistical Package for Social Sciences (SPSS) version 20. All variables results were given in the form of rates (%). Chi-Square test was used for categorical variables that measured the association among categorical variables using 3X3 contingency table. P-values less than 0.05 were considered significant.

Results

Distribution of HCV genotypes

Out of the 359 tested serum samples, the number of patients with HCV RNA negative was 53 (14.8%). Among total 306 typable subjects, genotype 3 was found the predominant (247, 80.7%), followed by unclassified (28, 9.2%), genotype 1a (14, 4.6%), genotype 1b (14, 4.6%), genotype 4 (2, 0.6%) and genotype 6 (1, 0.3%) (Figure1).

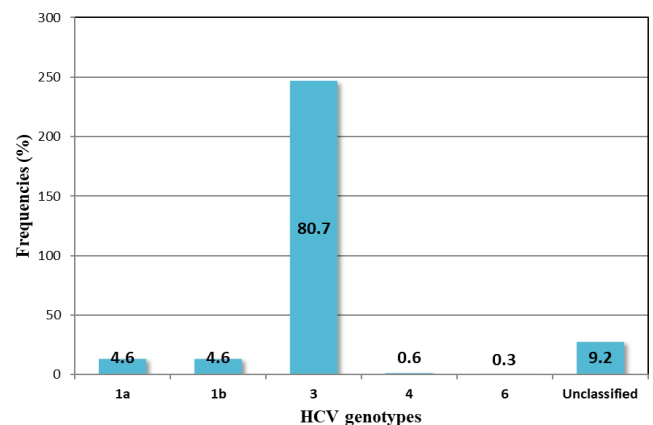


Figure-1. Frequencies of HCV genotypes among the total typable study subjects (N = 306).

Out of the 306 patients, about 63% were male, and 37% were female. The predominant genotype among males was 3 (75.5%), followed by combined other types (13%) and unclassified (11.5%). Similarly, the frequent genotype among the infected female patients was 3 (89.5%), followed by each combined other type and unclassified (5.25%) (Table 1).

Table-1. Distribution of HCV genotypes among males and females of the typable study subjects (N = 306).

Sex	Genotype			Total
	3	Others (1a, 1b, 4, and 6)	Unclassified	
Male	145 (47.4%)	25 (8.1%)	22 (7.2%)	192 (62.7%)
Female	102 (33.3%)	6 (2.0%)	6 (2.0 %)	114 (37.3%)
Total	247 (80.7%)	31 (10.1%)	28 (9.2%)	306 (100%)

Distribution of predominant HCV genotypes 3 in different age groups

Among 247 HCV genotypes 3 infected individuals, 85 (34.4%) were aged between 41 – 50 years, 61 (24.7%) between 51 – 60 years, 47 (19.0%) between 31 – 40 years, 28 (11.4%) between 21 – 30 years, 23 (9.3%) over 60 years and 3 (1.2%) under 20 years. It was found that predominant type was more frequent in the above 40 years of group compared to less than 40 years group (68.4% vs. 31.6%).

Association of the pre-treatment patients baseline HCV RNA loads with the HCV genotypes and subtypes

Among the total study subjects, 263 individuals were referred by the specialist physician to see the baseline viral load and

genotypes. According to Jensen et al.²², viral load was classified into three categories based on its levels. However, levels were kept not exactly the same, as different geographical region population might have different range of viral load. These individuals were stratified into three categories based on viral load levels such as low ($< 5 \times 10^4$ IU/mL), intermediate ($5 \times 10^4 - 5 \times 10^5$ IU/mL) and high ($> 5 \times 10^5$ IU/mL). The extent of HCV RNA viral load exhibited a significant difference among them. The majority of patients infected with HCV genotype other types (1a, 1b, 4, 6) (27/28, 96.4%) had intermediate to high viral load compared to those infected with genotype 3 (167/215, 77.7%) and unclassified types (11/20, 55.0%) ($\chi^2 = 15.41$; $p = 0.004$) (Table 2).

Table 2. Association of the pre-treatment viral load with the HCV genotypes/subtypes (N = 263).

Viral load (IU/ml)	HCV genotypes/subtypes		
	Unclassified	3	Other types
$< 5 \times 10^4$	9 (45.0%)	48 (22.3%)	1 (3.6%)
$5 \times 10^4 - 5 \times 10^5$	3 (15.0%)	90 (41.9%)	11 (39.3%)
$> 5 \times 10^5$	8 (40.0%)	77 (35.8%)	16 (57.1%)
Total	20 (100.0%)	215 (100%)	28 (100.0%)
	χ^2	15.41	
	p	0.004	

Percentages are of their respective column totals.

Discussion

This study aimed to investigate HCV genotypes' distribution and association of particular type with the viral load, age group, and gender. HCV genotype 3 was the predominant genotype circulating in Bangladesh. It was the predominant type among the males and females of the study subjects also. The pattern of HCV genotypes distribution is concordant to that reported from Southeast Asian countries like Thailand and Malaysia, India and Pakistan where the predominant genotype is 3 and discordant from northern Southeast Asian countries such as Myanmar, Laos, and Vietnam where genotype 6 is prevalent and Island nations of Singapore, Indonesia and

Philippines demonstrated the prevalence of genotype 1²³⁻²⁵. A prospective as well as retrospective cross-sectional observational study found genotype 3 was the commonest HCV genotype among the Bangladeshi population²⁶.

We found that the predominant HCV type 3 was more prevalent in the above 40 years of group compared to less than 40 years group. This pattern was not in agreement with the other study, which showed that for all HCV genotypes, the highest rate of prevalence was observed in the age group of ≤ 40 years²⁷.

We found that pre-treatment viral load was significantly associated with the genotypes other than 3. The correlation between HCV genotype and HCV viral load was found controversial in many studies. However, pre-treatment viral load, genotype, and age were shown as independent predictors for sustained HCV RNA response¹⁶. We strived to find out any association of the pre-treatment viral load with the genotype in light of this. We found that among HCV other types (1a, 1b, 4, 6) infected patients, 96.4% had intermediate to high baseline viral load. Among patients infected by HCV type 3, 77.7% had intermediate to high baseline viral load. Among patients infected by the unclassified types, 55% had intermediate to high baseline viral load. This finding and other¹⁸ intensify the need to determine HCV genotypes and basal viral load when therapeutic strategies against HCV are scheduled at the national level.

We found a good proportion (9.2%) of the study subjects remained as untypable HCV variants. Routine diagnostic laboratories in Bangladesh used to perform Real-time PCR technology to determine the HCV genotype and subtypes. This study also used the same technology where probes and primers specific for the 5'-UTR region of six types (1a, 1b, 2, 3, 5a, and 6) were used. The emergence of HCV quasispecies in patients with chronic infection is well recognized. Multiple factors contribute to the continuous generation of HCV variants that may have clinical significance²⁸. Although HCV genotyping targets a highly conserved region of the HCV genome, minor changes in this region result in the loss of detecting exact genotype by the method used. Besides, chronic HCV patients might have mixed variants that also remain undetectable. Analysis of the HCV genome by either Sanger Sequencing or Next Generation Sequencing (NGS) could be the most effective way of accurately identifying the changing pattern in HCV clades in Bangladesh and determining the course of standard interferon therapy. This strategic approach should be implemented in clinical diagnostic settings in Bangladesh to determine HCV genotypes and subtypes.

Conclusion

The study demonstrated HCV genotype 3 as the predominant strain circulating in Bangladesh. The majority of the HCV type 3 infected patients were in ages between 41 – 50 years. Baseline viral load was significantly high in patients infected by HCV other genotypes (1a, 1b, 4, and 6) compared to genotype 3 and untypable variants. Sequencing based investigation of HCV genotypes is recommended to accurately determine all possible variants of HCV circulating in Bangladesh and thereby facilitate treatment options and preventive strategies in the country.

Acknowledgements

This study was the part of research grants received from the Ministry of Science and Technology, Government of the People's Republic of Bangladesh (Special Allocation Grant for Science and Technology, No. BS-283, 2018-19) and Conference Travel and Research Grant Committee (CTRG), Office of Research, North South University (No. OR-NSU-CTRG-19/SHLS/06). We are greatly indebted for the support. We would also like to express our gratitude for the assistance

and support from DNA Solution Ltd. and Medinova Medical Services Ltd. staff to collect, preserve, and process blood samples from participants. We are grateful to all participants of the study who gave their kind consent.

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