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Original Article

Determination of HCV genotyping for therapeutic purpose in capital city Dhaka, Bangladesh

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ABSTRACT: Background: Hepatitis C is an infectious disease affecting the liver, caused by the hepatitis C virus (HCV). The hepatitis C virus is a small, enveloped, single-stranded, positive sense RNA virus with a large genetic heterogeneity. Hepatitis C virus is classified into six major genotypes with closely related isolates, which are grouped into many subtypes. Genotypes 1, 2 and 3 circulate around the world, while other genotypes are mainly restricted to particular geographical areas. Genotype determination of HCV is clinically valuable as it provides important information, which can be used in determining the type and duration of therapy and in predicting the outcome of the disease.

Results: Plasma samples were collected from one hundred and sixty-eight HCV RNA positive patients who were referred to the DNA Lab Limited, Dhaka, Bangladesh by specialists in order to determine genotypes for treatment purpose between January 2017 and December 2018. Plasma samples from patients were subjected to HCV genotype determination through the use of Real-Time PCR method. The frequency of HCV genotypes was determined as follows: genotype 3a (82.1%), 1a (11.3%), 1b (4.2%), 6 (1.2%), 4 (0.6%) and 5a (0.6%).

Conclusion: Genotype 3a is the most prevalent followed by the genotypes 1a & 1b and the less frequent genotypes are 4, 5 and 6 in capital city Dhaka, Bangladesh

Keywords: HCV, Genotypes, Prevalence, RT-PCR, Therapy.

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INTRODUCTION

Hepatitis C virus (HCV) is a small enveloped virus first isolated in 1989 and belongs to the family of flaviviridae.[1] Its genome is composed of a positive-sense, single-stranded RNA encoding a polyprotein comprising structural (core and envelope glycoproteins, E1 and E2) and non-structural (NS2, NS3a/b, NS4a/b and NS5a/b) proteins. Acute HCV infection is mild and often asymptomatic while chronic hepatitis C in an indolent course but may progress to cirrhosis.[2] This may lead to progressive liver disease, cirrhosis, liver failure and hepatocellular carcinoma within 20 to 30 years. Factors associated with disease progression following infection include the viral genotype, the patient's alcohol consumption and viral load.[3] HCV is a slowly progressive infection spreading primarily through intravenous drug users. It can also spread by sharing of toothbrushes, razors and contaminated needles, sexual relations, from mother to child etc. HCV RNA has not been detected in semen, urine, stool or vaginal secretion and whether it is present in saliva remains controversial[4]. HCV is classified into six major genotypes with closely related isolates, which are grouped over 100 subtypes.[5] In HCV, genome replication is occurred by RNA-dependent RNA polymerase, it has no proof-reading activity and mutation rate is high, so many genotypes and subtypes of HCV are easily emerged. That is why hepatitis C virus is called viral quasispecies. HCV genotyping is crucial for epidemiological and clinical analysis. Determination of the HCV genotype also provides clinically important information that can be used to direct the type and duration of anti-viral therapy and to predict the likelihood of sustained HCV clearance after therapy.[6, 7] Patients with HCV genotype 1 may benefit from a longer course of therapy and genotypes 2 and 3 are more likely to respond to a combination of interferon and Ribavirin therapy.[8] This study, first of its kind in capital city Dhaka, Bangladesh, has been performed to facilitate the health care providers and clinicians in designing the therapeutic strategies for predicting the likelihood of sustained HCV clearance after therapy.

MATERIALS AND METHODS

Patients and study design

Patients who were infected with hepatitis C virus were referred to the DNA Lab Limited, Dhaka, Bangladesh by specialists for determining genotypes for treatment purpose between January 2017 and December 2018. These patients were screened primarily for anti-HCV antibodies using a commercial ELISA kit - Hepanostika HCV Ultra (Beijing United Biomedical Co. Ltd, China). Those who were positive for anti-HCV antibodies were selected for HCV RNA detection using the reverse transcriptase real time PCR (RT-qPCR) technique. Out of 184 anti-HCV antibody positive patients, 168 were positive for HCV RNA. Patients who were positive for anti-HCV antibodies but were negative for HCV RNA were excluded from this study. All samples from HCV RNA positive patients were selected for this study and were subjected to HCV genotype determination.

Plasma samples

3 mL of venous blood samples were collected from each patient referred to the laboratory. Blood samples were collected in BD Vacutainer® K2EDTA tubes (BD, USA). Centrifuged and separated plasmas were immediately stored at -80^{0}C. To avoid HCV RNA degradation, aliquots were not thawed more than once prior to analysis.

HCV RNA detection

HCV RNA was extracted from plasma samples with QIAamp DSP Virus Kit (Qiagen, Germany). Extracted RNA samples were tested for the presence of HCV RNA using *artus* HCV RG RT-PCR Kit (Qiagen, Germany). The experiments were conducted according to the manufacturer's recommendations. Data was collected using Applied Biosystems™ 7500 Real-Time PCR System (Thermofisher, USA).

HCV genotyping determination

For determining the genotypes of HCV, AmpliSens® HCV-genotype-FRT kit (AmpliSens Biotechnologies, Russia) was used. The RNA was extracted again from plasma samples using RIBO-sorb kit (AmpliSens Biotechnologies, Russia) as the genotype kit was not optimized for QIAamp DSP Virus Kit. Reverse transcription step to make cDNA was done using Reverta-L kit (AmpliSens Biotechnologies, Russia). All experiments were conducted according to the manufacturer's recommendations. Data was collected using Applied Biosystems™ 7500 Real-Time PCR System (Thermofisher, USA).

RESULTS

Patients infected with hepatitis C virus and were referred to the DNA Lab Limited, Dhaka, Bangladesh by specialists for determining genotypes between January 2017 and December 2018 were subjected to the current study. These patients were firstly screened for the detection of anti-HCV antibodies in their plasmas as mentioned in materials and methods section. Among the one hundred and eighty-four anti-HCV antibody positive patients, one hundred and sixty-eight patients were positive for HCV RNA. These HCV RNAs were detected by RT-PCR mentioned in materials and methods section (Figure 1). Among the 168 patients, one hundred and seven subjects (63.7%) were male and sixty-one (36.3%) were female. The mean age of the HCV nucleic acid positive patients was 46.8 years and the age range were 11-80 years. The age between 40 and 60 years is more vulnerable for infection of HCV and from our

study, it has been found that 85 (50.6%) patients infected with HCV are within this age range (Table 1). The HCV infection is only 4.8% and 14.9% in people of <20 years and >60 years respectively. The current study also showed that 138 (82.1%) patients were infected with genotype 3a of HCV among the total cases (Fig.2). The next frequent identified genotype of HCV was type 1a, which accounted for 19 (11.3%) patients. The genotype 1b represented 7 (4.2%) of the total cases. Other less frequent identified genotypes were 4 (0.6%), 5a (0.6%) and 6 (1.2%) (Table 1).

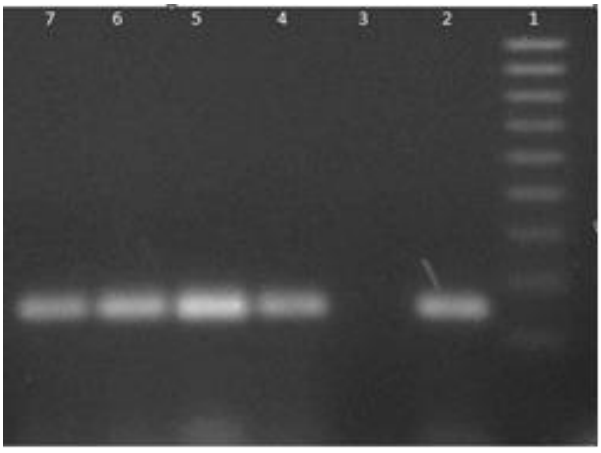

Figure 1. Agarose gel electrophoresis for the detection of HCV nucleic acid: Lane 1 represents the 100 bp marker (Bio-Rad, USA); Lane 2 represents the positive control (280 bp); Lane 3 represents the negative control; Lane 4 – 7 represents patients positive for HCV nucleic acid.

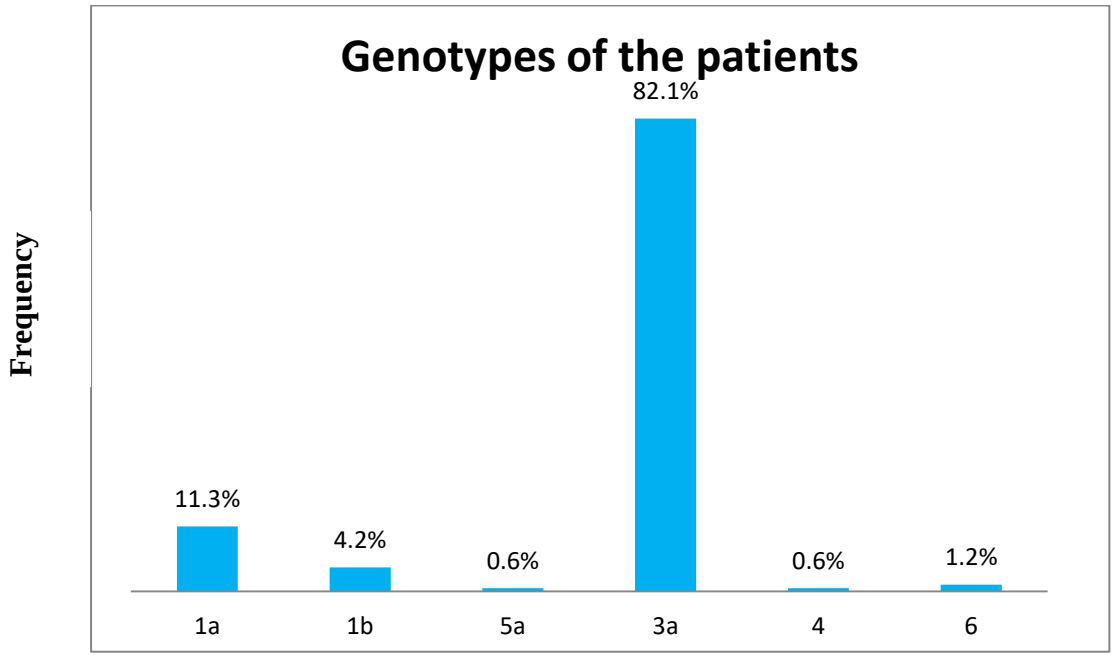

Figure 2. Different genotypes of hepatitis C virus

Table 1. Frequency of HCV genotypes among the patients

Variable	Genotypes of the patients						Total
	1a	1b	5a	3a	4	6	
Gender							
Male	13(68.4%)	4(57.1%)	0(0.0%)	87(63.0%)	1(100.0%)	2(100.0%)	107(63.7%)
Female	6(31.6%)	3(42.9%)	1(100.0%)	51(37.0%)	0(0.0%)	0(0.0%)	61(36.3%)
Age							
<20	3(15.8%)	0(0.0%)	0(0.0%)	5(3.6%)	0(0.0%)	0(0.0%)	8(4.8%)
20-40	7(36.8%)	2(28.6%)	0(0.0%)	41(29.7%)	0(0.0%)	0(0.0%)	50(29.8%)
40-60	9(47.4%)	4(57.1%)	1(100.0%)	69(50.0%)	1(100.0%)	1(50.0%)	85(50.6%)
>60	0(0.0%)	1(14.3%)	0(0.0%)	23(16.7%)	0(0.0%)	1(50.0%)	25(14.9%)

DISCUSSION

Global patterns for the distribution of different HCV genotypes are as follows:

Genotype 1a is mostly found in North and South America, also common in Australia. Genotype 1b is mostly frequent in Europe and Asia. Genotype 2a is the most common subtype in Japan and China. 2b is the most prevalent genotype in the US and North Europe. 2c is the most common genotype in Western and Southern Europe. 3a is highly prevalent in Australia and South Asia. 4a is highly predominant genotype in Egypt. 4c is highly frequent genotype in central Africa. 5a is highly prevalent only in South Africa. Genotype 6a is restricted to Hong Kong, Macau and Vietnam.[9]

HCV genotype determination is of epidemiological importance and knowledge of the genotype is one of the main independent factors that influence the outcome of therapy[10, 11, 12]. Hepatitis C infection may lead to a substantial health and economic burden over the next 10 to 20 years in the world[13]. One previous study reported that the genotype 3b is the most prevalent HCV genotype in Bangladesh[14]. Another recent study reported that HCV genotype 1 is the most prevalent worldwide and genotype 3 is the next most prevalent globally[15]. Genotype 3a is the most prevalent genotype in patients of all age groups[16]. Genotypes of hepatitis C virus in the Indian sub-continent, it has been found that genotype 3 is the common genotype in patients of all age groups[17].

From a public health perspective, the implementation of molecular tests as an integral part of the diagnostic and therapeutic management of infection with HCV should be imperative. Knowing the actual genotype is very important for taking proper therapy and its duration. Hence, nucleotide sequence analysis is regarded as the gold standard for the identification of different genotypes and subtypes of HCV. In this study, we used nucleotide sequence analysis for determining different HCV genotypes with high accuracy. This investigation was performed in the DNA Lab Limited, Dhaka, Bangladesh where patients from Dhaka and its surroundings were referred for determining the genotypes of HCV. HCV genotype 3a was found to be the most prevalent (82.1%), followed by genotype 1a (11.3%), 1b (4.2%), 6 (1.2%), 4 (0.6%) and 5a (0.6%). In comparison with studies made in Bangladesh's neighbor country, it can be understood that the most common genotype in Myanmar is type 6, followed by 3b[18]. Although genotype 6 is the most prevalent in Myanmar; this genotype is very uncommon in Bangladesh. A similarity has been found between Bangladesh and both India and Pakistan, in which genotype 3 is very prevalent and genotype 2 is rare[19, 20]. It could be due the high rate of migration of population among these countries. In our study, we have found that genotype 3a was the predominant HCV genotype in the capital city Dhaka, Bangladesh. This report also supported the previous one study completed in Bangladesh[21].

CONCLUSION

In conclusion, our results demonstrated the most prevalent HCV genotype 3a, followed by 1b and the very low frequency genotypes 4, 6 and 5a in the capital city Dhaka, Bangladesh. Even though this study had some limitation like relatively smaller sample size and shorter period of the study, it is the first of its kind of study in the capital city Dhaka, Bangladesh. This study will facilitate the healthcare providers and clinicians in designing the therapeutic strategies against the HCV infections.

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