

Brief Original Article

Hepatitis C virus genotype and its correlation with viral load in patients from Kathmandu, Nepal

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Abstract

Introduction: Knowledge about the distribution of hepatitis C virus (HCV) genotype and its correlation with viral load are important for the decision of treatment and the prediction of disease progression, however such information is very limited in Nepal. Here, we investigated the distribution of HCV genotypes and viral load for HCV-infected patients from Kathmandu, Nepal.

Methodology: Ninety-six patients with HCV infection and not on antiviral therapy were enrolled from three different medical centers in Kathmandu valley, Nepal. Demographics were recorded and blood samples were collected. Plasma was separated and HCV RNA was extracted. Reverse transcriptase PCR (RT-PCR) was performed to measure the viral load, and virus genotype was determined.

Results: Genotype 3a (n = 53, 55.2%) was the most prevalent, followed by 1b (n = 19, 19.8%), 1a (n = 18, 18.8%), 5a (n = 3, 3.1%), and mixed types (n = 3, 3.1%). The median viral load for HCV genotype 1a was 770,942 IU/mL (IQR, 215,268-3,720,075), 1b was 700,000 IU/mL (IQR, 431,560-919,000), 3a was 1,060,000 IU/mL (IQR, 641,050-6,063,500), 5a was 673,400 IU/mL, and mixed was 6,428,000 IU/mL. A correlation between genotype and viral load was observed (p = 0.02), of which genotype 3a showed a high viral load.

Conclusions: HCV genotypes 1a, 1b, 3a, and 5a were identified in Kathmandu, Nepal, and mixed genotype patients were observed in the patients studied. HCV genotype showed a correlation with viral load in patient plasma. This finding may contribute to the treatment and prevention of hepatitis C in Kathmandu, Nepal.

Key words: Hepatitis C virus; genotype; infection; viral load.

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Introduction

Hepatitis C virus (HCV) chronically infects ~71 million people worldwide with estimated annual mortality of 399,000. Majority of HCV-infected patients (70-80%) develops chronic hepatitis C, which increased the risk of cirrhosis and hepatocellular carcinoma [1]. Southeast Asia is one of regions with high prevalence of viral hepatitis, and it is estimated that more than 11 million people in this region are infected with HCV [2]. A recent study showed that prevalence of anti-HCV antibody among the general population of Nepal and the blood donors was 0.3%-1.7% [3]. The prevalence of HCV genotypes is found to differ across countries over the period of time, depending on the population studied, route of infection, and evolution of the virus. Genotype 3 is found to be

predominant in India, Pakistan, Bangladesh; genotype 1 in Turkey, Russia, Moldova, and Uzbekistan; genotype 4 in Saudi Arabia, Iraq, Qatar, Kuwait; and genotype 6 in China and South Asia [1]. Identification of HCV genotypes and viral load can provide clues to the clinicians for treatment and disease prognosis [1,3], and this is important and cost-effective in resource-limited countries and regions.

Studies have shown that the patients with lower pre-treatment viral load (< 800,000 IU/mL) were more likely to respond positively to antiviral therapy than those patients with high pre-treatment viral load (≥ 800,000 IU/mL) [4]. In developing countries like Nepal, identification of HCV genotypes and subtypes and its clinical management is poor due to lack of sufficient infrastructure and treatment protocols. Both

the diagnosis and treatment can be improved by genotyping, subtyping, and quantification of virus, which could be used as indicators for decision making of an optimal treatment regimen. In patients with HCV, both viral load and HCV genotype have clinical relevance, as virus load at the time of diagnosis shows a correlation to the treatment duration, especially in interferon (IFN)-based therapy [5,6]. Pegylated IFN- α plus ribavirin (PEG-IFN- α /RBV) is still the common treatment in a low-income and resource-limited settings.

Besides few studies on HCV infections in defined populations, distribution of HCV genotypes and subtypes and its association with viral load have not been studied for patients in Nepal. Here, we studied the HCV-infected patients from Kathmandu valley, Nepal.

Methodology

Subjects

The study was conducted among 96 patients tested positive for the HCV and negative for HIV and HBV. All patients were not taking any antiviral therapy at the time of study. Patients were recruited from three medical centers of Kathmandu valley, Nepal. A semi-structured questionnaire was used to collect demographic data (age and sex). The study was conducted from October 2016 to December 2017 using convenient time-frame sampling. Patients were informed about the study and they voluntarily consented to participate in the study. Ethical permission to conduct the study was obtained from Nobel Medical College.

Sample collection and molecular analysis

Blood collection

Blood samples of 5 mL were collected using EDTA vials, and each subject was tested positive for anti-HCV antibodies and negative for HIV and HBV. Plasma was separated and stored at -30°C until further analysis.

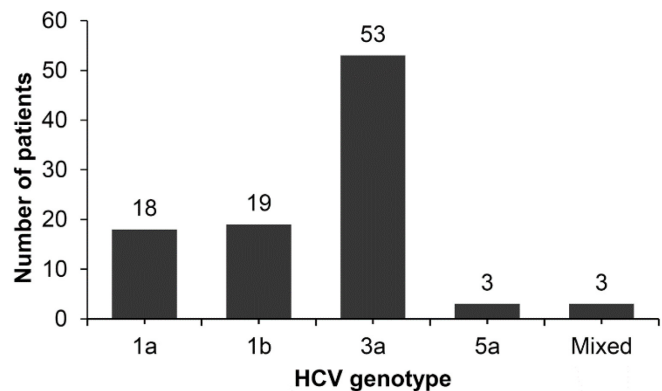
RNA extraction

Viral RNA was extracted using QIAamp Viral RNA Mini Kit according to the manufacturer's instructions (QIAGEN, Shenzhen, China).

Reverse transcriptase (RT)-PCR

RT-PCR was performed for the detection of HCV RNA using QIAGEN Artus HCV QS-RGQ kit (QIAGEN) by quantitative RT-PCR using Rotor Gene-Q System (QIAGEN). The HCV RT-PCR kit included reagents and enzymes for the reverse transcription and PCR amplification of HCV genome regions using a

Figure 1. Distribution of HCV genotype in the patients analyzed.



Plasma from total of 96 patients were analyzed for HCV genotype using AmpliSens® HCV-genotype-FRT PCR kit. Genotype 3 was predominantly prevalent among the samples. For the first time, genotype 5a and mixed were identified in Nepalese patients.

fluorescence dye FAM. TaqMan Fast Universal PCR Master Mix (2X) (Thermo Fisher Scientific, Shanghai, China) was added, which contained an optimized RT-PCR buffer, MgCl_2 , Taq DNA polymerase, and stabilizers along with quantitative standards QS1 to QS4 from the kit. The positive and negative control was included in parallel for each batch of analysis. The RT-PCR reaction was performed using the thermal cycles of cDNA synthesis at 50°C for 30 minutes, AmpliTaq Gold DNA Polymerase activation at 95°C for 15 minutes, and two-step amplification cycles comprising of denaturation at 95°C for 30 seconds and annealing and extension at 72°C for 30 seconds. The PCR products were detected by FAM and analyzed by Rotor Gene-Q System software package.

HCV genotyping

HCV RNA positive samples that had viral load > 1000 international unit per milliliter (IU/mL) were genotyped using AmpliSens® HCV-genotype-FRT PCR kit (Moscow, Russia). It was able to detect HCV genotypes 1a, 1b, 2, 3a, 4, 5a and 6 following the manufacturer's instructions. Briefly, 10 μL of each cDNA and 15 μL of each PCR mix (PCR-mix-1-FRT HCV 1b/3a, PCR-mix-1-FRT HCV 1a/2, PCR-mix-1-FRT HCV IC/4, and PCR-mix-1-FRT HCV 5a/6) were prepared on a MicroAmp® Optical 96-Well Reaction Plate (Thermo Fisher Scientific, Shanghai, China). The PCR reactions were analyzed in a Qiagen Rotor Gene-Q Cycler 5-plex HRM System with software version 2.3.1. The thermal cycles included 48°C for 2 minutes; 95°C for 20 minutes; and 45 cycles of 95°C for 10 seconds, 62°C for 30 seconds, and 56°C for 40 seconds.

Table 1. HCV genotype and subtype in different age and gender groups.

	HCV genotype and subtype				
	1a	1b	3a	5a	Mix
Age (years)					
< 20	2	0	4	0	0
20-39	15	19	44	2	3
> 39	1	0	5	1	0
Gender					
Male	16	17	52	3	3
Female	2	2	1	0	0

The analysis was completed within two hours by following the instructions.

The data was processed in Excel program and analyzed using IBM SPSS Statistics 21.0. Frequency of HCV genotypes and subtypes was determined along with the median viral load with interquartile range (IQR) for each genotype across gender and age groups. A high viral load was defined as a baseline HCV RNA $\geq 800,000$ IU/mL [7].

Results

A total of 96 HCV-infected patients from three medical centers in Kathmandu, Nepal were included in this study. The patients included 91 males and 5 females, and the mean age was 30.31 ± 6.68 years. As shown in Figure 1, 3a ($n = 53$, 55.2%) subtype was the most prevalent among these patients, followed by 1b ($n = 19$, 19.8%), 1a ($n = 18$, 18.8%), 5a ($n = 3$, 3.1%), and mix type ($n = 3$, 3.1%). Majority of patients (83/96, 86.45%) was in age of 20-39 years (Figure 1). The median viral load of 1a was 770,942 IU/mL (IQR, 215,268-3,720,075), 1b was 700,000 IU/mL (IQR, 431,560-919,000), 3a was 1,060,000 IU/mL (IQR, 641,050-6,063,500), 5a was 673,400 IU/mL, and in other mixed subtypes was 6,428,000 IU/mL. Distribution of different genotype and subtype with respect to gender and age (Table 1). Fifty-two of 91 male patients (57.11%) were 3a subtype, followed by 1b (17/91, 18.68%), 1a (16/91, 17.58%), 5a (3/91, 3.30%) and mix subtype (3/91, 3.30%). No genotype 5a and mix type-infected female patients was identified (Table 1). Here, we defined RNA copies of $< 800,000$

IU/mL as low viral load, while $\geq 800,000$ IU/mL was high viral load (Table 2). Overall, a correlation between HCV viral load and subtypes were observed ($p = 0.02$). The genotype 3a had a greater number of patients with high viral load ($n = 39$).

Discussion

In this study, we investigated the distribution of HCV genotypes prevalent in Nepalese patients attending to three different health centers at Kathmandu valley. Genotype 3a was the most prevalent accounting for more than half of HCV infections in the patients examined, followed by 1b, 1a, 5a, and mix genotypes. Majority of the HCV patients were at the age range of 20-39 years.

The presence of various HCV genotypes (1a, 1b, 3a) have also been reported earlier in studies from South Asian countries, such as India, Pakistan and Nepal, where 3a was also dominant genotype [8-10]. Genotype 3a was also found to be dominant in previously study and particularly prevalent among intravenous drug users [9,11]. We did not find genotypes 2, 4, and 6. Three patients were found with genotype 5a infection, and to our knowledge 5a was not identified in the previous studies from Nepal. The presence of 5a in Nepalese patients may indicate a shift of HCV genotypes. Genotype switch was also reported recently in neighbor country India during the span of 7 years [12]. Emergence of new genotype or a genotype switch in an area may require to pay attention in the diagnosis and treatment.

Table 2. HCV genotype and viral load.

HCV genotype	Viral load*		Significance (p value)
	Number (%) of $< 800,000$ IU/mL	Number (%) of $\geq 800,000$ IU/mL	
1a	9 (25.0)	9 (15.0)	0.02
1b	11 (30.6)	8 (13.3)	
3a	14 (38.9)	39 (65.0)	
5a	2 (5.6)	1 (1.7)	
Mix	0 (0.0)	3 (5.0)	

*, total of 96 patient samples were determined for viral load. HCV RNA of 800,000 IU/mL was defined as a baseline; $< 800,000$ IU/mL was low viral load, and $\geq 800,000$ IU/mL was high viral load.

We also identified mixed genotypes in three patients. Mixed genotypes were usually found to be higher in multiple exposure groups, such as hemophiliacs, injection drug users, and chronic hemodialysis patients. However, we did not measure any comorbidities in the patients studied. Mixed genotypes in a single patient may affect sustained virologic response (SVR) during therapy.

We found that most of HCV-infected individuals were in the age group of 20-39 years. This finding was in consistent with a nationally representative study from Nepal, in which the highest rate of HCV co-infection is concentrated in the age group of 30-39 years [11].

A significant association between HCV genotype and viral load were observed. High load ($\geq 800,000$ IU/mL) was observed with 3a patients (65%), while patients with other genotypes had relatively low virus load ($< 800,000$ IU/mL). A study from Pakistan also showed that viral load is significantly higher in genotype 3 patients than other genotype patients [13]. Pre-treatment viral load is an important predictive sign of an outcome of antiviral therapy. High pre-treatment viral load is reported to be associated with low response to standard interferon therapy, which increases the probability of relapse compared to those with low viral load [14]. Taken together, we conclude that timely detection of viral load is necessary in order to achieve a better SVR during treatment. This should be advised especially in the resource-limited areas, where viral load at one time point was repeated referenced during treatment course.

Conclusions

This study provides additional information on the distribution of HCV genotypes of pre-treatment HCV patients in Kathmandu, Nepal. Mixed and 5a HCV genotypes existed in Nepal. Genotype 3a was associated with high viral load. However, to understand a complete situation of HCV prevalence in Nepal, larger number of patients from various regions and longer time frame sampling should be included in the future study.

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