FREQUENCY DISTRIBUTION OF HEPATITIS C VIRUS GENOTYPES AMONG CHRONIC HCV PATIENTS IN PESHAWAR, PAKISTAN

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ABSTRACT

Objectives: To determine the distribution frequency of different genotypes of Hepatitis C Virus (HCV) at Gene Tech Laboratory, Peshawar.

Methods: A total of 385 patients were consecutively included in the study after meeting the inclusion criteria from January to December 2017. RNA was extracted from the serum of the patients by using Favor Prep TM viral RNA extraction Kit according to protocols of manufacturer. Complementary DNA (cDNA) was synthesized by incubating 10μl of the extracted RNA with core region specific primer, 200U of Moloney Murine Leukemia Virus reverse transcriptase (MMLV RTase), dNTPs and ddH2O at 37°C for 50 minutes. The synthesized cDNA was amplified with both sense and antisense primers for qualitative detection. Genotyping of HCV was carried out by using type specific method of HCV genotyping.

Results: All the tested 385 samples were positive for HCV RNA, in which 200 (51.9 %) were female and 185 (48.1 %) were male. We have detected only three genotypes with highest frequency of 3a (78.7%) followed by 1a (5.9%) and 3b (0.2%). Whilst, 58 cases (15.1%) were untypable by the given method.

Conclusion: The distribution frequency of different HCV genotypes was concluded with highest dominance of 3a, ensue by untypable genotypes, 1a and 3b.

KEY Words: Genotyping, Hepatitis, HCV.

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INTRODUCTION

Hepatitis C virus (HCV) was discovered by Choo et al in 1989 and accounted for severe destruction of liver.[1,2] It is the most frequent viral agent of chronic liver diseases, leading towards hepatocellular carcinoma and liver cirrhosis.[3] The global prevalence of HCV infections has been estimated 92-149 million, whilst Pakistan is imparting approximately 11 million of the total burden.[4] HCV is an enveloped virus containing single stranded non segmented ribonucleic acid (RNA) with positive polarity. The genome of HCV is about 9.6kb encoding a polypeptide of 3000 amino acids.[5] There are 11 major genotypes of HCV, with multiple subtypes denoted as a, b, c etc., but some researchers regarded genotype 7 through 11 as the variants of the genotype 6.[6] Genotype 1 (G1) is the most prevalent genetic variant of HCV around the world, tailed by G3, G2, G4, G6 and G5 respectively.[4] Geographically, G1 is predominant in United State and Europe, G2 in West Africa, G3 in Australia and Asia, G4 in Northern Africa and Middle East, G5 in South Africa and G6 in Asia after G3.[7] In Pakistan G3a is the predominant genotype of HCV followed by G1a and G3b.[6] Genetic heterogeneity of HCV has greater implications in epidemiology and response to antiviral therapy.[8,9] Therefore, the current study is conducted to determine the frequency distribution of different genotypes of HCV in Peshawar, Pakistan because very limited data is available in the region.

METHODS

This prospective cross sectional study was conducted at Gene Tech laboratory, Peshawar from January to December 2016. A total of 385 HCV chronic patients were consecutively included in the study after meeting the inclusion criteria. Patients of both genders, aged ≥ 15, were eligible to enroll in the study after fulfilling the two conditions i.e. positive for anti HCV and HCV RNA. None of the study patient had commenced the antiviral treatment. All the patients signed the informed consent and the study was approved by ethical committee of Gene Tech Laboratory, Peshawar.

RNA was extracted from the serum of the patients by using FavorPrep TM viral RNA extraction Kit (FAVORGEN Biotech Corporation, ping-Tung 908, Taiwan) according to the protocols of manufacturer. Complementary DNA (cDNA) was synthesized by incubating 10μl of the extracted RNA with core region specific primer, 200U of Moloney Murine Leukemia Virus reverse transcriptase (MMLV RTase), dNTPs and ddH2O at 37°C for 50 minutes. The synthesized cDNA was amplified with both sense and antisense primers for qualitative detection. Briefly, early denaturation was done for 5 minutes at 94°C, trailed by 45 cycles of denaturation, each of 45 seconds, at 92°C, annealing was done for 45 seconds at 55°C and 1

artery disease. [9-13]Furthermore, a change in mean platelet volume can potentially contribute significantly to the pathogenesis of several diseases such as myocardial ischemia, stroke, coronary atherosclerosis, diabetes mellitus, hypertension, obesity and endometriosis.[13-19]

minute extension at 72°C followed by final extension of 10 minutes at 72°C. The amplified DNA was visualized in ultraviolet transilluminator on 2% agarose gel, stained with ethidium bromide. HCV RNA was quantified in all PCR samples with positive results using Piko Real 24 Real time PCR system (Thermo Fisher Scientific, Lenexa, KS 66285, USA) according to the manufacturer's instructions. The lower and upper detection limits of the assay were 5.0 × 102IU/mL and 5.0 × 108 IU/mL respectively.

Genotyping of HCV was carried out by using type specific method of HCV genotyping. Briefly, 10μl of extracted RNA was reverse transcribed to cDNA using 200 U of M-MLV RTase at 37°C for 50 minutes, followed by first round amplification of the cDNA with primers specific for the core region of HCV. Furthermore, type specific primer for the HCV core region was used for the detection of most frequent (1a, 1b, 1c, 2a, 2b, 3a, 3b, 3c, 4, 5a, and 6a) genotypes. The type specific primers were divided into two groups, to discriminate the amplified products of equal length on agarose gel. Therefore, two second round nested PCR were performed, one with primer mix A and the other with primer mix B using first round products as template. Mix A was comprised of primers for genotypes 1a, 1b, 1c, 3a, 3c and 4, whilst, mix B contained primers for 2a, 2b, 3b, 5a and 6a. The amplified products of second round were subjected to electrophoresis on 2% agarose gel, and further visualized by staining the gel with ethidium bromide under UV transilluminator. A 100-bp DNA ladder (Fermentas, USA) was included in each run as DNA size marker and the HCV genotypes were determined by comparing the genotype-specific PCR band with DNA ladder.

RESULTS

All the tested 385 samples were positive for HCV RNA, in which 200 (51.9 %) were female and 185 (48.1 %) were male. We have detected only three genotypes including 1a, 3a and 3b in the studied patients. The most frequent genotype was 3a followed by 1a and 3b respectively. The frequency distribution of 3a was 78.7% (303/385), 1a was 5.9% (23/385) and 3b was 0.2% (1/385). Whilst, 15.1% (58/385) of the cases were untypable by the given method. The studied patients were stratified in four different group based on age, to elucidate the distribution of different genotypes into different age strata (Table 1).

Table 1. Distribution of Genotypes in gender and different age group

Age Group		15-34	35-54	55-74	75-94	Total
Gender	Male	48	105	30	2	185 (48.1%)
	Female	53	107	39	1	200 (51.9%)
Total		**101 (26.2%)**	**212 (55%)**	**69 (17.9%)**	**03 (0.7%)**	**385**
Genotype	3a	77	169	54	03	303 (78.7%)
	1a	06	11	06	00	23 (5.9%)
	3b	00	01	00	00	01 (0.2%)
	Untypable	18	31	09	00	58 (15.1%)

DISCUSSION

The frequency distribution of HCV genotype is considerably diverse among distinct geographical locales. The detection of HCV genotype across different regional zones has greater implications on management and preventive approaches.[10] Moreover, variations in the disease outcome and treatment response of HCV genotypes have been previously reported.[11]

Our results indicate that cases of HCV are higher in female (51.9%) then male (48.1%) which extend the previous information reported by Kumar et al in 2017,[12] but other researchers also reported higher incidence in male than female.[13] The results of present study report the dominance of genotype 3a and 1a in Peshawar city which validate the findings of the recently conducted studies in this region.[12,13] In contrast, a study reported shift in the distribution frequency of HCV genotypes in Peshawar with dominance of 3a followed by 1b and 1a is the least frequent reported genotype, but we did not detect 1b in the tested cases.[14] The current study shows that genotype 3b is the dominant genotype after 3a and 1a which is in concordance with the earlier studies.[13,14] We did not detect a mixed infection of different HCV genotypes and the same is reported by Ali et al but dual infection has been published in several other studies.[15,16,17] A large number of untypable HCV cases were detected in the studies which extend the previously documented data about untypable HCV.[16,18] These untypable mutants can be a challenge for future, because they can affect the management, prognosis and vaccine development of HCV.

CONCLUSION

Conclusively, the overall patterns of frequency distribution of different genotypes of HCV remain same with highest proportion of genotype 3a, both in Peshawar and Pakistan.

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