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Genotype distribution of hepatitis C virus in Khorasan Razavi Province, Iran

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Background/aim: Several types of the hepatitis C virus (HCV), with variations in different parts of the genome, have been isolated from different regions of the world. Based on heterogenic sequences in the isolated genome, HCV is classified into different genotypes and subtypes. Data on distribution of HCV genotypes in a certain region could be important to patient management. Therefore, this study was conducted to determine the distribution of HCV in Mashhad, Northeast Iran.

Materials and methods: This cross-sectional study was conducted on 103 patients with HCV infections in Mashhad. Among the participants, at least 22 (21.4%) were intravenous drug users. HCV seropositivity was determined by an enzyme-linked immunosorbent assay and was confirmed by reverse transcriptase polymerase chain reaction. HCV-positive samples were selected for HCV genotyping using genotype specific primers.

Results: Of 103 subjects, 43 (41.7%) and 34 (33.0%) had genotypes 1a and 3a, respectively. Other genotypes including 1b, 2a, 2b, 3b, and 5a were found in 4 (3.9%), 1 (1.0%), 3 (2.9%), 4 (3.9%), and 1 (1.0%), respectively. Coinfections with 2 genotypes were also observed in 11 (10.7%) patients. Genotyping for 2 (1.9%) of 103 samples did not produce any results.

Conclusion: Genotypes 1a and 3a were found to be the most prevalent HCV genotypes in Mashhad, Iran.

Key words: Hepatitis C virus, genotype, Khorasan Razavi Province, Iran

1. Introduction

The infection caused by the hepatitis C virus (HCV) is one of the most serious health problems in the world (1). In many countries, this infection is the most common cause of malignant and severe liver diseases. About 3% of the world's population suffers from HCV infection (1,2), among which 80% exhibit a chronic form of hepatitis. Twenty percent of patients with chronic hepatitis C are likely to progress to cirrhosis and 1.5%–4% may develop hepatocellular carcinoma (3,4).

Several studies highlight the importance of clinical and laboratory diagnostic tests in determining HCV genotypes. Epidemiology (5), pathogenesis (6,7), response to antiviral treatments (7,8), and duration of standard HCV treatment with PEG interferon and ribavirin for reaching a sustained virological response are in part dependent on the HCV genotype (9,10). Genotype 1 is considered to be the most antiviral-resistant genotype of HCV. In addition, patients

suffering from chronic infection with HCV subtype 1b, when compared with other HCV subtypes, are more likely to develop severe liver diseases (11,12).

In the past, blood transfusions and blood products were the main culprits for HCV transmission. Recently, however, intravenous drug usage has become the most common form of transmission (13). According to our previous study, the prevalence of HCV infection is low ($\leq 1\%$) among the general population of Mashhad, Iran (14). However, due to common needle injection, the frequency of this infection is high among intravenous drug users (IDUs).

This study was performed to determine the frequency of HCV genotypes among HCV-positive subjects in Mashhad, Iran.

2. Materials and methods

This cross-sectional study was performed between October 2009 and October 2011 on 103 patients with HCV infection

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[serum-positive patients confirmed by reverse transcriptase polymerase chain reaction (RT-PCR)] in Khorasan Razavi Province. Included in this study were patients referred to the clinical diagnostic laboratory of Qaem Hospital and other clinical diagnostic laboratories, as well as IDUs referred to the Addiction Research Center of Imam Reza Hospital. This sample included at least 22 (21.4%) IDUs. After informed consent was obtained from participants, a 10-mL blood sample was taken from each patient. Samples that were positive by enzyme-linked immunosorbent assay (ELISA) and confirmed by RT-PCR were considered HCV infections. HCV antibody was determined by ELISA (Delaware Biotech, USA) and HCV RNA was investigated by RT-PCR. In brief, HCV antibody positive sera were selected for viral RNA extraction using a commercial Viral RNA Extraction Kit (QIAGEN, USA). Next, reverse transcription (using random hexamers) and PCR were performed with an ASTEC pc818 Thermal Cycler using an HCV detection kit (STRP Hepatitis C Virus Detection Kit, CinnaGen, Iran) following the manufacturer's recommended procedures. Using the method of Ohno et al., HCV isolates of all positive samples were genotyped (13) and 2 rounds of PCR were performed on cDNA samples to determine HCV genotypes. Core specific primers Sc2 (5'-GGGAGGTCTCGTAGACCGTGCACCATG-3') and Ac2 (5'-GAG(AC)GG(GT)AT(AG)TACCCCATGAG(AG)TCGGC-3') were used for the first round of PCR and 2 mixtures of primers were used for the second round of PCR. Mix 1 primers were used for the detection of HCV genotypes 1b, 2a, 2b, and 3b while mix 2 primers were used for the detection of HCV genotypes 1a, 3a, 4, 5a, and 6a. The PCR product was visualized on a 2% agarose gel by Green-Viewer staining (Pars Tous, Iran) and UV photography.

3. Results

Of 103 HCV RNA-positive patients, 86 (83.5%) were male and 17 (16.5%) were female. Patient age ranged from 18 to 65 years and the mean was 39.6 ± 11.1 years.

The authors were not able to perform genotyping for 2 (1.9%) of the 103 samples. HCV genotypes 1a with 43 (41.7%) and 3a with 34 (33.0%) had the highest frequencies. Genotypes 1b, 2b, and 3b were reported from 4 (3.9%), 3 (2.9%), and 4 (3.9%) patients, respectively. One (1.0%) patient was found positive for both genotypes 2a and 5a. Eleven (10.7%) were reported to have coinfections with 2 different genotypes. Coinfections with genotypes 1a-1b,

1a-3b, and 1b-3a were observed in 3, 2, and 2 individuals, respectively. However, only 1 patient each was found with other coinfections, including 2b-3a, 5a-3a, 2a-1a, and 1b-1a (Table). This sample of 103 patients included at least 22 (21.4%) IDUs. Other patients may have had a history of drug usage but did not report it. Five cases (45.5% of 11 cases of coinfection) were observed among patients with addiction.

4. Discussion

Various virus genotypes and subtypes with different frequencies have been reported from different countries (15). In the current study, the Ohno et al. method was used for HCV genotyping. The results obtained based on this method are in conformity with the gold standard method of HCV genotyping (sequencing). A PCR of the core region of HCV genotypes 1a, 1b, 2a, 2b, 3a, 3b, 4, 5a, and 6a with specific primers is the basis of the Ohno et al. method. Ohno et al. also suggested that due to a rather limited number of samples available for genotypes 3 to 6 in their study, their method requires further confirmation for these genotypes (16).

In many European studies, genotype 1 and subtype 1b seem dominant, and in Germany, subtype 3a had the highest frequency (46%) in patients with drug addiction. However, in considering all the subjects, subtype 1b was the most prevalent one (17). Among 236 patients with HCV infection in Belarus, 127 (53.8%) and 58 (28.8%) were found with genotypes 1b and 3a, respectively (18). A study among French hemodialyzed patients reported a rate of 77% for genotype 1b (19). Subtype 1b (54%) had the highest frequency in Vienna, Austria, and surrounding areas (20). A retrospective study conducted on 373 Italian children with HCV noted the dominance of genotype 1b (41%), followed by 1a (20%) (21).

Two studies in Brazil in the states of Rondônia (22) and Alagoas (23) noted a higher prevalence of genotype 1b, followed by 1a. However, unlike Europe and South America, in the United States, genotype 1a is considered to be dominant. In Appalachia (USA), genotype 1a (66%) was by far the most prevalent genotype (24). Another study in the United States performed in Galveston, Texas, also reported the dominance of 1a (62.8%) over other genotypes (25).

It has been suggested that 2 trends exist in the Middle East. Genotypes 4 and 1 are generally considered to have the highest prevalence in Arab countries (apart

Table. Percentage of different genotypes among the 103 participants.

1a	3a	Coinfections	1b	3b	2b	Not determined	2a	5a
43 (41.7%)	34 (33.0%)	11 (10.7%)	4 (3.9%)	4 (3.9%)	3 (2.9%)	2 (1.9%)	1 (1.0%)	1 (1.0%)

from Jordan) and non-Arab countries (Iran, Turkey, and Israel), respectively (26). In Iran, genotypes 1a and 3a predominate. A study by Amini et al., which was performed on 116 samples representing the whole country, showed that genotypes 1a (61.2%) and 3a (25.0%) were the most prevalent ones (27). The authors observed that the frequency of genotypes 1a and 3a were 41.7% and 33.0%, respectively, in the city of Mashhad. Several studies were conducted among HCV-positive patients and also among different disease groups in Tehran. Kabir et al. reported the frequency of 37.8% and 28.9% for genotypes 1a and 3a, respectively, in Tehran (28). Another study in Tehran reported similar results (39.7% for 1a and 27.5% for 3a) (29). This finding was also confirmed by 2 other studies in this city (30,31). In Isfahan (Central Iran), however, genotype 3a (61.2%) was more prevalent than 1a (29.5%) (32). The frequency observed in Shiraz was 44.1% for 1a and 42.0% for 3a (33). For Golestan Province, in the north of Iran, 3b (24.7%), followed by 1b (19.5%) and 1a (19.5%), was predominant (34). In Shahrekord, in West Iran, the prevalence rates of 54.26% and 27.66% were reported for 1a and 3a, respectively (35). Among hemodialytic patients in Guilan Province, in the north of Iran, 1a (59.38%) and 3a (40.62%) were the most prevalent genotypes (36).

Although Turkey and Pakistan border Iran, their genotype distribution differs from that of Iran. Turkey seems to follow the distribution pattern of other European countries with a high prevalence of genotype 1b. In a study in Turkey of 365 patients with chronic HCV, 306 (84%) were of genotype 1b (37). It is noteworthy that in a study conducted in Northwest Iran, a rate of 71.4% was found for 1a, followed by 14.2% for 1b (38). This finding contrasts with those in many other cities in Iran in which only 1a and 3a were the prevailing genotypes. Therefore, it could be concluded that in the mentioned geographical area (which is near Turkey), genotype 1b is more or less dominant. Pakistan is Iran's neighbor to the east. In the Khyber Pakhtunkhwa area of Pakistan, a rather different trend was observed with 2a (39%) and 3a (31%) being the most prevalent genotypes (39), while in another study performed on 1000 samples from remote cities in Pakistan,

3a, followed by 3b, was reported to be dominant (40). Regarding other regions of Asia, a study on 138 patients with HCV infection in Korea suggested that 1b (71%) was the most prevalent genotype, similar to many European studies (41).

Several molecular epidemiological studies suggested a clear relationship between intravenous drug usage and the prevalence of infection with genotype 3 (42,43). A study by Samimi-Rad et al. in 2012 among IDUs in Iran reported the prevalence rate of 58.0% for genotype 3a and 42.0% for genotype 1a (44). The authors observed that among the studied group, genotype 1a had the highest frequency, both among patients known to be IDUs and others. Among our patients, coinfection with 2 different genotypes of HCV was more common among IDUs. Previous studies proposed several reasons for the existence of 2 or 3 genotypes in 1 patient. One reason included an unspecific band resulting from the interaction of the specific primers of a genotype with the sequences of another genotype and/or exposure to 2 different HCV genotypes (45,46). In our study, common needle injections among patients with addiction might have had a role in infection with different HCV genotypes.

In the current study, the participants' extensive medical histories were not available. Further studies with larger sample sizes that include complete patient records during a certain period are encouraged for the city of Mashhad.

Our results from Mashhad (a major city of the Khorasan Razavi region) confirmed that genotypes 1a and 3a are prevailing, which is similar to the study of Vossughinia et al. (47). However, contrary to that study, which reported almost the same proportion of 40% for 1a and 3a, we observed that genotype 1a was more common than 3a (41.7% vs. 33.0%). The slight difference observed may have been due to the nature of the sample or the fact that the sample sizes of both studies were not large enough.

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