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Hepatitis C Virus Genotypes in Patients Referred to Educational Hospitals in Zahedan (2009 – 2013)

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Abstract

Background: The Hepatitis C virus (HCV), a single strand, linear RNA virus, is one of the most important hepatotrope agents worldwide. At least 6 distinct genotypes of HCV and more than 50 subgroups have beendetected. It has been demonstrated that HCV genotypes vary in their distribution, which may indicate the route of acquisition and affect the clinical outcome and response to therapy.

Objectives: This study investigated the distribution of HCV genotypes and their relationship to risk factors in Zahedan, the capital of Sistan and Balouchestan province, in southeast Iran

Patients and Methods: This cross-sectional study enrolled all patients with positive or indeterminate anti-HCV tests who were referred to Zahedan university of medical sciences (ZAUMS) educational hepatitis clinics. To determine viral infection and analyze genotypes,the study applied nested polymerase chain reactions (PCRs) and restriction fragment length polymorphisms (RFLPs).

Results: The study found that 72 patients, 43 males and 29 females, had documented HCV infections. Age's ranged from 20 - 75, with a mean of 48. The largest number of patients had the 1a genotypes (41 patients = 56.9%), followed by 3a (25 patients = 34.7%), and 1b and 4a each of them (3 patients = 4.2%).

Conclusions: The study showed that genotypes 1a and 3a are the most common genotypes in patients with chronic HCV infection in Sistan and Balouchestan province, in southeast Iran.

Keywords: Hepatitis C Virus, Genotype, Risk Factor

1. Background

It is estimated that more than 185 million peopleworldwide are infected by the Hepatitis C virus (HCV) (1), which is an important cause of chronic liver diseases.Rates of infection among populations vary in various regions of the world, including in Iran. HCV causes acute hepatitis in 10 - 15% of cases, and the remainder becomes chronically ill. Various studies have shown that in the US, 70% of all chronic liver diseases, 40% of liver cirrhosis, 60% of hepatocellular carcinoma, and 30% of liver transplants are HCV related (2-6). In Western countries, HCV is the main cause of cirrhosis and liver cancer (7, 8).

HCV is a single strand, linear virus that has 9600 nucleotides. At least 6 or 7 distinct genotypes of HCV and more than 50 subgroups within genotypes have been distinguished by using nucleotide rows. Appearance of these genotypes and various pseudo-species in the defective HCV shows a high rate of mutation, which causes humoral immunity interference and escape (4-6).

In addition, distribution of HCV genotypes differs in various parts of the world. Genotype 1 is the most common genotype worldwide; although genotypes 2 and 3 are also widely distributed worldwide. In the US and Europe, the most common genotype subtypes are 1a and 1b (9, 10).

Genotype 4 is the most prevalent HCV genotype in North Africa and the Middle East (11), and in South Africa and Southeast Asia, the most prevalent genotypes are 5 and 6,respectively (12).

In Iran, the most common HCV genotypes are 1a (47%) and 3a (36%) (13). In Turkey, Russia, Belarus, Moldova, Uzbekistan, Lithuania, and Latvia, the predominant HCV genotype is 1b (14-18), and in Pakistan, it is genotype 3a (11, 19).

In addition, there is known to be a relationship between the HCV genotype and the response to treatment. For example, treatment of genotypes 2 and 3 has been more successful, but treatment of genotype 1has been more difficult. Progression of liver diseases is more likely in patients with genotype 1 of chronic HCV (5).

Genotypes 2 and 3 are reported to respond favorably, with sustained virologic response rates of more than 70%. However, genotypes 1, 4, 5, and 6 generally respond much more

poorly and require a longer treatment time (20). This variation in response to treatment should be consistent among various populations and in various geographical regions. Genotyping is important to predict treatment response and determine treatment duration. HCV genotyping has proven beneficial for prognosis, treatment planning, and developing new treatments. In the future, subtyping may be used to determine the genetic factors that are involved in the drug resistance of various subtypes (21).

2. Objectives

This study attempted to determine the common genotypes of HCV in infected patients who were referred to hepatitis clinics at the educational hospitals of the Zahedan University of Medical Sciences (ZAUMS) and to assess their relationship to risk factors.

3. Patients and Methods

This cross-sectional study included all patients with positive or indeterminate anti-hepatitis C (83 patients) who referred to ZAUMS Educational Hospitals between 2009 and 2013. The study obtained informed consent from all patients and was approved by the ZAUMS Ethics Committee. To detect HCV-RNA in positive and indeterminate cases, the study used RT-PCR with the qualitative AMPLICOR HCV Test v. 2.0 (Roche Molecular Systems; Branchburg, NJ, US).

HCV genotyping/subtyping was performed by two RT-PCR assays: 1) restriction length poly-morphism analysis (RFLP) of the 5'noncoding region (5'NCR); and 2) nested PCR with type-specific primers following primary RT-PCR with primers designed for the core region of the HCV genome.

For each patient, the study recorded demographic data and risk factors for HCV infection, such as transfusion, tattooing, prison history, positive familial history, IV drug use, and unsafe sexual behaviors. Finally, for each patient, the HCV genotype and its relationship to their risk factors was examined by Fisher's exact test. A P value < 0.05 was considered statistically significant.

4. Results

Of the 83 patients who had positive or indeterminate anti-hepatitis C statuses, 75 had documented HCV infections, and 72 had HCV genotypes identified by RFLP, while 3 could not be subtyped using this method. Of the 72 patients, 43 were males and 29 were females. Age's ranged from 20 - 75, with a mean of 48.

The largest number of patients had the 1a genotype (41 patients = 56.9%), followed by 3a (25 patients = 34.7%) and 1b and 4a each of them (3 patients = 4.2%).

Table 1 shows the frequency distribution of various HCV genotypes.

The Fisher's exact test showed no significant differences in distribution of HCV genotypes based on age, sex, transfusion history, tattooing, prison history, IV drug use, or familial history of HCV infection.

As Table 2 shows, the most prevalent risk factor in patients with HCV infection was tattooing followed IV drug addiction.

Table 1. Frequency Distribution of Various HCV Genotypes

Genotype	Frequency, %
1a	41 (56.9)
1b	3 (4.2)
2	0
3a	25 (34.7)
3b	0
4a	3 (4.2)
Mixed	0
Total	72 (100)

Table 2. Distribution of Risk Factors in Patients With Hepatitis C Infection[a]

Risk Factor	Genotype				Total
	1a	1b	3a	4a	
Tattooing					
Yes	8 (50)	1 (6.3)	6 (37.5)	1 (6.3)	16 (100)
No	33 (58.9)	2 (3.6)	19 (33.9)	2 (3.6)	56 (100)
Transfusion					
Yes	7 (53.8)	1 (7.7)	4 (30.8)	1 (7.7)	13 (100)
No	34 (57.6)	2 (3.4)	21 (35.6)	3 (3.4)	59 (100)
IV drug use					
Yes	8 (57.1)	0	5 (35.7)	1 (7.1)	14 (100)
No	33 (56.9)	3 (5.2)	20 (34.5)	2 (3.4)	58 (100)
Prison history					
Yes	1 (50)	0	1 (50)	0	2 (100)
No	40 (57.1)	3 (4.3)	24 (34.3)	3 (4.3)	70 (100)
Familial history					
Yes	4 (57.1)	0	3 (42.9)	0	7 (100)
No	37 (56.9)	3 (4.6)	22 (33.8)	3 (4.6)	65 (100)

[a]Values are presented as No. (%).

5. Discussion

This study examined 72 hepatitis patients and their risk factors. The largest number of patients hadgenotype1a, followed by 3a. One of the most interesting findings was the frequency of genotype 4a (4.2%), which is higher than in other regions of Iran, where it is near lyzero. Farivar et al. studied 52 patients with HCV infections in Zahedan from July 2007 to April 2009. Their results showed that of the 52 samples, 53.84% had genotype 1, 3.88% had genotype 2, 23.08% had genotype 3, and 13.4% had genotype 4. Mixed infections with combinations of genotypes 1 and 3 were seen in 5.77% (22). Their reported frequency of genotype 4 (13.4%) was higher than the frequency reported by the present study. It is known that genotype 4 has been reported in Arab countries, including Iraq (23), Yemen (24), Saudi Arabia, Kuwait (25), Syria, and Lebanon (17).Therefore, this higher frequency of genotype 4in the present study may be explained by cross-border travel, especially from the southern part of the province, to Persian Gulf countries for work or close contact, probably unsafe sex, with those from Arab countries.

Shahraki et al. conducted a study of 20 multi-transfused patients suffering from β-thalassemia major and chronic HCV infectionbetween2005 and 2009 in ZAUMS. They found that the most prevalent HCV genotype was 3a (40%), followed by 1b (25%) and 1a (15%). These results differ from those of the present study (26). This difference may be explained by the use of different populations. The present study included only seven patients with histories of transfusions, which does not compare statistically with the population used for the Shahraki et al. study (26).

Using a Fisher's exact test, the present study found no significant relationship among any HCV genotype and risk factors such as age, sex, transfusion history, tattooing, prison history, IV drug addiction, or positive familial history.

A study by Amini et al. (2009) used 116 serum samples of patients with HCV infection from various areas in Iran, including central, north, northwest, west, southwest and east, but excluding the southeast (Sistan and Balouchestan province). Results showed that genotypes 1a, 1b, and 3a were more dominant than other genotypes. In addition, there was no significant relationship between these genotypes and demographic status and risk factors (27). The results matched those of the present study. A 2007study by Keyvani also indicated the prevalence of these three genotypes in Iran (28). It reported that the largest number of subjects had genotype 1a (39.7%), followed by 3a (27.5%), and 1b (12.1%). Genotypes were determined in mixed in 1.6% of the subjects. The study found a significant relationship between genotype 1b and age; with genotype 1b being more common in older subjects. The current study did not find this relationship.

A study by Kabir et al. of (2006) 156 patients with anti-HCV and positive HCV-RNA in Iran found a prevalence of HCV genotype 1 in 87 (55.8%) cases. In addition, 45 (28.8%) patients were infected with genotype 3, two patients (1.3%) with genotype 4, and one patient (0.6%) with a mixed infection of genotypes 1 and 3. The distribution of HCV genotype subtypes related to age, sex, and source of infection showed that 40% of IV drug addicts had genotype 1a and 37.8% had genotype 3a (29). In contrast, the present study did not find any significant relationship between genotypes and risk factors.

A study of 85 HCV patients in Kerman, Iran, found that genotypes 1a (38.8%) and a3 (29.4%) were prevalent and that there was no relationship between genotype and age. However, genotype 3a was more prevalent in IV drug addicts and those with a history of tattooing (30).This agrees with the results of the present study, which found that genotypes 1 (56.9%) and 3 (34.7%) were prevalent and that there were no significant relationships between genotype and age or sex (30).

Khodabandehloo et al. (4) conducted a systematic review in 2014 of the prevalence of HCV genotypes in Iranian patients. Results were completely compatible with those of the present study for the Iranian population.

A study in Pakistan (2008) of 3351 HCV patients found that 94% of them (3150) had infection with a single genotype. The largest number of patients (49.5%) was infected with genotype 3a, followed by 3b (17.66%). More than 8% of patients infected with genotype 3a and 72% of patients infected with genotype b3 had histories of frequent injections. Both major and minor surgeries were more prevalent in those infected with genotypes both 1a and 1b, and those infected with genotype 2b,2a,or an indeterminable genotype were infected sporadically (31).

A study of 1688 HCV patients in Brazil (2005) showed that 64.9% of them were infected with genotype 1 and that various genotypes were distributed in various parts of Brazil (32). This distribution of HCV genotypes in different geographic areas was variable and changed over time, probably due to different study populations, different methods of infection, and viral mutations (6, 33).

The most prevalent HCV genotypes in countries near Iran have been reported as 1b in Turkey, Russia, Moldova, and Uzbekistan (34), 4 in Saudi Arabia, Iraq, Qatar, Bahrain, Kuwait, and Yemen (35), and subtype 6a in China (33). The predominant subtype is 3a in both Pakistan (31) and India (36).

Unfortunately, unlike Hepatitis B, there is no effective vaccine against HCV, and this lack of an effective vaccine, expensive treatment regimens, and increased high-risk behaviors, such as intravenous drug use, have led to increased HCV infections in Western societies that will probably soon follow in developing countries. In Iran, the prevalence of HCV in the general population is 0.25 - 0.5% and the prevalence of Hepatitis B is 1.5% (37). Therefore, an understanding of transmission routes and the most important risk factors in Iran and similar countries may help to prevent HCV infections.

The present study found that most HCV patients were infected with genotype1a, which responds more slowly to treatment and is more likely to progress to chronic hepatitis or liver diseases, thus confirming that genotyping is crucial to selecting the proper treatment and its duration.

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Footnotes

Authors' Contribution: Alireza Bakhshipour, Narjes Sargolzaie, Malek Kiani and Fatemeh barazesh had equal role in design, Practical work, statistical analysis, and manuscript writing.

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