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Original Article

Distribution of Hepatitis C Virus Genotypes in Yazd, Central Province of Iran: Increasing the Mixed Genotypes

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Abstract

Background and Aims: Different hepatitis C virus (HCV) genotypes have characteristic geographical distribution. Identification of HCV genotype is an important factor in the progression, clinical outcome and therapy of HCV infection. The aim of this study was to determine the prevalence of HCV genotypes among HCV-RNA positive patients in Yazd, Iran.

Materials and Methods: In this cross-sectional study, 150 HCV-infected individuals with detectable plasma HCV RNA levels were enrolled from January to August 2015. HCV-RNA was extracted from plasma samples and retro-transcribed to c-DNA. Then HCV genotypes 1, 2, 3a, 4 were determined using a PCR based genotyping kit.

Results: A total of 150 HCV-positive patients with mean age 40.45±11.83 were enrolled in the study. 89.3% of participant were males and 10.7% were females. The most common genotype was 3a (52%), followed by 1a (28%). Mixed-genotype infection was 20% and the most prevalent mixed genotype was 3a/1a (83% of mixed genotypes). The other genotypes were 1a/1b/3a in 10%, 3a/2 and 1a/2/3a both in 3% of patients with mixed HCV genotypes.

Conclusions: Unlike other regions of Iran, Genotypes 3a was predominant in HCV-RNA positive patients in Yazd province. Also, HCV mixed-genotype infections were more common than previously estimated in other studies from different parts of the country.

Keywords: Genotype, Hepatitis C virus, Iran.

Introduction

Hepatitis C virus (HCV) can cause both acute and chronic hepatitis infections (1). About 130–150 million people globally have chronic hepatitis C infection. Approximately 500000 patients die each year from HCV diseases (2). HCV genome is a positive-sense, single-stranded RNA and shows a high rate of mutations. The RNA polymerase of HCV has not efficient proofreading ability, therefore HCV genetic heterogeneity happens in infected individuals (3).

Hepatitis C virus is classified into seven distinct genotypes and more than 65 subtypes (4). HCV genotypes have distinct geographic distributions in worldwide; 1a and 1b are the most prevalent genotypes in Europe, USA, and Japan. Genotype 3 is prevalent in India, Southeast Asia, and Indonesia, genotype 4 in North Africa and the Middle East, and genotype 5 has been reported from South Africa. Genotype 6 appears to predominate in the Middle East. Genetic variants of HCV in these populations may be due to diversity in

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the race, routes of transmission and socioeconomic status (5, 6).

HCV genotypes have been shown to differ in their clinical outcome, prognosis, and response to antiviral therapy (7). HCV genotypes 1a and 4 may lead to chronic infection, more severe disease, re-infection, and poor response to therapy, compared to genotypes 2 and 3 (1). In addition, patients infected with genotype 1b have been reported to have a higher risk for hepatocellular carcinoma and lower response to interferon therapy (8).

HCV genotyping is currently used for determining dose of the antiviral agents, duration and estimating therapeutic response. For physicians, knowing the genotypes of Hepatitis C is helpful in making a therapeutic recommendation (9). Individuals with genotypes 2 and 3 are almost three times more than individuals with genotype 1 likely to respond to therapy with alpha interferon or the combination of alpha interferon and ribavirin (10).

The most common genotypes in north-west and south of Iran are 3a and 1a, respectively, while genotype 1a is typically dominant in all parts of Iran (11). Different HCV genotypes may be associated with risk factors and the mode of HCV transmission. Thus, the genotypic patterns can be used to pursue the modes of transmission (12). After 1996, systemic screening of blood for anti-HCV in Iran has led to a remarkable decrease of HCV by blood transfusion (11). The major modes of transmission HCV infection in developed countries is through the use of intravenous drugs (13). Past studies have shown that the main risk factor for infection by genotypes 1a and 3a is intravenous drug use in Iran. The main risk factors for infection by genotypes 1 are transfusion of blood and blood products, hemodialysis and organ transplant. Genotype 4 is the most prevalent in patients enduring hemodialysis (14).

In the previous studies, HCV genotype 3, 1a and 1b were the predominant in Yazd, central province of Iran (15). Determination of HCV genotypes in different parts of the country is necessary to facilitate preventive strategies and treatment options. The prevalence of HCV genotypes in an area may alter over the time because of changing in route of infection (16, 17), therefore repeating of HCV genotyping tests in an area every few years is helpful. The present study aimed to evaluate distribution of HCV genotypes in the Yazd province of Iran in 2015.

Methods

Patients and sampling. In this cross-sectional study, blood samples from 150 HCV-RNA positive patients- referred to Yazd hospitals- were collected from January to August 2015. Plasma was separated from each blood sample and stored at -20ºC. The informed consent form was signed by each patients. Ethic committee of Yazd University of Medical Sciences was approved this study. All samples were primarily checked for the presence of HCV genome by RT-PCR method, therefore an inclusion criterion for patients was to be positive for HCV-RNA. The patient's exclusion criteria were negative RT-PCR test for HCV-RNA and lack of informed consent.

RNA extraction and cDNA synthesis. HCV-RNA was extracted from plasma samples using RIBO-prep extraction kit (AmpliSens, Russia). According to the manufacturer's instructions, 300µl of the warm lysis buffer was added to each 100µl of the sample, and extraction was performed using standard protocol of the kit. The RNA was then reverse transcribed into complementary DNA (cDNA). One microliter random hexamer primer, 4 µL of 5X reaction buffer, 1 µL of RiboLock RNase inhibitor (20 U/µL), 4 µL of dNTP mix (10 mM), 1 µL of M-MuLV (200 U/µL), ddH2O and 1µg of total extracted RNA were utilized for cDNA synthesis (total volume: 20 µL) according to RevertAid First Strand cDNA Synthesis kit protocol (Thermo Scientific, USA). cDNA was stored at -20 °C until the HCV genotyping test was carried out.

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Table 1: Demographic Parameters and distribution of hepatitis c virus genotypes in patients

Parameters		Male	Female	Total	P value
Number (%)		134 (89.3)	16 (10.7)	150 (100)	0.003
Age (Mean ± SD)		39.85±11.49	45.50 ±13.81	40.45±11.83	0.07
Genotypes (%)	1a	39 (26)	3 (2)	42 (28)	
	1b	0	0	0	
	2	0	0	0	
	3a	73 (48.7)	5 (3.3)	78 (52)	
	4	0	0	0	
	1a/3a Mix	19 (12.66)	6 (4)	25 (16.66)	
	1a/1b/3a Mix	1 (0.67)	2 (1.33)	3 (2)	
	3a/2 Mix	1 (0.67)	0	1 (0.67)	
	1a/2/3a Mix	1 (0.67)	0	1 (0.67)	

HCV genotyping method. HCV genotypes were determined using a commercial HCV genotyping kit (AmpliSens, Russia). The specificity and analytical sensitivity of the kit were 100% and 103 IU/ml, respectively. This kit could detect the genotypes 1a, 1b, 2, 3a (which are most prevalent in Iran) by generating PCR products with different size. The PCR testing was performed using a hot-start protocol. For each sample, 2 reactions were performed in two separate tubes containing master mix, 5 µl of cDNA and specific primers for either genotypes 3a+2 or genotypes 1a+1b. The PCR reaction was performed in a thermal cycler (SensoQuest, Germany) with a temperature profile as follows: initial denaturation at 95 ℃ for 5 min, followed by 42 cycles of 95 ℃ for 30 s, 68 ℃ for 30 s, 72 ℃ for 30 s and a final extension at 72°C for 10 min. PCR products were run on 2% agarose gel containing DNA safe stain, then visualized by a transilluminator. The expected PCR product sizes for genotypes 1a, 1b, 3a and 2 were 338, 395, 227 and 286 bp, respectively. Negative and positive controls were used in every PCR experiment. In addition, 5 µl of Internal Control during the RNA extraction procedure directly was add to the each sample/lysis mixture.

Statistical analysis. The Chi-square test and Student's t-test were performed using SPSS statistics software version 18 (Chicago, IL, USA). Data are expressed as absolute number, percentage and mean ± SD.

Results

A total of 150 HCV-positive patients with mean age 40.45±11.83 in range of 19-65 years were enrolled in the study. 89.3% of participant were males with mean age of 39.85±11.49 years and 10.7% were females with mean age of 45.50 ±13.81 years. The mean age of the men was statistically lower than women (T-test, P = 0.07) (Table 1). A significant difference was observed in HCV genotypes distribution between male and female (P=0.003). There was no statistically significant difference between age and distribution of HCV genotypes (P=0.63).

The highest frequency of HCV infection was observed in patients 31 to 40 year old with the mean of 35.35±2.83 (Fig 1). The HCV genotype distribution varied between different age groups, as shown in Figure 1. It was found that 52% (n=78) patients were infected with genotype 3a, 28% (n=42) with HCV genotype 1a and 20% (n=30) with HCV mixed genotypes. No patients were found infected with genotype 1b, 2 and 4. The prevalence of HCV mixed genotypes in the study population were like that: genotype 1a/3a was 16.66% (n = 25), genotype 1a/1b/3a was 2% (n=3) and both of genotype 3a/2 and 1a/2/3a were 0.67% (n =1) as presented in Table 1.

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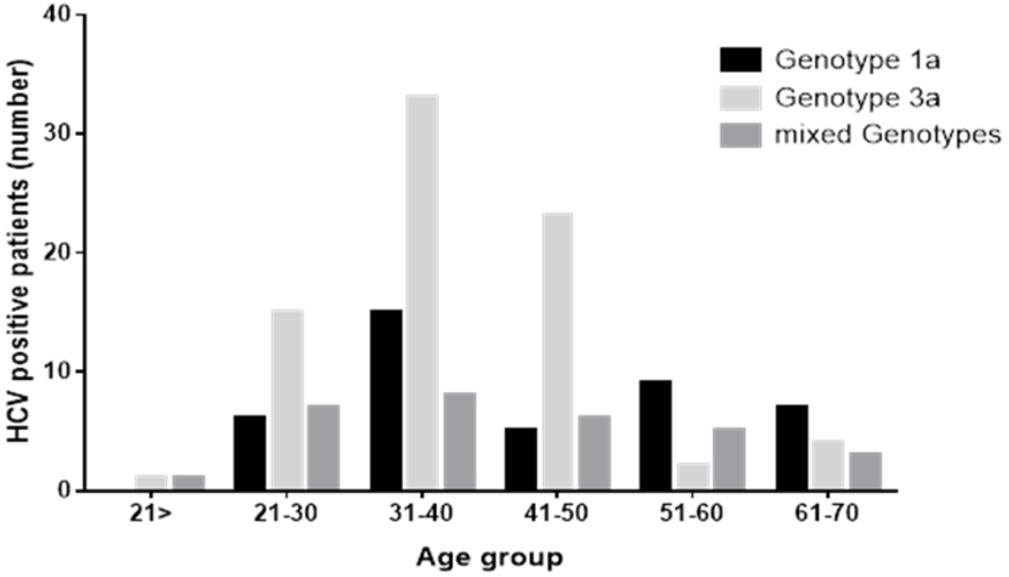

Fig. 1. HCV genotype distribution among age groups in positive patients

Discussion

The present study aimed to evaluate distribution of HCV genotypes in Yazd province, Iran. Our results indicated that Genotype 3a (52%) was the most prevalent genotype and genotype 1a (28%) was less common in this area. Genotypes 1b, 2 and 4 were not observed in this study. The remaining patients had a combination of two or more different HCV genotypes. The majority of these patients with mixed genotypes had 1a/3a genotypes (16.66), followed by the 1a/1b/3a genotypes (2%), 3a/2 genotypes (0.67%), and 1a/2/3a genotypes (0.67%). Another study conducted by Molaabedin et al. in Yazd revealed that the most common genotype was 3a (65%), followed by 1a (35%). They could not find other genotypes and mixed genotype infection (18). Also Nedoushan et al. found genotypes 3a (50.3%), 1a (38.7%), 1b (6.8%), 2 (1.6%) and mixed genotypes 1a /3a, 1a/1b (2.6%) in this province (15). Similar genotypes have also been detected in populations from other neighboring province of Yazd. In Isfahan, Shanehsazzadeh et al. identified most samples with genotype 3a followed by 1a and 1b in this population (19). In Mashhad, the prevalence of 3a, 1a, 1b, 2a and mixed genotypes in 278 samples was reported as 49.6%, 36.3%, 12.6%, 0.4% and 1.4% respectively(20). Genotypes 1a was previously predominant in Tehran followed by 3a and 1b, but an increase in genotype 3a took place from 2003 to 2011 (11). This might be relevant to changes in the main route of transmission. The high prevalence of genotype 3a occurred more often among intravenous drug users (13). High frequency of genotypes 3a and 1a are observed among IDUs in Iran. Since HCV infections by transfusion of blood and blood products in the country have declined dramatically as a result of universal HCV screening, most new HCV infections are associated with needle sharing among IDUs, leading to a replacement of genotype 1b with 3a (21). We also encountered a relatively high rate of genotype 3a, probably because Iran received many immigrants from the Eastern neighbors. This genotype accounts for 61.4% and 39% in Pakistan and Afghanistan, respectively (22).

Multiple encounters to HCV might lead to coinfection with two or more HCV genotypes in some patients (23). Reports of HCV mixed genotype infections can be used to pursue risk factors modifications and dynamic change of

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HCV subtypes in a population. In this study, the prevalence of mixed genotype infections was 20%. Mixed infections with HCV genotypes 1a and 3a accounted for 83.4% of the total mixed infections. Prevalence of HCV mixed genotype infections has been reported with estimates ranging from 0% to 29.73 % by previous studies from Iran (24-26). Although most prior studies in Iran found a lower prevalence of mixed genotypes than our findings (13, 21). Differences in mixed genotype frequencies may, in part, be due to variation in study population and different HCV genotyping methods. The multiple infection with two or more genotypes are more common in patients with hemophilia, thalassemia and injecting drug users (27). To the best of our knowledge, the highest HCV mixed genotype prevalence in Iran was found among IDUs in Mazandaran by Rafiei et al. (25). However, the prevalence of HCV mixed genotype infections was lower in other studies from Iran than the present study, implying that HCV infection with mixed genotypes in Iran may have specific geographic distribution and epidemiological features. It seems that the route of transmission is very important for the HCV mixed genotype distribution, but unfortunately we could not investigate this association since information on the route of infection could not be obtained from study population. Repeated exposures may have resulted in an increased risk of co-infection with different genotypes for many injection drug users. Epidemiologically, injection drug user is the predominant mode of transmission for HCV infection in Iran (21). That is probably why mixed genotype infection with 1a and 3a among HCV-positive patients is dominant in our study. Some studies have suggested that patients with multiple HCV genotypes play a role in the changes in predominant genotype over time as a result of immunologic pressure, genetic interaction between virus and host, and treatment intervention (28).

Conclusion

In conclusion, our data suggest that the dominate HCV subtype among patients with HCV infection in Yazd is 3a. HCV genotype distribution pattern is changing with the decrease in subtypes 1a and 1b. According to the prevalence of mixed 1a and 3a genotype infections in our study, we propose that greater attention should be paid to the mixed genotype screening in the management of HCV-infected patients and evaluation of future treatment options.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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