

Hepatitis C virus in Iran; transmission routes, growth in 3a genotype distribution, and lack of liver marker relation with genotypes

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Background: The hepatitis C virus (HCV) outbreak in Iran is increasing. This study investigated the dissemination and transmission routes of HCV genotypes in different regions of Iran. The relationship between serum biochemical markers and viral genotypes was also assessed to find whether liver enzymes level can be considered as the markers for HCV genotypes. **Materials and Methods:** HCV-infected patients from different provinces of Iran (from August 2017 to March 2019) were enrolled. Nested reverse transcriptase polymerase chain reaction (PCR)-restriction fragment length polymorphism and real-time PCR were used to discover the genotypes. The infection transmission routes in the study population were investigated and recorded. Serum samples with equal viral load from the patients without other liver disorders were recruited to explore the association between the genotypes and the liver biochemical markers. **Results:** One thousand serum samples positive for the HCV genome were recruited. Genotype 3a was the most prevalent in the north, while genotype 1a was dominant at the center. In total, genotype 3a was the dominant genotype closely followed by 1a. Needle sharing by addicts was the most common transmission way of infection in Iran. This way was also the most for genotype 3a dissemination, and genotype 1a was transmitted mostly between family members. No significant association ($P > 0.05$) was observed between biochemical marker titers and HCV genotypes. **Conclusion:** A shift in the distribution profile of HCV genotypes, related to the transmission routes, has happened over time. Public awareness of the main routes of HCV transmission can break the cycle of transmission. Liver enzyme values in HCV-infected patients showed no relation with genotypes and only represented hepatocellular dysfunction.

Key words: Genotype, hepatitis C virus, Iran, transmission

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INTRODUCTION

Hepatitis C virus (HCV) is one of the major worldwide causes of an infectious disease that affects the liver.^[1] About 170 million people are infected worldwide (<http://www.who.int>), and annually, over 350,000 deaths are reported due to HCV-related disorders.^[1] HCV-positive sense and single-stranded RNA encodes core and envelope glycoproteins E1, E2 (structural), as well as NS2, NS3a/b, NS4a/b, and NS5a/b (nonstructural) proteins.^[2] According to genome sequence analysis of nonstructural proteins and 5' untranslated region, HCV isolates are classified into at least 11 genotypes with a

30%–50% difference in the RNA genome. Genotypes 1–6 are the major ones, and among them, genotypes 1, 2, and 3 have distributed worldwide.^[2]

HCV genotypes show variation in the different geographical areas, and effective treatment can vary based on the dominant genotype.^[3] By the introduction of direct-acting antivirals (DAAs), HCV genotyping may not be necessary for the treatment management in the near future;^[4] however, considering the price of interferon-free regimens for low/middle-income countries including Iran and the shortage of clinical and pharmacological data about drug–drug interactions and resistant variance in DAA regimen,^[5] genotyping

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before treatment stands a necessity in Iran. This country, with about 1% HCV infection prevalence in its population, can be categorized in low-frequency HCV infection countries.^[6] However, the disease prevalence with variations in genotypes is rising in different parts of Iran.^[7,8] Risk factors for infection outbreak are infected blood/blood products, syringe sharing in drug abusers, unsupported sexual intercourse, interfamily, and mother-to-infant transmissions.^[9] As no vaccine is designed against HCV infection,^[10] the knowledge of the HCV infection distribution in Iran and accurate information about disease transmission routes may provide a rationale for preventive strategies. To the best of our knowledge, there is no study on HCV genotype transmission routes in Iran. Hence, the present study aimed (1) to provide a recent epidemiological report from the HCV genotype outbreaks and (2) to investigate the routes of viral transmission in patients with different HCV genotypes and in different regions of Iran.

Another concern about hepatitis C is the association between HCV genotypes and serum biochemical markers (platelet count, bilirubin, hyaluronic acid, collagen, and elevated alanine aminotransferase/aspartate aminotransferase [ALT/AST]), and conflicting results have observed in this regard.^[11-13] Elevated liver enzymes reflect the inflammation/damage to cells in the liver, and for HCV infection, it is mainly related to the patient's immune response to the viral infection in the liver and can fluctuate.^[14] Further, many factors such as serum HCV RNA load,^[12] HIV infection, co-infection with another hepatitis virus, or having another liver disease can change the liver enzyme balance.^[15] Hence, this present study, as the third aim and for the first time in Iran, investigated the association between the level of liver enzymes with HCV genotypes to find whether enzymes level not only can be considered as markers for each genotype and disease severity but can also be used as an indicator for treatment management. For this purpose, serum enzyme levels were measured and compared in patients infected with different HCV genotypes.

MATERIALS AND METHODS

Study population

A cross-sectional study performed on serum samples positive for anti-HCV antibodies from the patients referred to Firoozgar Hospital and two other hospitals of Tehran and Rajaei Hospital of Karaj (Alborz province) from August 2017 to March 2019. Samples were from public hospitals to which HCV-infected patients are referred from all over Iran for a more accurate diagnosis of infection. Patients were from different provinces of Iran including Mazandaran that classified as the northern region; Tehran, Qom, and Semnan as central; Kermanshah and Lorestan as western; Khuzestan

as southwest; and Kerman and Sistan and Baluchestan as southeast of Iran. After the detection of HCV-RNA, samples subjected to HCV genotyping. Using a questionnaire, all participants asked about a possible way of infection and the prescribed drugs (if treatment is initiated). For the assessment of serum biomarkers association with HCV genotypes, patients with equal viral load selected; those with other liver diseases, hepatitis B virus (HBV) infection, HIV infection, HIV/HCV or HBV/HCV coinfection, hepatitis D virus infection, hepatocellular carcinoma, autoimmunity disease, drug or alcohol abuse, and diabetes; and women with pregnancy were excluded.

Informed consent

The study was conducted following the approved institutional guidelines of the Islamic Azad Medical University in Tehran (Code: IR.IAU.PS.REC.1398.193), and volunteer's data were anonymized before analysis.

Hepatitis C virus genome amplification

Viral RNA extraction from the plasma samples was performed using a high pure viral nucleic acid kit (Roche Diagnostics GmbH, Mannheim, Germany), according to the manufacturer's instructions. The extracted purified RNAs were treated with DNase and were reverse transcribed to cDNA using a commercial cDNA synthesis kit (Takara, Japan), according to the manufacturer's manual. The cDNA stored at -70°C until use as DNA templates in the reverse transcriptase-polymerase chain reaction (RT-PCR) reactions.

Viral load determination and genotyping

Viral load estimation (IU/mL) was performed on a 100 μL serum sample of the selected population using the HCV RNA quantification kit (Artus HCV QS-RGQ) with a linear range of 67.6 to 17,700,000 IU/ mL. The real-time PCR was performed on the Rotor-Gene Q instrument (QIAGEN Artus HCV QS-RGQ kit handbook 2010). HCV genotyping was done using restriction fragment length polymorphism (RFLP) assay on the RT-nested PCR products, according to Pohjanpelto *et al.*,^[16] and real-time PCR was done using HCV-genotype-FRT Kit (AmpliSens, Russia) on Rotor-Gene 3000/6000 instrument. Genotyped isolates (by sequencing) from the Keyvan Laboratory of Firoozgar Hospital were used as the positive controls.

Biochemical markers assessment

Serum samples with equal viral load belonged to the patients without other liver disorders were employed. Biochemical markers level was assessed to survey the association with HCV genotypes. ALT, AST, and alkaline phosphatase (ALP) levels were measured by an autoanalyzer (Hitachi 912, Japan). The studied population was analyzed and compared in three grouping forms: (1) whole selected population; (2)

treated and untreated population separately; (3) males and females.

Statistical analysis

Quantitative data were presented by the rate (%) and mean \pm standard deviation. Chi-square test and Student's *t*-test (SPSS version 19, SPSS version 19, Chicago, IL, USA) were used to measure the association between categorical variables. Statistical tests were conducted at the $P < 0.05$ significance level.

RESULTS

Demographic characteristics

One thousand positive HCV genome serum samples were detected, of which 712 were from Tehran hospitals and 288 were from Rajaei Hospital of Alborz province. The population study originated from north, center, west, southwest, and southeast of Iran. Table 1 represents the demographic data for the studied population. 200 serum samples containing 73 known to contain HCV genotype 1a, 77 genotype 3a, and 50 genotype 1b (with an equal viral load (4×10^5 IU/mL) and from patients without other liver diseases) were selected for the third phase of the study. As mentioned in Materials and Methods, the studied population was analyzed in three grouping forms and the final number of HCV genotypes in each group is shown in Table 2.

Transmission route in different regions of Iran and genotypes

Probable transmission route was asked from each patient and analyzed to find the most common way of transmission in each region and find the most probable transmission route of each genotype. The results are shown in Figures 1 and 2. Needle sharing between drug abusers with 26.8% was the most common way of infection followed by unsupported sexual behavior (24.2%) and intrafamily (22.8%). Blood transfusion caused 11% of infection transmission. Needle sharing by addicts was the most probable way of infection in the west, southwest, and southeast of Iran, while unsupported sexual intercourse and interfamily transmission were the major causes of infection in the north and center of Iran, respectively. The most common way of 1a genotype transmission was found transmission between family members, while needle sharing between drug abusers was the most probable way of 3a HCV genotype transmission.

Genotypic finding

Using RFLP assay on the RT-nested PCR products and real-time PCR, five HCV genotypes including 1a, 3a, 1b, 2, and 4 were detected. The frequency of the HCV genotypes in different locations, age, and gender groups is presented

Table 1: Genotyping study population (n=1000)

Parameter	n (%)
Age group	
30-40	414 (41.4)
41-50	308 (30.8)
>50	278 (27.8)
Gender	
Male	608 (60.8)
Female	392 (39.2)
Location	
North*	288 (28.8)
Center**	367 (36.7)
West#	157 (15.7)
Southwest [§]	75 (7.5)
Southeast [†]	113 (11.3)
HCV treatment history	
Naive	443 (44.3)
IFN-based treatment ^ε	540 (54)
DAA -based treatment	17 (1.7)
Total	1000 (100)

*Mazandaran; **Tehran, Karaj, Qom and Semnan; #Kermanshah, Kordestan and Lorestan; §Khuzestan; †Kerman and Sistan and Baluchestan; ^εThe recommended regimen was PegIFN + ribavirin for 24-48 weeks depending on the HCV genotype. DAA=Direct acting antivirals; IFN= Interferon; PegIFN=Pegylated IFN; HCV=Hepatitis C virus

Table 2: Biochemical markers assessment study population

Grouping criteria	HCV genotypes (n)
1 (genotype): Whole (n=200)	
1a	73
3a	77
1b	50
2 (treatment history)	
Treated (n= 100)	
1a	33
3a	39
1b	28
Naive (n=100)	
1a	40
3a	38
1b	22
3 (gender)	
Female (n=111)	
1a	36
3a	44
1b	31
Male (n=89)	
1a	37
3a	33
1b	19

HCV=Hepatitis C virus

in Table 3. Genotype 3a was the most abundant HCV genotype (41.5%) followed by 1a (39.6%). Genotype 1b, 4, and 2 were detected with lower frequency in 12.5%, 4.4%, and 2% of the participants, respectively, and HCV genotypes 3b, 5, and 6 were not identified. Totally, the distribution of

Table 3: Hepatitis C virus genotypes distribution profile (n=1000)

Variable	HCV genotypes, n (%)					Total, n (%)
	1a	3a	1b	4	2	
Location						
North	96 (33.3)	134 (46.5)	35 (12.1)	16 (5.5)	7 (2.4)	288 (28.8)
Center	174 (47.4)	136 (37)	31 (8.4)	18 (4.9)	8 (2.1)	367 (36.7)
West	39 (24.8)	76 (48.4)	29 (18.4)	9 (5.6)	4 (2.5)	157 (15.7)
Southwest	39 (52)	22 (29.3)	13 (17.3)	1 (1.3)	0	75 (7.5)
Southeast	48 (42.4)	47 (41.5)	17 (15)	0	1 (0.8)	113 (11.3)
Age group						
30-40	122 (40.6)	120 (40)	35 (11.6)	19 (6.3)	4 (1.3)	300 (30)
41-50	130 (41.2)	128 (40.6)	45 (14.2)	8 (2.5)	4 (1.2)	315 (31.5)
>50	144 (37.4)	167 (43.3)	45 (11.6)	17 (4.4)	12 (3.1)	385 (38.5)
Gender						
Male	234 (38.4)	256 (42.1)	76 (12.5)	28 (4.6)	14 (2.3)	608 (60.8)
Female	162 (41.3)	159 (40.5)	49 (12.5)	16 (4)	6 (1.5)	392 (39.2)
Total	396 (39.6)	415 (41.5)	125 (12.5)	44 (4.4)	20 (2)	1000 (100)

HCV=Hepatitis C virus

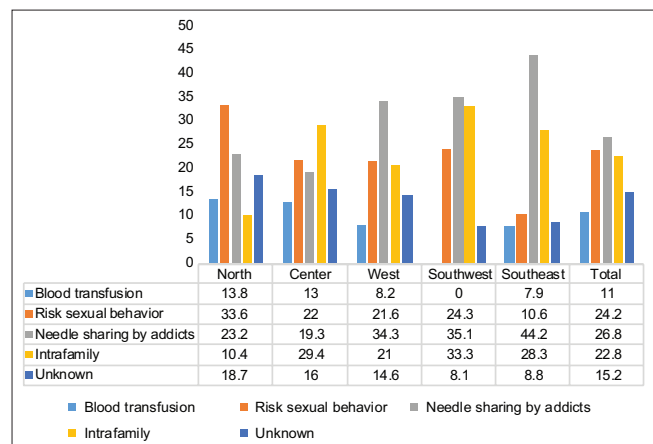


Figure 1: Transmission route in different regions of Iran (%)

HCV in the center (36.7%) and north (28.8%) was higher than the west (15.7%), southwest (7.5%), and southeast (11.3%) of Iran. Genotype 3a was more frequent in the north and west of Iran, and the dominant genotype in the center and southwest of Iran was 1a. In the southeast, the prevalence of HCV genotypes 1a and 3a was relatively equal. More HCV distribution was observed in males (60.8%) than females (39.2%), and while genotype 3a was dominant in males, genotype 1a was more detected in female patients. Between different age groups, the category >50 captured the highest rate of HCV infection [Table 3]. HCV mix genotype (1a/3a) was observed in 11 patients (1.1%) from the north.

Serum markers and its correlation with hepatitis C virus genotype

According to the ALT, AST, and ALP values, differences in enzyme values in serum samples with different HCV genotypes were not significant ($P > 0.05$) in type 1 population grouping. In the second type of grouping analysis, a significant difference was observed in the enzyme

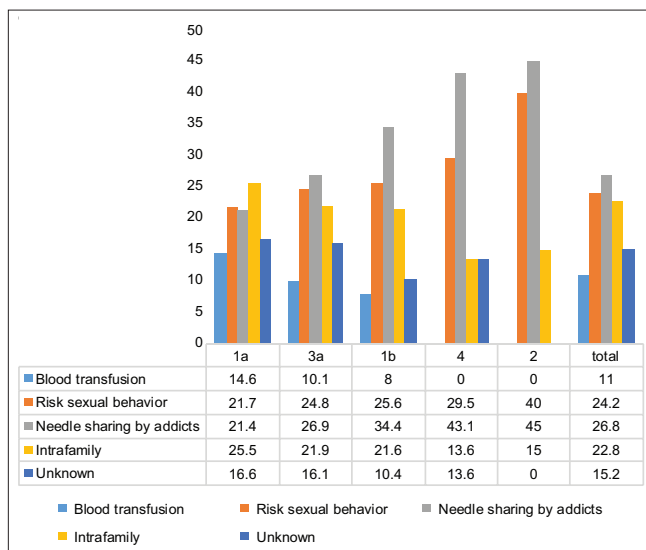


Figure 2: Transmission route of different hepatitis C virus genotypes (%)

levels between treated and naïve groups in three tested genotypes ($P < 0.05$). In this type, genotype 3a showed significantly more decrease in enzyme levels in response to treatment than 1a and 1b. Enzymes level was not significantly different among male and female groups (the third type of grouping) in three studied genotypes ($P > 0.05$).

DISCUSSION

The distribution of HCV genotypes varies in different areas.^[3] As different HCV genotypes show different responses to antiviral drugs, genotyping before the determination of optimal therapy duration and treatment is of importance.^[2,3] This study provides a recent report from HCV genotype distribution and its probable way of transmission in Iran. The prevalence of HCV genotypes in the present study population was 41.5%, 39.6%, 12.5%, 4.4%, and 2% for genotypes 3a, 1a, 1b, 4, and 2, respectively. Studies have done in Iran to

show the frequency of HCV genotypes. In a study by Amini *et al.*^[17] on 116 HCV RNA-positive patients from different parts of Iran, the authors reported genotype 1a (61.2%) as the predominant genotype, and the most frequent genotype reported in a study on 514 hemodialysis patients in Guilan (North) was 1a (59.4%).^[18] Further, in 2013 in a survey on 11561 HCV-infected patients,^[19] and in 2018 on 83 HCV-infected cases,^[20] it was shown that genotype 1a prevails other genotypes and is the most abundant HCV genotype. In Amini *et al.*'s study^[17] the frequency distribution of 3a in the north, center, west, southwest, and east was 20%, 17.6%, 27.3%, 31.3%, and 21.4%, respectively (whole-studied regions: 25%), while at the present study, the figures are 46.5%, 37%, 48.4%, 29.3%, and 47% (whole-studied regions: 41.5%). In line with our findings, several studies in Pakistan, India, and Malaysia showed HCV genotype 3a as the predominant genotype.^[18,21-23] The high rate of travel or immigration between Iran and countries above can be a justification for the growth in 3a genotype dissemination in Iran especially because the prevalence of HCV infection in Pakistan is higher than Iran.^[9] By comparison of our results with previous studies, we can conclude that during the last 10 years, the frequency of genotype 3a has grown in Iran, and similar to other studies, genotypes 1b, 2, and 4 are less frequent.^[17-19]

The prevalence of mixed genotype infections (1a/3a) in this study was low (1.1%) which was mostly belonged to the addicts. This is because multiple infections with two or more genotypes are mainly common in injecting drug users and patients with hemophilia and thalassemia.^[10]

Variation in the epidemiology of HCV genotypes roots from differences in the transmission ways,^[24] which in this study is investigated for each HCV genotype and region of Iran. According to the recorded information [Figure 1], blood transfusion shows the lowest rate in transmission risk factors, and needle sharing in drug abusers and unsupported sexual behavior include the most. These results show conformity with the distribution pattern of genotypes thereof, sexual and drug abuse show a higher role in 3a genotype expansion [Figure 2]. Iran shows a high frequency of injecting drug addicts,^[25] and considering the results of the present study in which needle sharing by drug abusers consists of the highest rate of transmission rout, we should expect more frequency of HCV infection in Iran in the future. The change in the genotype pattern of HCV infection from 1a dominance to 3a observed in this study may be related to the mentioned dominant transmission route. Blood transfusion plays the least role in HCV distribution, and according to the recorded data of this study, this way shows more contribution to 1a distribution than 3a. Recently, Iran Blood Transfusion Organization implies restrict rules for screening and assessment of blood

donors,^[26] but the risk of HCV transmission by blood/blood products remains. This can be because of asymptomatic or occult HCV infected blood donors.^[27] In this study, HCV infection transmission between family members stands after sexual contact and needle sharing by addicts and was the most probable way of genotype 1a transmission.

Regionally, the results showed that unsupported sexual intercourse is the most common cause of infection transmission in the north, which can be defined as mucosal contact to infectious blood or mucosal secretions.^[28] However, based on the results of the present study [Figure 1], the risk factor that should be considered as a stronger factor in the acquisition of HCV infection in Iran is needle sharing by addicts. Because this factor has a high percentage of the disease transmission routs in the west, southwest, and southeast of Iran. Differences in the transmission routes of infection in different genotypes of HCV and regions of Iran should be updated and considered to apply better strategies for the prevention and treatment management of HCV infection in Iran.

To the best of our knowledge, few or no studies in Iran show the relation of HCV genotypes to serological markers. In the third part of the study, we evaluated and compared the level of serum markers including ALT, AST, and ALP in different genotypes in three types of the grouping of the participants. To avoid the enzymes level fluctuation interference, 200 serum samples from patients without any other liver disease or disorders were recruited. The results of the present study showed no significant relationship between HCV genotypes and studied liver enzyme levels. However, a significant difference was observed in the enzyme levels between treated and naïve groups in three tested genotypes ($P < 0.05$). The difference in the levels of serological markers was not significant between male and female patients infected with different HCV genotypes ($P > 0.005$). On the flip side, Riaz *et al.*^[13] observed significantly ($P < 0.005$) higher ALT, AST, and ALP titers in infection with genotype 1a than 3a, and in a study on the Caucasian population, HCV patients with genotype 1b showed significantly higher ALT levels in comparison with other genotypes.^[12] Conflicting results may be related to the applied criteria in the study population selection and/or the number of included serum samples. Absence of persistent relation between serum transaminase activity and fibrosis progression rate^[29] and fibrosis or active inflammation occurrence in individuals with chronic HCV but normal ALT level^[30] showed in previous studies^[29,30] are other confirmations for the inapplicability of liver enzyme consideration in HVC infection management. Accordingly, serum aminotransferase levels that reflect hepatocellular injury cannot be used as markers for differentiation of HCV genotypes and to predict the severity of liver damage by each HCV genotype.

CONCLUSIONS

Our findings showed remarkable growth in genotype 3a distribution. The transmission routes vary between regions of Iran and can affect the HCV genotypes distribution pattern. Liver enzymes levels cannot be considered as a marker for the formulation of HCV treatment or disease severity prediction, and more precise diagnostic methods are needed for superior control of HCV dissemination.

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Conflicts of interest

There are no conflicts of interest.

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