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**Determining hepatitis C virus genotype distribution among high-risk groups in Iran using real-time PCR**

Jamalidoust M *et al*. Determining HCV genotypes in Iran

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**Abstract:**

**AIM:** To assess hepatitis C virus (HCV) genotypes pattern among Iranian risk groups, using rt-RT-PCR assay.

**METHODS:** In this study, we evaluated the distribution of different HCV genotypes among injection drug users and other high-risk groups over a 4-year period (from 2009 to 2012) using Real-time PCR. Sera from 888 HCV-infected patients residing in southern and southwest Iran were genotyped using Real-time PCR with common primers and specific probes. These patients were grouped into distinct exposure categories. Illicit drug users constituted the primary group and were further evaluated for the HCV genotype distribution, age range and *etc*.

**RESULTS:** In this study, 62% of the examined HCV-infected patients were victims of substance abuse, although the route of transmission could not be determined for approximately 30% of the patients. HCV genotyping revealed that Gt1 was the most prevalent genotype among the drug users as well as among patients with thalassemia, haemophilia, solid organ recipient and haemodialysis. Mixed infections were only seen in addict groups, where Gt2 genotype was also found. The highest frequencies of HCV-positive addict patients were observed in the age group of 31–40 years. Our research also showed that the addiction age has increased, whereas the addiction rate has dropped in this region. Most illicit drug users were with more than one risk factor such as tattoo and/or a history of prison.

**CONCLUSION:** This study revealed that the most common HCV-infection route andHCV**-**genotype in southern and southwest of Iran was illicit drug abuse and Gt1 respectively.

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**Key words**: Hepatitis C virus genotype distribution; Injection drug users; Real-time PCR; Iran

**Core tip:** The primary tool for hepatitis C virus (HCV) treatment, determination of evolution pathways and assessment of epidemiological status, knowledge of HCV genotypes distribution among high risk groups such as the addicts are so important. We assessed the different HCV genotypes among illicit drug users and other high risk groups, during a 4-year period from 2009 to 2012 by real-time PCR assay. We determined the most affected high risk groups to be illicit drug users and specified respective age distribution and risk factors. An important valuable finding in this research was the genotype pattern shift from 3 to 1, especially among addicts.

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**INTRODUCTIONS**

The increasing use of different types of injections, including herbal injections, particularly chemical drugs, in many developed and developing countries, including Iran, and the lack of an effective hepatitis C virus (HCV) vaccine have resulted in HCV infection becoming a major public health concern[1,2]. A lower prevalence of HCV infection has been reported in Iran compared to other parts of the world, particularly the Middle East[[1](#_ENREF_1),[2](#_ENREF_2)]. Given the fact that Injection Drug Users (IDUs) constitute the major group of HCV patients, planning for their treatment is essential for community health[[3](#_ENREF_3),[4](#_ENREF_4)].

HCV genotyping is the most significant predictor of treatment duration and evaluation of the course of infection, with different genotypes showing different treatment responses and varying epidemiological as well as virological features[[5](#_ENREF_5)]. HCV has a single-stranded RNA genome and at least six different genotypes, more than 90 subtypes and many types of quasi-species variants based on scattered and local variations of its genome. This genetic heterogeneity is due to the lack of fidelity of viral RNA-dependent RNA polymerases[[6](#_ENREF_6)]. HCV-RNA diversity is concentrated in the E1 and E2 glycoprotein-coding regions of the genome, while the least heterogeneity is found in the regions encoding core and NS3 proteins, which represent structural and non-structural proteins, respectively. In total, the nucleotide sequences of the different genotypes differ from each other by 30%–35%, with a divergence of 92% in the 5′-UTR regions[[1](#_ENREF_1)].

The HCV genotype pattern varies among different groups of infected individuals, particularly IDUs, in different areas. For example, the predominant genotypes are Gt3a and Gt4 in European countries and Gt3a in Argentina[[1](#_ENREF_1),[7](#_ENREF_7)-9]. Mahfoud *et al*[1[0](#_ENREF_9)] showed that HCV Gt3 was distributed among Lebanese IDUs with 57% frequency. The aim of this study was to assess HCV genotype distribution among drug-addicted HCV-infected patients in Iran and compare this distribution with other high-risk groups of infected patients using Real-time PCR, which simultaneously detects target HCV genomes with genotype-specific primer sets and probes.

**MATERIALS AND METHODS**

From March 2009 to December 2012, a total of 888 HCV-RNA positive patients from southern and southwest Iran were enrolled for HCV genotyping in this cross-sectional study. These patients underwent a preliminary inquiry, which allowed the identification of individuals with parenteral risk factors. Among them, 550 (61.9%) IDUs with the ages 18-74 years (mean ± SD, 37.89 ± 10.192 years) were identified, including 546 (99.27%) males and 4 (0.72%) females. None of the patients under study had been previously treated for HCV infection. Ethylenediaminetetraacetic acid (EDTA) plasma was prospectively collected, and was aliquots stored in 1.5 mL vials at -70°C. HCV infection was reaffirmed by the detection of antibodies using an immunochromatographic assay, manufactured by Artron (Burnaby, BC, Canada).

Viral RNA was isolated from 200 μL aliquots of serum samples using the InviTrap® Spin Blood RNA Mini kit (Invitec, Berlin) as per the manufacturers’ instructions, and eluted using 50 μL of nuclease-free water. The concentration of the isolated HCV-RNA was determined for the various samples and then genotyped, followed by Real-time PCR using commercially available HCV kits (Genome Diagnostics Pvt. Ltd., Hague, Netherland) for determining HCV genotypes Gt1 to Gt4. All tests were carried out in accordance with the manufacturers’ instructions. The PCR conditions were as follows: 50°C for 25 min and 95°C for 10 min, followed by 50 cycles of 94°C for 10 s, 55°C for 32 s and 72°C for 25 s. The reactions were performed in a 7500 Real-time PCR system (Applied Biosystems, United States).

***Statistical analysis***

This study performed using SPSS for Windows systems (Version 16.0, 2007, SPSS Inc, Chicago, IL, United States). Comparison of HCV genotypes, their distribution among different high-risk groups and among different age groups of addicts were analysed by *χ*2 test.

**RESULTS**

A total of 888 HCV RNA-positive samples, collected between 2009 and 2012 from patients with chronic HCV infection, were included in this study [787 (83%) men and 99 (11.2%) women, 2 misplaced]. Of them, 738 (83%) HCV-positive serum samples could be genotyped successfully using HCV genotype Real-time PCR kits from Applied Biosystems. The frequency of HCV infection among various high-risk groups and HCV genotype distribution among the patients included in this study are presented in Table 1 and 2.

As shown in Table 1, HCV infection was most prevalent among the victims of substance abuse (62.2%), with the highest frequency (> 99%) found among male drug users. The high prevalence of HCV infection can also be attributed to unknown factors compromising approximately 31% of the cases. The other high-risk groups for HCV infection include patients with thalassemia, haemophilia and dialysis as well as recipients of solid organs.

Overall, the most prevalent HCV genotypes were Gt1 (51.1%) and Gt3 (30.1%), whereas Gt4 (0.5%) and Gt2 (0.3%) were less prevalent. Mixed HCV genotypes were reported only in 8 cases of addicts under study that included Gt1/3 and Gt1/2 co-infections with 0.7% and 0.2% prevalence, respectively (Table 2).

***Distribution of HCV genotypes among addicts***

A past or current history of illicit drug use was the predominant risk factor for HCV infection. A total of 550 addicts [546 (99.27%) men and 4 (0.72%) women] were subjected to HCV genotyping; 483 (87%) addicts among them could be genotyped, and included 475 (87.8%) patients with a single HCV genotype and 8 (12.2%) patients with a mixed genotype distribution. The most prevalent HCV genotype was Gt1, found in 283 addicts (51.5%), followed by Gt3 in 192 (34.9%), Gt1/3 in 6 (1.1%) and Gt1/2 in 2 (0.4%) addicts. Single or mixed genotypes of Gt2 and Gt4 were not detected in any of the addicts.

The prevalence of HCV infection and HCV genotype distribution varied between different age groups; as shown in Table 3 and Figure 1, the highest frequency of HCV-positive patients were observed in the age group of 31–40 years.

**DISCUSSION**

Since the most significant route of HCV infection in both developed and developing countries is addiction and the use of illicit drugs[[10](#_ENREF_10),[11](#_ENREF_11)], consideration of issues related to HCV infection such as viral load, genotyping, treatment response rate and epidemiological status are very important[[2](#_ENREF_2)].

HCV genotyping among a defined population serves as a useful tool for determining HCV evolution in different geographical regions and in identifying the respective high-risk groups[[12](#_ENREF_12)]. Specified HCV genotypes have also been reported as the primary tool for determining infection course and assessing the duration of treatment[[13](#_ENREF_13)]. however, certain investigations have implicated body mass index, IL28 genotype, gamma glutamyl transpeptidase, triglycerides and the level of miR-122 as other predictors of treatment length[[14](#_ENREF_14)].

Unfortunately, even in developed countries, only a small proportion of IDUs receive HCV treatment[[15](#_ENREF_15)], and clinicians are often reluctant to treat them. The reasons for this trend may include high risk of reinfection, concomitant excessive alcohol intake and high rates of concurrent mental health problems[[16](#_ENREF_16)].

Various studies suggest that the SVR rate among IDUs is comparable to that among non-IDUs and that it is not significantly related to IDU status[[10](#_ENREF_10),[17](#_ENREF_17)].

In clinical trials where peg interferon and ribavirin were prescribed, the median SVR rate among IDUs with chronic HCV infection was 54.3% (range, 18.1%–94.1%), comparable with 54%–63% seen in their non-IDU counterparts, whereas it was 68.5% in IDUs with acute HCV infection as opposed to 81.5% among non-IDUs[[18](#_ENREF_18),[19](#_ENREF_19)].

HCV prevalence rate and genotype distribution among Asian and Middle Eastern countries is very diverse. Although Iran is surrounded by countries with high prevalence of HCV of various genotypes, HCV prevalence in Iran (< 2%) is much lower than that of its neighbouring nations, Pakistan (5.31%) and Egypt (14.7%)[[20](#_ENREF_20)]. Genotypes 1, 2 and 3 (Gt1–Gt3) have been found to have a global prevalence while Gt4–Gt6 have a restricted pattern[[20](#_ENREF_20)]. Gt4 has been chiefly detected in Egypt, some Arab countries such as Saudi Arabia, Syria and United Arab Emirates, and recently, also in certain parts of Europe[[21](#_ENREF_21)]. Gt5 is restricted to South Africa, and Gt6 is primarily found in Southeast Asian countries such as China, Hong Kong and Taiwan [22,23].

Different studies have shown that Gt4 and Gt1 are the predominant genotypes in Arab and non-Arab countries, respectively, in the Middle East region[[21](#_ENREF_21)]. A recent study carried out in a neighbouring country, Pakistan, identified Gt3 as the predominant HCV genotype[[22](#_ENREF_22),[23](#_ENREF_23)]. Several investigations have also been conducted in different parts of Iran for determining HCV genotype distribution. The latest studies have indicated that HCV Gt among IDUs in south, southeast and north Iran (Mazandaran province), Gt1 in northeast and Gt3 in Isfahan province in central Iran, are the predominant genotypes[24,29].

The current study was performed to determine the distribution of HCV genotypes based on the route of infection, and revealed a significantly high prevalence (approximately 62%) of HCV infection among drug addicts. many studies have been carried out in different region of Iran in IDUs. Generally in Iran, HCV seropositivity in addict was higher than general population (30%-90% *vs* 0.2%-2.0%).

 Our data also shows that Gt1 is the most common genotype among thalassemia, haemophilia, solid organ recipient and haemodialysis patients as well as IDUs. A discrepancy between HCV genotype distribution among the addict and non-addict groups was seen, with Gt3 frequency in IDUs being lesser than in non-IDUs (Table 4). Among IDUs, Gt2 and Gt4 were rarely seen; the latter was not detected in any IDU patient while Gt2 was detected in just 2 patients with mixed infections of Gt1/2. Overall, among the 888 patients, mixed HCV genotypes were detected in 8 serum samples of only the addicts and not the non-addict groups. This could be due to multiple injections taken by the addict group.

A comparison of these results with our previous study[32] revealed an impressive shift in the distribution of HCV genotypes, from the dominant prevalence of HCV Gt3a seen previously[[24](#_ENREF_24)] to an increased prevalence of Gt1, particularly among IDUs, in the current study.

As shown in Table 3, most of the HCV-infected addicts belonged to the 31–40 age group and accounted for 35% of the infected addicts, followed by the 21–30, 41–50 and 51–60 age groups accounting for 25%, 19% and 12% infected addicts, respectively. These results indicate that unlike the study conducted in northern Iran[[25](#_ENREF_25)], the addiction age may not be reduced in southern and southwest Iran.

According to table 5 although all IDUs in this study had an addictive- drug using history in a short or long time between a few months to a several years but 72% of them had more than one risk factor. Among them, more than 70% had a history of imprisonment of variable lengths. Tattoos were other crucial risk factor that was seen only in patients who had a history of imprisonment. Drinking alcohol and having multiple sex partners were the other risk factors, there was a little data in these regards.

We found that the genotype distribution also varied with respect to underlying conditions among the patients from the same geographical area, and it is noteworthy that the least prevalent unknown genotypes were found among IDUs (Table 4), probably due to high mean quantitative viral load.

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**COMMENTS**

***Background***

In this study, hepatitis C virus (HCV) genotype patterns among different high risk groups were determined using real-time PCR, as a very sensitive and fast assay during 2009 to 2012.

***Research frontiers***

In this research, a large number of HCV sufferers (888 patients) in different high risk groups were subjected to genotyping. Genotype 1 was determined to be the predominant genotype in both non addict (thalassemia, hemophilia, and hemodialysis patients) and addict sufferers, the most prevalent high risk group.

***Innovations***

This study is the first report on HCV genotypes among Iranian sufferers with different exposure categories, residing in south and southwest of Iran, where genotype 1 was found to be the most frequent genotype. Compared to the results of our previous study, we found that addiction age increased. An important valuable finding in this research was the genotype pattern shift from 3 to 1, especially among addicts.

***Application***

This study demonstrated changing HCV genotype patterns in our region and it can serve as a basic research for prospective studies on varying HCV genome. This study could also be helpful for health authorities and decision makers.

***Peer review***

In the present study, authors examined HCV genotype in 888 Iranian patients by real-time PCR assay and evaluated the relation between infective root and HCV genotype.

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**Figure 1 Hepatitis C virus genotypes distribution among different addict’s age groups.**

**Table 1 Frequency of hepatitis C virus infection among high risk groups *n* (%)**

|  |  |  |
| --- | --- | --- |
| High Risk group | Frequency | M/F |
| Addict | 550 (62.2) | 546/4 |
| Thalassemia | 38 (4.3)  | 17/21 |
| Hemophilia | 8 (0.9) | 7/1  |
| Kidney graft | 4 (0.5) | 2/2 |
| Dialysis | 6 (0.7) | 5/1 |
| Liver graft | 3 (0.3) | 1/2 |
| Unknown | 277 (31.3) | 209/68  |
| Total | 886 (100.0) | 787/99 |

**Table 2 Distribution of hepatitis C virus genotypes among Iranian hepatitis C virus infected patients according to route of infection*****n* (%)**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| HighRisk group | Frequency  | Undetectable | Gt1  | Gt2 | Gt3  | Gt4 | Gt1/2 | Gt1/3  |
| Addict | 550 | 67 (12.2) | 283 (51.5) | 0 (0.0) | 192 (34.9) | 0 (0.0) | 2 (0.4) | 6(1.1) |
| Thalassemia | 38 | 15 (39.5) | 17 (44.7) | 0 (0.0) | 6 (15.8) | 0 (0.0) | 0 (0.0) | 0(0.0) |
| Hemophilia | 8 | 2 (25.0) | 5 (62.5) | 0 (0.0) | 1 (12.5) | 0 (0.0) | 0(0.0) | 0(0.0) |
| Kidney graft | 4 | 1 (25.0) | 2 (50) | 0 (0.0) | 1 (25.0) | 0 (0.0) | 0(0.0) | 0(0.0) |
| Dialysis | 6 | 1 (16.7) | 4 (66.7) | 0(0.0) | 0 (0.0) | 1 (16.7) | 0(0.0) | 0(0.0) |
| Liver graft | 3 | 1 (33.3) | 2 (66.7) | 0(0.0) | 0 (0.) | 0 (0.0) | 0(0.0) | 0(0.0) |
| Unknown | 277 | 64 (23.1) | 140 (50.5) | 3(1.1) | 67 (24.2) | 3 (1.1) | 0 (0.0) | 0 (0.0) |
| Total | 886 | 151 (17.0) | 453 (51.1) | 3(0.3) | 267 (30.1) | 4 (0.5) | 2 (0.2) | 6 (0.7) |

**Table 3 Comparison of illicit drug user age-groups in terms of hepatitis C virus genotypes *n* (%)**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Undetectable | Gt1 | Gt2 | Gt3 | Gt4 | Gt1/2 | Gt1/3 | Total |
| < 20 | 0 (0.0) | 1 (33.3) | 0 (0.0) | 2 (66.66) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 3 (0.57) |
| 21-30 | 14 (11.02) | 59 (46.45) | 0 (0.0) | 53 (41.73) | 0 (0.0) | 0 (0.0) | 1 (0.78) | 127 (24.47) |
| 31-40 | 23 (10.95) | 113 (53.80) | 0 (0.0) | 69 (32.85) | 0 (0.0) | 2 (0.95) | 3 (1.42) | 210 (40.46) |
| 41-50 | 18 (16.36) | 58 (52.72) | 0(0.0) | 33 (30) | 0 (0.0) | 0 (0.0) | 1 (0.9) | 110 (21.19) |
| 51-60 | 6 (11.53) | 25 (48.07) | 0(0.0) | 20 (38.46) | 0 (0.0) | 0 (0.0) | 1 (1.92) | 52(10.01) |
| > 60 | 1 (5.88) | 10 (58.82) | 0 (0.0) | 6 (35.29) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 17 (3.27) |
| Total | 62 (11.9) | 266 (51.25) | 0 (0.0) | 183 (35.26) | 0 (0.0) | 2 (0.38) | 6 (1.15) | 519 (100.0) |

**Table 4 Comparison of hepatitis C virus genotypes in the addicts and non-addict high risk groups**

|  |  |  |
| --- | --- | --- |
| **Genotype** | **Non-addict** | **Addict** |
| Gt1 | 50.7% | 51.5% |
| Gt2 | 1.0% | 0.0% |
| Gt3 | 22.0% | 34.9% |
| Gt4 | 1.3% | 0.0% |
| Gt1/2 | 0.0% | 0.4% |
| G1/3 | 0.0% | 1.1% |
| Unknown  | 25.0% | 12.2% |

**Table 5 Most important risk factors in Iranian illicit drug users *n* (%)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **HCV suffers** | **+Imprisonment and tattoo** | **+Imprisonment** | **+ No other risk factor** | **Total** |
| Injection Drug Users | 70 | 235 | 134 | 439 (80) |
| non-Injection Drug Users | 63 | 23 | 25 | 111 (20) |
| Total | 133 (24) | 265 (48) | 159 (28) | 550 (100) |