

## Distribution of hepatitis C virus genotypes in Arak city, central province of Iran

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### ABSTRACT

**Background and Objectives:** Hepatitis C virus (HCV) infection is a worldwide concern and it is the major cause of liver disease. Several genotypes of the HCV have been reported from different regions of the world. The determination of the HCV genotypes is important for the prediction of response to antiviral treatment and clinical outcomes. So, HCV genotyping in each region is of great importance. This investigation was performed to determine the distribution of HCV genotypes in Arak city, Central province of Iran.

**Patients & Methods:** In this cross sectional study, 174 cases with chronic HCV infection from Arak city were enrolled. HCV infection was confirmed by positive results in HCV antibody (anti-HCV) and HCV-RNA tests. HCV genotypes were determined using a PCR based genotyping kit.

**Results:** A total of 174 HCV infected patients with mean age of  $37.5 \pm 10.24$  years were enrolled. 97.7% of cases were male and 2.3% were female. The main route of HCV transmission was injection drug use (IDU) which was observed in 59.8% of cases. Genotyping results demonstrated that subtype 3a (52.9%) was the most prevalent HCV type in Arak, followed by subtype 1a (22.9%) and subtype 1ab (17.8%).

**Conclusion:** This study showed that HCV subtype 3a was the most prevalent HCV type, followed by subtype 1a and subtype 1ab in Arak, central province of Iran. Investigation of HCV genotypes in different parts of the country is needed to facilitate treatment options and preventive strategies.

**Keywords:** Hepatitis C virus (HCV), Genotype, Arak, Iran

### INTRODUCTION

Hepatitis C virus (HCV) is a small, enveloped positive sense, single stranded RNA virus that belongs to the genus Hepacivirus and the family Flaviviridae. It is estimated that 3% of the world's population (170 million people) are infected with HCV (1) and

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its prevalence ranges from 0.2% to 40% in different countries (2, 3). HCV is a major cause of chronic viral hepatitis, liver cirrhosis and hepatocellular carcinoma. Therefore it has important clinical, epidemiological and economic outcomes throughout the world especially in developing countries (4, 5).

Genotyping of hepatitis C virus is important because different genotypes have different infectivity and influencing the rate of progression of HCV infection to cirrhosis and hepatocellular carcinoma. Besides various HCV genotypes would also result different responses to anti-viral treatment. For example, genotypes 1 and 4 are more resistant to interferon based therapies than genotypes 2 and 3 (6- 8).

Currently, HCV is classified into eleven genotypes (1-11) with sequence differences range from 30% to 50% that six of them are the major genotypes (1 to 6) (6, 9). Within HCV genotype, several subtypes (named as a, b, c, etc.) can be defined by sequence differences range from 15% to 30% (10, 11).

The distribution of HCV genotypes and subtypes is geographically different. Genotype 1 is the most prevalent globally, followed by genotype 3 and 2. Types 1a and 1b are the most common, accounting for about 60% of global infections. They predominate in Northern Europe and North America, and in Southern and Eastern Europe and Japan, respectively. HCV genotype 2 is particularly prevalent in West Africa. Genotype 3 is primarily found in Australia and South Asia. Genotype 4 is principally detected in the Middle East, Egypt, North and Central Africa. Type 5 is mostly found in Southern Africa and genotypes 6-11 are distributed in Asia (12-16).

The determination of the HCV genotypes is important for the prediction of response to antiviral treatment. So, HCV genotyping in each region is of great importance. The distribution of HCV genotypes was reported from various regions of Iran (17-20); however, such studies were not conducted in Arak city. Therefore, this investigation was performed to determine the distribution of HCV genotypes in Arak, Central province of Iran.

## PATIENTS AND METHODS

**Study population.** In this retrospective study, 174 cases with chronic HCV infection [ positive result of hepatitis C antibody (anti-HCV) more than 6 months and positive HCV-RNA] referred to Infectious center

of Valiasr Hospital, Arak; Central province of Iran were enrolled. HCV infection was confirmed by positive HCV antibody (anti-HCV) and HCV-RNA tests. All the cases were negative for hepatitis B surface antigen (HBsAg) and Human Immunodeficiency Virus (HIV) antibodies. A questionnaire was used to collect data such as age, sex and possible route of HCV transmission. The project was approved by Arak University of Medical Sciences ethical committee. Informed consent was obtained from each subject before participation.

**Sample collection and serological tests.** A peripheral blood sample from each patient was collected in an EDTA containing sterile tube. Plasma was separated by centrifugation and stored at  $-80^{\circ}\text{C}$  for further tests.

Anti-HCV and HBsAg were tested by enzyme-linked immunosorbent assay (ELISA). The commercial enzyme immunoassay kits used were as follows: HBsAg (Hepanosticka Biomerieux, Boxtel, The Netherlands) and anti-HCV (Biorad, Segrate, Italy). Recombinant immunoblot assay (RIBA Inno-genetics, Ghent, Belgium) was employed to confirm anti-HCV reactivity.

Anti-HIV was determined by ELISA (MP Bio-medicals, Illkirch, France); with positive tests confirmed by Western blot assay (Diaplus, San Francisco, USA).

**RNA extraction.** Viral RNA was extracted from plasma sample using High Pure Viral Nucleic Acid Kit (Roche Diagnostics GmbH, Mannheim, Germany), according to the manufacturer's instructions.

**HCV genotyping.** HCV genotyping was carried out using a commercial kit (Sacace, Italy) according to manufacturer's instruction. This kit is designed for the detection of genotypes 1a, 1b, 2, 3a (which are most prevalent HCV genotypes in Iran) by generating different size PCR products. The specificity and sensitivity of the kit are 100% and 1000 viral particles/ml respectively. For each patient two sets of PCR amplifications were performed in two separate tubes containing primers for either genotypes 1a+1b or genotypes 2+3a. 5  $\mu\text{l}$  of cDNA was subjected to PCR amplifications using two sets of mixed primers included in the kit. The PCR profile was an initial denaturation at  $95^{\circ}\text{C}$  for 5 min, followed by 42 cycles of  $95^{\circ}\text{C}$  for 1 min,  $68^{\circ}\text{C}$  for 1 min,  $72^{\circ}\text{C}$  for 1 min and a

final extension at 72°C for 10 min. Genotype 1a generates a 338 bp PCR product, genotype 1b, 2 and 3a generate 395, 286 and 227 bp PCR products respectively. The PCR products were electrophoresed in a 2% agarose gel and stained with ethidium bromide.

**Treatment of the patients.** After determination of HCV genotypes, patients with genotypes 2 or 3, received 800 mg Ribavirin (RBV) daily in two divided doses for 24 weeks. Patients with genotype 1, received 1000 mg RBV daily if they weighed less than 75 kg and 1200 mg if over 75 kg for 48 weeks. All participants received 180 µg of Pegaferon weekly. Sustained virologic response (SVR) which is defined as undetectable HCV-RNA using a highly sensitive assay (real time polymerase chain reaction) was determined in patients after 24 weeks of the end of treatment.

**Statistical analysis.** The Chi-square was used with the SPSS 16 Package program for statistical analysis (Chicago, IL, USA). Data are presented as mean ± SD or, when indicated, as an absolute number and percentage.

## RESULTS

A total of 174 patients with chronic HCV infection with mean age 37.5±10.24 (range: 18-76 years) were enrolled in the study. 170 (97.7%) of cases were male and 4 (2.3%) were female. The main route of HCV transmission was injection drug use (IDU) which was observed in 104 (59.8%) of cases. Other presumed routes of HCV transmission were needle stick in 12 (6.9%), blood and blood products transfusion in 5 (2.9%), incarceration in 4 (2.3%), heterosexual contact in 1 (0.6%), tattooing in 1 (0.6%), IDU and tattooing in 7 (4%), IDU and blood transfusion in 7 (4%), IDU and incarceration in 6 (3.4%), IDU and heterosexual contact in 3 (1.7%), tattooing and heterosexual contact in 2 (1.2%), IDU, tattooing and incarceration in 2 (1.2%), IDU, tattooing and heterosexual contact in 3 (1.6%) and in 17 (9.8%) the route of HCV acquisition was not identified.

Genotyping results demonstrated that subtype 3a 92 (52.9%) was the most prevalent HCV type in Arak, followed by subtype 1a 40 (22.9%) and subtype 1ab 31 (17.8%). Table 1 showed distribution of HCV genotypes and subtypes in Arak city. HCV genotypes

distribution was not significantly associated with age and gender (P=0.07 and 0.06 respectively).

Sustained virologic response was observed in 78 (83.8%) of cases. SVR was detected in 42/4 (91.3%) of patients infected with genotype 3a versus 17/22 (77.3%) of those infected with genotype 1a and 9/13 (69.2%) of cases infected with genotype 1ab.

## DISCUSSION

Epidemiological studies in different region of the world show the wide variation and regional differences in the distribution of HCV genotypes (21). HCV genotyping is an important factor in clinical HCV treatment and investigation of HCV genotypes distribution is essential as prognostic factor in chronic HCV infection (11, 22). HCV genotypes are studied in different parts of Iran (17-20). In this survey which is the first study of HCV genotypes distribution in Arak city, Central province of Iran, we showed that subtype 3a (52.9%) was the most prevalent HCV type, followed by subtype 1a (22.9%) and subtype 1ab (17.8%).

An investigation by Hajia et al. (23) on specimens from 16 provinces of Iran revealed that type 3a was the most frequent HCV type (46.6%), followed by type 1 with rate of 43.2% (including 1a and 1b with 25.73% and 17.47% for each respectively).

Khodabandehloo et al. reviewed the prevalence of HCV genotypes in Iranian patients and reported that HCV subtype 1a was maximum in East Azarbaijan and Guilan respectively but minimum in West Azarbaijan. Frequency of subtype 3a was variable in different cities of Iran. It is maximum in Guilan and Isfahan and minimum in West Azarbaijan. Prevalence

**Table 1.** Distribution of HCV genotypes and subtypes in Arak city

HCV Genotype and subtype	Number (%)
3a	92 (52.9)
1a	40 (22.9)
1ab	31 (17.8)
1b	4 (2.3)
2a	4 (2.3)
1a & 2a	1 (0.6)
1a & 3a	1 (0.6)
1ab & 3a	1 (0.6)

of subtype 1b was 25%, 25% and 23% in Kermanshah, Zahedan and Hormozgan respectively. Prevalence of genotype 2 was 0.7% in Tehran and 17.2% in Kermanshah (24).

In a study by Esmaeilzadeh et al. (17) in Zanjan (the Northwest of Iran), subtype 3a was the most current subtype in this city. In another study by Omrani et al. (25) HCV genotype 3a with the prevalence rate of 48.1% was the most prevalent HCV type in West Azerbaijan, Northwest of Iran. A study in Shiraz, a city in Southern part of Iran revealed that the highest level of HCV infection belonging to type 3a followed by 1a (26). Additionally genotype 3 was the most common genotype in plasma and peripheral blood mononuclear cells (PBMCs) of HCV infected patients in Jahrom city (another city in Southern part of Iran) (27). Besides, genotype 3a is the most frequent HCV genotype in Isfahan province, Iran. HCV subtypes 3a and 1a were determined in the hemodialysis patients living in Tehran as the prevalent subtypes (28).

On the other hand genotype 1a was the more frequent HCV genotype in Khorasan Razavi Province (Northeast of Iran) (29). In another survey in Southeast of Iran, genotype 1 was the more prevalent HCV type followed by genotype 3, 4 and 2 (30). Genotype 1a (41.7%) followed by 3a, 2 and 4 were reported as most frequent HCV genotypes in Khuzestan province, Southwestern Iran (18).

Jahanbakhsh Sefidi et al.(31) showed that in 2003, genotype 1a was the most common HCV genotype in Iran (47.8%) but it decreased over time and HCV genotype 3a was increased in this period of time (30.1% in 2003 which was increased up to 39.6% in 2011. Salehi Moghadam et al. (32) also reported that while HCV subtype 1a is predominant among HCV infected Iranian subjects, subtype 3a is predominant among Iranian injecting drug users.

Our results are in accordance with a recent study from Yazd city, another town in Central province of Iran which showed that HCV genotype 3a was the predominant genotype followed by the subtypes 1a and 1b (33) and it was also compatible with the results reported from another parts of Iran (17, 25-28). On the other hand, genotype 3a is significantly associated with HCV transmission through injection drug use (34), which is the main route of HCV transmission in our participants that was observed in 59.8% of cases.

In our study, SVR was more observed in patients infected with genotype 3a versus those infected with genotype 1a and 1ab, which was in accordance to this

issue that genotype 3a revealed a great SVR among interferon-treated patients, compared to genotype 1(6-8). This study had limitations like small sample size and lack of the correlation between HCV genotypes and the severity of the disease among the studied population.

## CONCLUSION

This study showed that HCV subtype 3a was the most prevalent HCV type, followed by subtype 1a and subtype 1ab in Arak, central province of Iran. Determination of HCV genotype distribution could affect the HCV therapeutic methods and duration and could increase the chance of successful treatment. Investigation of HCV genotypes in different parts of the country is needed to facilitate treatment options and preventive strategies.

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