

# Epidemiological Distribution and Genotype Characterization of the Hepatitis C Virus Among HIV Patients in Kashan, Iran

Hasan Afzali,<sup>1</sup> Mansooreh Momen-Heravi,<sup>2,\*</sup> and Asefeh Farokhzad<sup>3</sup>

<sup>1</sup>Associate Professor, Department of Infectious Disease, School of Medicine, Kashan University of Medical Sciences, Kashan, IR Iran

<sup>2</sup>Associate Professor, Department of Infectious Disease, Social Determinants of Health (SDH) Research Center, Kashan University of Medical Sciences, Kashan, IR Iran

<sup>3</sup>Infectious Disease Specialist, Department of Infectious Disease, School of Medicine, Kashan University of Medical Sciences, Kashan, IR Iran

\*Corresponding author: Mansooreh Momen-Heravi, Associate Professor, Department of Infectious Disease, Social Determinants of Health (SDH) Research Center, Kashan University of Medical Sciences, Kashan, IR Iran. Tel: +98-9133611017, E-mail: momenheravi1346@gmail.com

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## Abstract

**Background:** Parenteral transmission is a common route of transmission for both human immunodeficiency virus (HIV) and hepatitis C virus (HCV); therefore, hepatitis C viral infection is highly prevalent among people infected with HIV.

**Objectives:** This study was designed to examine the epidemiology and describe the clinical manifestation as well as the HCV genotypes in patients from the city of Kashan, Iran, who are coinfecting with HIV and HCV.

**Patients and Methods:** This descriptive study was conducted in 2014 in the city of Kashan. The population consisted of all the HIV-infected patients who were referred to the behavioral counseling center and jail in Kashan. Demographic information and HCV- and HIV-related risk behaviors were obtained through the use of an interviewer-assisted questionnaire. After the participants gave written informed consent to participate, 10 cc venous blood samples were collected. The serum samples were screened for HCV infection using an enzyme-linked immunosorbent assay (ELISA). In the event of a positive test for HCV, the RNA was then amplified by polymerase chain reaction (PCR) amplification. The HCV subtypes were determined via the direct sequencing of the amplicons. All data analysis was performed using SPSS version 16.0 for the descriptive statistics, and then the chi-square test and Pearson coefficient were performed for additional analysis.

**Results:** The results of the analysis indicated that 54 (85%) of the 63 HIV-infected patients were males who were also HCV positive and who had less than a high school level education. There was a significant association between HCV infection and both occupation ( $P < 0.0001$ ) and level of education ( $P < 0.05$ ). All the HIV/HCV coinfecting cases had a history of illicit drug use, while 92.6% had a history of imprisonment and 40.7% had high risk sexual contacts. Overall, genotype 1 was found in 75.9% of HCV patients, while genotype 3 was found in 24.1%. Some 94.4% of HCV patients had subtype A. There were no clinical symptoms of chronic hepatitis C.

**Conclusions:** The majority of HIV-infected persons in the city of Kashan were also HCV positive. Genotype 1 was the predominant type, alongside subtype A. Considering the high prevalence of HCV among the HIV-infected persons, as well as the impact of occupation, education, illicit drug use, and imprisonment on the incidence of both infections, health policy makers must introduce health programs and plans to reduce the prevalence of these infections.

**Keywords:** HIV, Hepatitis C Virus, Genotype

## 1. Background

The human immunodeficiency virus (HIV) continues to be a major public health concern worldwide, having claimed more than 34 million lives so far. HIV targets the immune system and weakens people's defense systems against infections and some types of cancer. As the virus destroys and impairs the function of cells involved in the immune response, infected individuals gradually become immunodeficient. Immune function is typically measured using the CD4 cell count. Immunodeficiency results in increased susceptibility to a wide range of infections and diseases that people with healthy immune systems are able to fight off (1, 2).

Parenteral transmission is a common route of transmission for both HIV and hepatitis C virus (HCV); therefore, coinfection is common. An estimated 5 - 10 million individuals in the Western world are infected with both viruses. The majority of people acquire HCV via injection drug use and, to a lesser extent, through blood transfusion and blood products (3).

Among those persons with HIV who also have parenteral exposure, for example, injection drug users and recipients of blood products, the prevalence of chronic HCV infection is high, ranging from 60% to 95% worldwide. HIV coinfection adversely affects the natural history of HCV, since it enhances HCV replication and accelerates the progression of hepatic fibrosis, presumably due to HIV-related

immunosuppression. On the other hand, conflicting reports exist as to the impact of HCV infection on the natural history of HIV and, for most persons, HCV does not adversely affect the progression of HIV or its treatment with antiretroviral therapy (ART). In the era of effective HIV treatment, HCV infection has emerged as an important cause of morbidity and mortality in patients with HIV (4). Chronic HCV infection is now the leading cause of death, after AIDS-related complications, among HIV-infected individuals in areas where highly active antiretroviral therapy (HAART) is available. HIV coinfection exacerbates HCV, thereby increasing the likelihood of cirrhosis and HCV-related mortality (5-9).

There are reports showing the coinfection rate in countries such as Italy, Ukraine, Brazil, and the United States to be 49%, 70%, 54%, and 25% - 40%, respectively (10). The coinfection rate in Iran has been unofficially reported to be 8.7% to 94% (11-13).

The significance of these two infections lies in their common routes of transmission. As a result of infection with HIV and HCV, the liver is impaired and death may occur. The risk of mortality from liver disease is inversely associated with the CD4 cell count. An increasing rate of hepatocellular carcinoma and hepatotoxicity due to the use of antiretroviral medication has been reported. For this reason, it is necessary to examine the hepatic function of HIV patients using different methods, including biopsy (14-18).

The identification and determination of the HCV genotype is important when examining different aspects of hepatitis C, since there are associations between the genotype and the pathogenesis, response to antiviral drugs, and epidemiology of the disease. Presently, six major genotypes and more than 150 subtypes of the virus have been identified. The major genotypes have different geographic distributions; however, both genotype 1 and genotype 2 have a worldwide distribution (19). Several studies have been conducted to show the clinical and laboratory significance of the HCV genotype. These studies highlight the important role of identifying the virus genotype with regard to different aspects, including the epidemiology, pathogenesis, and response to treatment with antiviral medication of HCV, as well as evaluating the appropriateness and effectiveness of different methods for the diagnosis and screening of infected samples (20-22).

## 2. Objectives

There is currently insufficient knowledge available regarding the genotype of HCV in HIV-infected patients in the city of Kashan, Iran. Research in this area is necessary in order to reduce the social, economic, and psychological impact of the spread of HCV in the city. Therefore, this

study was designed to determine the epidemiological, clinical, and laboratory description, as well as to identify the HCV genotype, in HIV-infected patients.

## 3. Patients and Methods

### 3.1. Study Population and Setting

This cross-sectional study was conducted in 2014 in the city of Kashan, Iran. Kashan is a city in the Isfahan province with a population of approximately 500,000. The city has an independent university of medical sciences. According to Iran's health system policy, every medical university should have a referral behavioral counseling center (i.e., a triangular clinic) that is responsible for identifying, counseling, and treatment of HIV-infected patients.

Using a census strategy, all 63 HIV patients who were identified by the behavioral counseling center of Kashan University of Medical Sciences, who had open medical records, and who were visited by an infectious disease specialist once a month were included in our study.

The ethical approval code for this research is p/29/5/1/3294 (date: 2014/10/11).

All participants were provided with information about the objectives of the study, and written informed consent was obtained prior to the start of the study. The interviewer read out the informed consent form to those who could not read. After consent was obtained, 5 cc venous blood samples was collected and sent to the laboratory, where the serum was separated and the remaining part of the sample was tested with an anti-HCV ELISA. Serological assays, including for the hepatitis C antibody, were used to categorize the hepatitis status of the patients. The HCV antibody was tested using a third generation EIA (DIA.PRO Diagnostic, Bioprobes Srl, Milan, Italy). Flow cytometry was used to determine the CD4 cell count (Partec, GmbH Munster, Germany).

In addition, a structured questionnaire was employed to record participants' demographic information, high risk behaviors, clinical signs, and physical findings through both a direct interview and a physical examination. All the patients who were tested positive according to the ELISA method underwent additional testing for alanine aminotransferase (ALT), aspartate aminotransferase (AST), complete blood count (CBC), and CD4. Their genotype was determined using the real-time PCR method. There was one observer for all the tests.

### 3.2. Testing Procedures

The sample used for testing was 1.5 to 2 cc of plasma isolated from the blood samples drawn from the patients and placed in tubes for CBC testing with an anticoagulant.

The samples were separated from the blood using the centrifugation method. They were extracted from the blood according to the instructions provided with the kits purchased from Tekapost Co. Since the hepatitis C virus is an RNA virus, it is necessary to convert it into a DNA virus prior to testing. The kit necessary for performing this procedure was purchased from SinaClon BioScience Co. (Tehran, Iran). The temperature program and duration for the procedure, including the sequencing, were based on the instructions provided by the manufacturer: First stage, 33 cycles 30 seconds at 90 degrees centigrade, 40 seconds at 55 degrees, 30 seconds at 72 degrees, 20 minutes at 42 degrees, and 2 minutes at 93 degrees; second stage: 30 cycles 30 seconds at 72 degrees, 60 seconds at 93 degrees, 30 seconds at 93 degrees, and 35 seconds at 55 degrees.

### 3.3. Statistical Analysis

Using the statistical package SPSS version 16, all descriptive statistics, including the mean, standard deviation, and frequency, were calculated. The analytical statistics were determined using the chi-square test for the qualitative variables, while the Pearson coefficient was used to detect the association between the quantitative variables. The significance level was set to 0.05.

## 4. Results

The results of the analysis indicated that of the population of 63 HIV patients, 54 were also afflicted with hepatitis C; thus, the prevalence rate of the disease was 85%. The mean age of the HIV/HCV coinfecting patients was  $34.91 \pm 9.7$  years. All of the HIV/HCV coinfecting patients were male, and 46.3% were workers. There were significant differences among the job categories ( $P < 0.0001$ ). The results of the analysis also showed that 44.4% of the patients were single. No significant differences were found in terms of marital status ( $P > 0.05$ ). As 37% of patients had a 9<sup>th</sup> grade education level, there was a significant association between coinfection and education level ( $P < 0.05$ ). All the HIV patients were current drug addicts or had a history of drug addiction. The results also showed that 92.6% of the patients had a history of imprisonment ( $P < 0.05$ ). Some 40.7% of the HIV patients with HCV coinfection claimed they exhibited high risk sexual behavior; however, there was no significant association between this type of behavior and HIV infection ( $P > 0.05$ ). Table 1 presents the frequency distribution of the demographic variables according to the HCV results.

Some 75.9% of the HIV/HCV patients had genotype 1a, while 18.5% had 3a and 5.6% had 3b. Further analysis revealed that 94.4% of the HIV/HCV coinfecting patients had

**Table 1.** Frequency Distribution of Demographic Variables of the HIV Patients According to Their HCV Results<sup>a</sup>

| Variables                        | HCV+      | HCV-     | P Value      |
|----------------------------------|-----------|----------|--------------|
| <b>Sex</b>                       |           |          | $P < 0.0001$ |
| Male                             | 54 (100)  | 7 (77.8) |              |
| Female                           | 0 (0)     | 2 (22.2) |              |
| <b>Job</b>                       |           |          | $P < 0.0001$ |
| Unemployed                       | 9 (16.7)  | 2 (22.2) |              |
| Worker                           | 25 (46.3) | 3 (33.3) |              |
| Free job                         | 11 (20.4) | 2 (22.2) |              |
| Driver                           | 9 (16.7)  | 2 (22.2) |              |
| <b>Marital status</b>            |           |          | $P > 0.05$   |
| Single                           | 24 (44.4) | 1 (11.1) |              |
| Married                          | 16 (29.6) | 4 (44.4) |              |
| Widowed                          | 14 (25.9) | 4 (44.4) |              |
| <b>Education level</b>           |           |          | $P < 0.05$   |
| Illiterate                       | 6 (11.1)  | 2 (22.2) |              |
| Elementary                       | 19 (35.2) | 3 (33.3) |              |
| Guidance                         | 20 (37)   | 3 (33.3) |              |
| Diploma                          | 9 (16.7)  | 1 (11.1) |              |
| <b>Addiction history</b>         |           |          | $P < 0.05$   |
| Have                             | 54 (100)  | 9 (100)  |              |
| Have not                         | 0 (0)     | 0 (0)    |              |
| <b>Prison history</b>            |           |          | $P < 0.05$   |
| Have                             |           |          |              |
| Have not                         |           |          |              |
| <b>High risk conduct history</b> |           |          | $P > 0.05$   |
| Have                             | 22 (53.7) | 3 (33.3) |              |
| Have not                         | 20 (40.7) | 6 (66.7) |              |
| Unknown                          | 3 (5.6)   | -        |              |

<sup>a</sup>Values are expressed as No. (%).

type a. There was a significant difference between the proportions of the subtypes of the HIV patients coinfecting with hepatitis C. There were significant differences among the types of genotypes ( $P < 0.05$ ). In addition, there was a significant association between the CD4 and TLC ( $P < 0.0001$ ); however, no significant associations were found between the other variables ( $P > 0.05$ ) (Tables 2, 3).

## 5. Discussion

The results of this study showed that 54 (85%) of the 63 HIV patients were coinfecting with HCV. In the study

**Table 2.** Mean and Standard Deviations of the CD4, TLC, ALT, and AST of the HIV Patients According to Their Hepatitis C Status

| HCV                       | TLC      | CD4     | AST     | ALT    |
|---------------------------|----------|---------|---------|--------|
| <b>Positive</b>           |          |         |         |        |
| Frequency                 | 54       | 54      | 54      | 54     |
| Mean                      | 2397.22  | 398.80  | 58.50   | 50.67  |
| Standard deviation        | 1231.725 | 202.680 | 39.925  | 33.713 |
| <b>Negative</b>           |          |         |         |        |
| Frequency                 | 9        | 9       | 9       | 9      |
| Mean                      | 2882     | 480.33  | 101.76  | 46.78  |
| <b>Standard deviation</b> | 1005.130 | 167.522 | 123.208 | 15.920 |

**Table 3.** Correlation Coefficients of the CD4, TLC, ALT, and AST of HIV Patients According to Their Hepatitis C Status

| Correlation Coefficients | TLC    | CD4                | AST                | ALT                | Age    |
|--------------------------|--------|--------------------|--------------------|--------------------|--------|
| <b>TLC</b>               |        |                    |                    |                    |        |
| Pearson correlation      | 1      | 0.999 <sup>a</sup> | -0.071             | -0.084             | -0.047 |
| Sig. (two-tailed)        |        | 0.000              | 0.579              | 0.513              | 0.716  |
| <b>CD4</b>               |        |                    |                    |                    |        |
| Pearson correlation      | 0.999a | 1                  | -0.070             | -0.084             | -0.050 |
| Sig. (two-tailed)        | 0.000  |                    | 0.584              | 0.511              | 0.699  |
| <b>AST</b>               |        |                    |                    |                    |        |
| Pearson correlation      | -0.071 | -0.070             | 1                  | 0.517 <sup>a</sup> | 0.073  |
| Sig. (two-tailed)        | 0.579  | 0.584              |                    | 0.000              | 0.569  |
| <b>ALT</b>               |        |                    |                    |                    |        |
| Pearson correlation      | -0.084 | -0.084             | 0.517 <sup>a</sup> | 1                  | 0.056  |
| Sig. (two-tailed)        | 0.513  | 0.511              | 0.000              |                    | 0.663  |
| <b>Age</b>               |        |                    |                    |                    |        |
| Pearson correlation      | -0.047 | -0.050             | 0.073              | 0.056              | 1      |
| Sig. (two-tailed)        | 0.716  | 0.699              | 0.569              | 0.663              |        |

<sup>a</sup>Correlation is significant at the 0.01 level (two-tailed).

conducted by Moradmam Badie involving 365 infected patients from the voluntary counseling and testing center of Imam Khomeini Hospital in Tehran, 129 (35.3%) patients were coinfecting with HIV/HCV (23). In the study by Grzeszczuk of adult patients infect with HIV-1 in a Polish HIV/AIDS reference center, of the 457 patients, anti-HCV antibodies were detected in 325 (71.1%) individuals (7).

Kerubo conducted a study in Nairobi, Kenya, and found that of the 268 (20.4%) HIV-1 positive participants, 56 (4.26%) had HBV, while 6 (0.46%) had HCV. The odds of getting hepatitis infection were higher in the HIV-1 participants (24). In another study from Nairobi, of 300 HIV-1 infected individuals, some 15.3% (46/300) were co-infected

with either HBV, HCV, or both, while 10.3% (31/300) were coinfecting with HIV-1 and HCV and 6% (18/300) were coinfecting with HIV-1 and HBV. However, only three individuals (1%) were coinfecting with all three viruses (HIV/HBV/HCV) (25).

The prevalence of HCV coinfection differs depending on the route of HIV transmission (6). There are common routes of transmission for HIV and HCV; therefore, coinfection with both viruses frequently occurs. The most efficient means of HCV transmission is percutaneous exposure to blood, with the transmission efficiency being ten times higher for HCV than for HIV. The principal route of HCV transmission is injection drug use (IDU), and the rate

of HCV coinfection is often higher than 90% among HIV-infected persons with a history of injection drug use (26, 27).

In our study, the mean age of the HIV/HCV coinfecting patients was  $34.91 \pm 9.7$  years, and all the HIV/HCV coinfecting patients were male. In the study by Moradmand Badie, 188 of the infected patients (51.5%) were aged between 21- and 40-years-old (23). In the study by Grzeszczuk, the median age of the cases was 38 years (range 23 - 72 years), and the majority (76.6%) were male. In the Middle East, the HIV infection rates are much higher for men than for women, and most of the reported AIDS cases occur in persons aged 25 to 44 years (7). Hence, the results of this study are in agreement with reported statistics.

However, the findings of the present research showed that there was an association between addiction and infection with HIV/HCV. It should be mentioned that a high percentage of the population of this study were drug addicts. Some other studies have shown that most patients infected with HIV/HCV acquired the HIV infection through sexual contact or parenteral transmission, whereas drug addicts made up only a small proportion of their study population (28, 29). This discrepancy may be due to the fact that in most countries sexual contact without protection results in HIV infection, whereas in Iran injection drug use is the most common route of HIV transmission. Thus, in most HIV/HCV coinfecting patients, the parenteral route is the most common route of transmission for both HIV and HCV (30, 31).

The results of our analysis indicated that 90.5% of the patients coinfecting with HIV/HCV had a history of imprisonment. Considering this result and the results of other investigations, it seems that the likelihood of infection with hepatitis B and C and HIV increases in prison. The main cause of this is the crowding of individuals in a closed environment who live together and frequently commit high risk behavior. It seems that the majority of drug addicts in prison inject drugs. It is likely that imprisonment (with a longer duration and a higher number of prisoners) increases the chance of high risk behavior and eventually results in infection (32, 33).

In this study, 75.9% of the coinfecting HIV/HCV patients had genotype 1a, while 18.5% had 3a and 5.6% had 3b. Further analysis revealed that 94.4% of the HIV/HCV coinfecting cases had subtype a, while 5.6% of them had subtype b. There was a significant difference between the proportion of the subtypes of the HCV and HIV patients afflicted with hepatitis C. There were also significant differences among the genotypes ( $P < 0.05$ ).

According to the results of a meta-analysis, the most common subtypes of HCV in Iran were 1a, 3a, and 1b. A literature review of papers reporting the HCV genotypes in Ira-

nian patients conducted by Khodabandehloo et al. showed that of 22,952 HCV-infected cases, subtype 1a was the most common with a rate of 39% (95% CI: 34% - 44%), followed by subtype 3a with a rate of 32% (95% CI: 26% - 39%), subtype 1b with a rate of 13% (95% CI: 10% - 15%), genotype 4 with a rate of 5.18% (95% CI: 3.27% - 7.5%), and genotype 2 with a rate of 3.6% (95% CI: 1.6% - 8.3%) (34). Hadinedoushan reported that the HCV genotype 3 was the predominant genotype (50.3%) in the Yazd province of Iran, followed by subtypes 1a (38.7%) and 1b (6.8%) (35).

In a study conducted by Wahdat and associates in Bushehr, a province in the south of Iran, it was found that 36.7% of hepatitis patients had subtype 1a, while 38.3% had subtype 3a and the rest had a non-typing genotype. The highest risk factor in patients was drug addiction, followed by blood transfusion and dental treatment. Genotype 3a was clearly associated with drug injection, while genotype 1a was correlated with dental treatment ( $P < 0.05$ ) (33).

In addition, a study conducted by Samimi-Rad et al. in Tehran and five other towns in Iran examining anti-HCV positive cases reported that genotype 1a was the dominant genotype (47%). The prevalence of genotypes 3a, 1b, and 4 was 36%, 8%, and 7%, respectively (36). In summary, researchers in Iran have concluded that the HCV genotypes within the country are as follows:  $1a < 3a < 1b < 2 < 4$  (36).

In another study conducted to determine the genotype of the hepatitis C virus in anti-HCV positive cases in Golestan, a province in the north of Iran, the following genotypes were recorded: 19.5% were 1a, 19.5% were 1b, 15.6% were 3a, 24.7% were 3b, 2.6% were 2a, 7.8% were 4, and the rest (6.58%) were a combination of 1 and 3 (33). The results of the present study are in agreement with these results (37).

Ahmadipour et al. stated that the HCV types and subtypes exhibit complex patterns of geographic distribution, relative prevalence and modes of transmission. The epidemic group including subtypes 1a, 1b, 2a, 2b and 3a are distributed globally and account for the majority of HCV infections worldwide. The rapid spread and worldwide distribution of these subtypes resulting from their efficient transmission via percutaneous blood exposure such as injecting drug use. Subtypes 1b and 2a are more strongly associated with transmission by the infected blood products and the relative prevalence of these subtypes has decreased in recent years due to improved blood screening. Subtypes 1a and 3a most often infect IDUs and appear to be increasing in prevalence (38).

In the present study, the mean value of the CD4 cells was  $410 \pm 199$  (cells per microliter), which was close to the illustration reported by Mohammadnejad and associates (39).

In addition, Korner and associates examined the asso-

ciation between HCV and apoptosis in the CD4 cells of AIDS patients. They concluded that HCV alone may not lead to an increase in apoptosis in CD4 cells, although the presence of AIDS can severely reduce the number of CD4 cells. There was a close association between the number of CD4 cells and the HIV viral load, whereas there was less of an association between the HCV viral load and the number of CD4 cells (40).

In a study by Ajayi et al. involving 273 HIV seropositive patients, two (0.7%) patients tested positive for serum anti-HCV antibodies. The CD4+ T lymphocytes cell count ranged between 5 and 1050 cells/ $\mu$ L, with a mean of  $286.19 \pm 233.31$  cells/ $\mu$ L. The majority of patients (71.8%) had a CD4+ T lymphocytes cell count  $< 350$  cells/ $\mu$ L (41). In the study by Tremeau-Bravard et al., of the 443 HIV/AIDS positive individuals, ten patients were coinfecting with the hepatitis C virus (2.3%). Remarkably, an overall lower CD4 count was seen in the coinfecting population (205 cells/ $\mu$ L versus 243 cells/ $\mu$ L), with the lowest count seen for the triply infected individuals (97 cells/ $\mu$ L) (42).

In the present study, the level of ALT in patients infected with HCV was  $50.67 \pm 33.713$  u/L, while in the patients without HCV, this value was  $47.78 \pm 15.92$  u/L. In addition, the level of AST in patients with HIV and HCV was  $58.50 \pm 39.92$ , whereas this level for the patients without HCV was  $101.67 \pm 123.208$ . Kyrlagkitsis and associates examined 91 patients infected with chronic HCV and compared the individuals with a high level of ALT and those who had a normal level of ALT. They concluded that individuals who have a normal level of ALT have less fibrosis than those with a high level of ALT; however, none of studied individuals had normal histology. In this study, 15 patients had a normal level of ALT, inflammation, or noticeable fibrosis (43). Therefore, it is not useful to make the decision to treat a patient based on the high level of ALT, since this variable is not a good predictor of fibrosis, response to treatment, or complications associated with advanced liver disease.

### 5.1. Conclusion

Considering the high prevalence of hepatitis C infection in patients coinfecting with the HIV virus, as well as the effects of variables such as unemployment, history of imprisonment, injection drug use, and a low level of education on the occurrence of this disease, it is necessary to establish programs aimed at intervening to prevent and treat the disease, in addition to increasing the awareness and knowledge of drug addicts.

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### Footnotes

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