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Distribution of Hepatitis C Virus Genotypes Among Chronic Infected Injecting Drug Users in Tehran, Iran

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ABSTRACT

Background: Hepatitis C virus (HCV) is the main cause of infection that has the potential to cause chronic liver disease. Injecting drug users (IDUs) have a key role in HCV transmission in Iran. Knowledge of the distribution of various genotypes is essential for successful future research and control strategies.
Objectives: The aim of this study was to identify HCV genotypes among chronic infected injecting drug users (IDUs) in Tehran, Iran.
Patients and Methods: In this cross sectional study, we investigated HCV genotypes among 36 plasma samples from HCV infected IDUs (35 male and 1female, mean age: 33.67, and age range 20-62 years), referred to Research Center of Iranian Blood Transfusion Organization(IBTO) in Tehran from December 2008 to March 2009.HCV Genotyping was performed using type-specific primers.
Results: Genotypes 3a, 1a and 1b were found in 58.3 %, 25% and 16.7 % patients, respectively.
Conclusions: Our study demonstrated the high prevalence of genotype 3a among injecting drug users, which is also found in Europe and United states.

Keywords: Hepatitis C virus; HCV Genotypes; Injecting Drug Users; Iran

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Implication for health policy/practice/research/medical education:
High prevalence of genotype 3a among injecting drug users (IDUs) due to the possibility of the changing of dominant circulating viruses in our community.

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1. Background

Hepatitis C virus (HCV) infection is a worldwide problem in health. HCV is a major cause of chronic liver disease, hepatocellular carcinoma, and the single most common indication for liver transplantation. Approximately 2-3 percent of the world's population is infected with HCV (1, 2).The most recent WHO estimate of the prevalence of HCV infection is 2% (ranging from 0.6% to 2.3%) in North America, Northern and Western Europe, and Australia. The prevalence of infection in healthy blood donors ranges from 0.01-0.02% in Northern Europe, and 1-1.5% in Southern Europe, to 6.5% in parts of equatorial Africa (3).

The prevalence of HCV infection is about 0.12% in blood donors in Iran (4). It seems that the prevalence of HCV infection is less than 1% in our general population (5), but the infection is emerging due to injecting drug users (IDUs) and needle sharing among addicts. HCV is a blood-borne pathogen and can be transmitted through blood products and infected syringes, and infection rates are typically high among IDUs. Nowadays, injecting drug using is the major risk factor for HCV infection (6).The incidence of HCV infected IDUs varies from 31% to 89% in different parts of the word (7, 8). A study reported that the rate of HCV antibody positivity among IDUs is 52% in Tehran and it is still expanding (9).

A recent international standardization of HCV nomenclature proposed a classification into 6 genotypes (1 to 6) and more than 70 subtypes. The different genotypes display up to 70% sequence similarity, whereas subtypes vary by more than 20 % (10). Genotyping is useful tool for investigating outbreaks and for understanding the epidemiology of the infection. Clinically, genotyping of HCV is important for predicting treatment responses and for determining the duration of antiviral therapy. Response to interferon (IFN)-based therapies in patients infected with HCV genotype 1 and 4 is much lower than in genotypes 2 and 3 (11).Infection with genotype 1 may proceed rapidly to severe form of chronic hepatocellular, cirrhosis, and hepato cellular carcinoma, when compared with genotypes 2 and 3 (12). Although genotypes 1, 2 and 3 are responsible for more than 90% of the infection in North and South America and Japan, prevalence and distribution of HCV genotypes are linked to geographical region and the route of viral transmission (12-14).

In a study, all isolated from Iranian patients have been classified to five groups: 1a (52.88%), 1b (14.01%), 3a (27.57%) , 2a (2.1%), and 4 (3.44%) (15). A similar study on Iranian patients revealed that 1a, 1b and 3a were predominant genotypes with an overall rate of 61.2%, 13.8%, and 25%, respectively (16). Sharifi *et al.* showed that the prevalence of HCV genotypes in 103 blood donors and 64 patients are as following: The highest frequency was for genotype 1a, with 53 and 34 (51.5% versus 53.1%) of subjects in blood donors and patients respectively (17). Genotype 3a and 1b were the other frequent genotypes with 4 and 16 (3.9% versus 25%) and 39 and 10 (37.9% versus 15.6%) subjects, respectively. Molecular epidemiology studies have shown that genotype 3a is significantly associated with transmission through injecting drug use in industrial countries (18). Although there are many reports describing the distribution of HCV genotype in different parts of Iran, but it seems that there are few published information about the distribution of HCV genotype in different groups of Iranian HCV infected patients such as IDUs within the different reports. For example, Samimi Rad *et al.* in a phylogenetic analysis of NS5B region testing showed younger IDUs had more frequently subtype 3a (54%) (19). Kabir *et al.* tested HCV genotyping in IDUs, and showed that genotypes 1a with 18 (40%) and 3a with 17 (37%) are the most frequent in this group of Iranian patients (20).

2.Objectives

Due to the possibility of the changing of dominant circulating viruses in community, it is important to determine HCV genotype in different geographical regions and routes of transmission, for both epidemiological purposes and patients' management. In this study we investigated HCV genotypes among HCV-infected IDUs, referred to Research Center of Iranian Blood Transfusion Organization (IBTO), Tehran, Iran.

3. Patients and Methods

This is a cross-sectional study of 36 HCV infected IUDs (35 male and 1 female, mean age 33.67), referred to IBTO, Tehran from December 2008 to March 2009. HCV infection in patients had been confirmed by positive results in HCV-Ab and HCV-RNA tests. Blood samples were centrifuged and plasma samples were aliquoted and stored at -70 °C before testing. All samples were tested for HCV genotyping by primer specific method as explained below.

3.1. RNA Extraction

HCV-RNA was isolated from 250 μl of plasma using TriPure Isolation Reagent (Roche Applied Science, Mannheim, Germany) accoriding to manufacturer's instruction. The RNA was eluted in 20 μl elution buffer and then 5 μl were used for cDNA synthesis.

3.2. HCV Genotyping

Genotypes were determined by performing PCR, using specific primers for the target core region of the HCV genome, with two separate reaction tubes containing different primer mixes. This method helps for the determination of genotypes 1a, 1b, 2a, 2b, 3a, 3b, 4, 5a, and 6a in separate reaction tubes (21). For the reverse transcription -PCR, 1 μg of the extracted nucleic acid, 1.5 mM $MgCl_2$, 1X PCR buffer containing 10 mM Tris-HCl,50 mM KCl (pH 8.3) 10mM DTT, 10 nmol of each dNTP, and 25 μM of outer primers in a total volume of 10 μl were used for the reac-

tion. The reaction mixture was incubated at 95°C for 5 min before the addition of 20 U Rib nuclease inhibitor (Roche Molecular Biochemical) and 20 U of reverse transcriptase from avian myeloblastosis virus (Roche Molecular Biochemical Company, Mannheim, Germany). After 60 min at 42°C, the reaction was heated for 5 min at 95°C. Briefly, 2 μl of the cDNA was amplified in a 50 μl reaction volume containing 1.5 mM MgCl2, 10 mMTris–HCl, 50 mM KCl, and 2.5 μM of both sense and antisense outer primers.

The first round of amplification was performed under the following conditions: twenty cycles of amplification at 94°C for 1 min, 45°C for 1 min, and 72°C for 1 min; followed by an additional 20 cycles of 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min. 1 μL of first round product was taken as input for the second round PCR. The products of the second round PCR were run into a gel electrophoresis with a 2% agarose gel. Samples were assigned to their genotypes based on the band size of the final amplified product as recommended (21). The plasma with different genotypes of HCV (BIO Quality control reagent Rijswijk, The Netherlands) was used as positive control for detection of different genotypes of HCV.

3.3. Statistical Analysis

Descriptive statistics including mean age and genotype frequency distributions were computed. All analysis were done using SPSS version 16.0.

4. Results

A total of 36 patients were studied, 97.2% of them were male and 2.8% of them were female, and aged between 20 and 62 years (mean age 33.67). In this present study, only 3 subtypes were detected. There was not any patient with mixed infection or undetected genotype. 21 (58.3%) HCV infected IUDs were infected with genotype 3a, therefore 3a was predominant, followed by genotype 1a with 9 (25%), and genotype 1b with 6 (16.7%).

5. Discussion

Epidemiological studies in different region of the world show the wide variation in HCV prevalence patterns (2). IDUs have now become the predominant source of HCV infection in developed and developing countries (18). Therefore for controling HCV infection in general populations, we need to manage and control the infection in these groups of patients.

In this present study we showed that genotype 3a (58.3%) was the predominant genotype followed by 1a (25%) and 1b (16.7) among IDUs. Our results were similar to the results of another report from Iran that revealed the predominant HCV genotype in Iranian IDUs to be 3a(54%) (19). similar Another study in Iran showed that HCV genotypes 1a (40%) and 3a (37%) were the most frequent in Iranian IDUs (20). A recent similar study in Lebanon showed that HCV genotype 3 was predominant (57.1%), followed by genotype 1 (21.4%), and genotype 4 (17.9%) in Lebanese IDUs (22).

Genotype 3a is significantly associated with transmission through injection of drug in industrialized countries, and this explains the prevalence of 3a in many North and South American and European countries , where injection of drug use is common (23). The molecular epidemiology of HCV-3a in IDUs in these countries suggested that HCV-3a has been spread from a common origin through a unique worldwide epidemic, which rapidly spread among the drug user communities transmitting HCV-3a throughout the world (24).

Our results showed that it seems that there is high similarity between the pattern of HCV genotype in IDUs in Iran and Europe as it had showed in the report from Lebanon (22). Lebanese authors suggested that HCV genotype 3a could have been introduced to Lebanese IDUs population from Europe, whereas some of American and European authors reported that the HCV genotype 3 originated from Asia, and then it has widely spread among IDUs (24). The data that indicate the predominant HCV genotype among IDUs in neighboring Middle –East countries is still limited and controversial (19, 20, 22).

The geographical origin of HCV-3a is not clear, because sera archived in the last decades are not available. Also worldwide prevalence of HCV-3a and mechanisms of its transmission are not accurately defined, therefore, epidemiologic studies are needed for better understanding of this issue. The main route of HCV transmission is through IDUs, who are very spread in our population. It is very likely to change the frequency of specific genotype in HCV- infected patients from 1a to 3a, as another study suggested (19), but further studies on large number of IDUs are necessarily needed.

In conclusion, our study showed that in chronic HCV infected IUDs in Tehran,the subtype 3a is the most frequent .This information could have epidemiological significance and would be added to Middle-East regional network. Considering that HCV anti-viral therapy varies significantly with the genotype, this result may affect disease treatment and control.

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Authors' Contribution

None declared.

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