Short Communication

Frequency of hepatitis C virus genotypes in infected patients in Charmahal-O-Bakhtiari Province, Iran

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In Iran, limited information exists on the epidemiology of hepatitis C virus (HCV) genotypes in different districts of the country. In Charmahal-O-Bakhtiari Province, there was no datum on the mentioned issue. In this study, serum samples were collected from 80 clinical patients (suspected to have HCV infection between 2009 and 2010) and tested in a laboratory in Hajar Hospital of Shahre-Kord, Charmahal-O-Bakhtiari Province, Iran; they were analyzed for HCV ribonucleic acid (RNA). The most frequently found genotype was genotype 3a, present in 11 to 17 (64.7%) patients, followed by genotype 1a. In conclusion, the most frequent HCV genotype in Shahre-Kord was 3a, which was different from that of Iran's general population, an indication that the epidemiology of HCV infection in the region is drug abuse predominated. More studies are needed to confirm this conclusion.

Key words: Hepatitis C virus genotyping, Iran, epidemiology.

INTRODUCTION

Hepatitis C virus (HCV) is the major causative agent of non-A, non-B viral hepatitis. Acute HCV infection is often asymptomatic or has minimal symptoms; however majority of these cases have progress to chronic hepatitis. Chronic hepatitis due to HCV infection can lead to disastrous outcomes like cirrhosis, liver failure, and hepatocellular carcinoma within 20 to 30 years. Several factors that plays role in a progressive liver disease in HCV infected patients includes: viral genotype, the patient's alcohol consumption and viral load. In the recent era, efforts are to be made to establish therapeutic strategies based on specific HCV genomes. For this reason, in addition to quantitative polymerase chain reaction (PCR), genotyping has become increasingly important in routine laboratory diagnostics. The most effective way to obtain data on these two parameters

would be to use the same amplicons for diagnostic HCV RNA detection and for genotyping (Davis et al., 1997). Since different HCV genotypes have been associated with several individual transmission predictors, disease features and response to therapies, determining HCV genotypes provides clinically important information that can be use to direct the type and duration of antiviral therapy and to predict the likelihood of sustain HCV clearance after therapy as well as installation of preventive strategies.

More than 60 genotypes and subtypes of HCV are distributed worldwide, and their therapeutic response rates are different. Therefore, accurate and efficient genotype determination is critical both in therapeutic and epidemiologic settings (Ross et al., 2000). It was reported that patients with HCV genotype 1 are more likely to need a longer course of therapy, but the combination of genotypes 2 and 3 benefit interferon-ribavirin therapy, efforts had been focused to distinct genotype 1, 2 and 3 (Ross et al., 2000) and other HCV genotypes that are less commonly seen in the general population. The US

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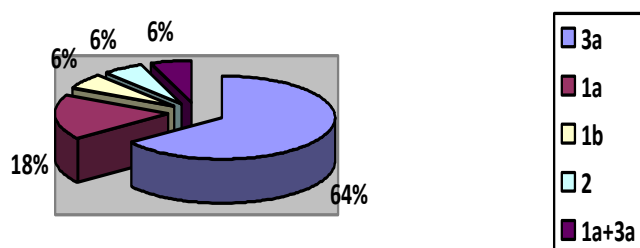
Figure 1. Distrubution of different HCV subtypes in Shahre-Kod, Iran.

had received quite lesser attention and insufficient existing data, regarding the therapeutic response of HCV genotypes 4, 5, 6 and 7. In Iran, limited data exist on the epidemiology of HCV genotypes in different districts of the countries but in Charmahal-O-Bakhtiari Province of our country there is no data on the mentioned issue. Regarding lack of epidemiologic data on HCV genotyping in our region, the aim of this work was to determine HCV genotypes in patients from the major Hospital of Shahre-Kord, Center of Charmahal-O-Bakhtiari Province of Iran.

MATERIALS AND METHODS

The aim of this study was to determine HCV genotypes in a group of patients from Shahre-Kord, Iran. In this cross-sectional study, a total of 80 serum samples were collected from patients suspected with Hepatitis C infection that were referred to Hajar Hospital of Shahre-Kord, and were analyzed for HCV RNA. The sera were collected in tubes, immediately stored at -80℃ and subsequently analyzed for HCV RNA. Afterwards, HCV RNA genotyping were performed.

HCV RNA detection

The serum samples were tested for HCV genome with general primers, using the conventional RT-PCR commercial kit (Qiagen, Germany) which is able to detect all different HCV genotypes. Tests were performed according to the manufacturer's recommendations and are briefly given as follows: 50 µl plasma was added to 5.6 µl RNA carrier and 560 µl of lysis buffer, incubated at room temperature for 10 min. Then 560 µl of ethanol (96 to 100%) was added. 630 µl of the solution was transferred to the Minispin column, centrifuged at 6000 g for 1 min and then the spin column was placed into a clean 2 ml collection tube, the tube containing the filtrate being discarded. Subsequently 500 µl of washing buffer was added and centrifuged at 6000 g for 1 min; again the tube containing the filtrate was discarded. 500 µl of elution buffer was added and centrifuged at full speed for 3 min.

Finally, 60 µl of dissolving buffer was added to the last spin column to collect RNA. Small aliquots of isolated RNA were tested to assess the quality and quantity of RNA; using a spectrophotometer (Eppendorf, Germany). 2 µg of RNA was reverse-transcribed into complementary deoxyribonucleic acid (cDNA) using a master mix of 5XRT mix, M-MLV reverse-transcribe enzyme, RNAse inhibitor, dNTPs and ddH$_2$O, and placed on a thermal cycler (Eppendorf, Germany) at 37℃ for 30 min . 5 µl of cDNA was subjected to PCR amplification using general primers included in the kit, which is able to amplify all different genotypes of

HCV. The PCR program is as follows: initial denaturation at 95℃ for 5 min, 45 cycles at 95℃ for 30 s, 57℃ for 15 s, 72℃ for 30 s and a final extension at 72℃ for 5 min. PCR products were subjected to electrophoreses and separation on 2% agarose gel, being visualized under UV light after ethidium bromide staining.

Statistical analyses

Software SPSS 17.0 (SPSS Corp.; IL; Chicago; USA) was used for data analyses. Non parametric methods were used. A P value of <0.05 was considered significant.

RESULTS AND DISCUSSION

HCV PCR products were found for 17 of the 80 (21.25%) patients studied. The mean age of all the patients studied was 33.4 ± 14 and that of the HCV infected was 31.13 ± 15 years. 15 (88.2%) were males and 2 (11.8%) were females. There was no significant relationship between HCV positive and the sex and age of the patients studied (P > 0.1 for all) Figure 1. The most frequently found genotype was, genotype 3a present in 11 of 17 (64.7%). 3 of 17 (17.6%) were corresponded to genotypes 1a. 1 of 17 (5.8%) were corresponded to genotypes 1b and 2. 1 of 17 (5.8%) was noticed to be infected with mixed genotypes of 1a+3a (Figure 1). HCV undergoes large genetic variability and has major genetic groups called genotypes that are categorized from 1 to 6, as well as more than 50 subtypes. The knowledge on genotypes of HCV infection in different geographical areas is of high relevance due to its role in epidemiological studies and outbreak investigation.

Moreover, due to differences in the treatment protocols suggested for different HCV subtypes, genotype distribution has a significant influence on the total costs of HCV disease. On the other hand, there is an astonishing similarity in the epidemiology of different HCV subtypes related to various transmission routs. For example, Genotypes 3 and 1a are more prevalent in IV drug abusers both in Iran and Western countries (Mcomish et al., 1993; Zarkesh-Esfahani et al., 2010).

This fact evidently shows us, the importance of epidemiology in HCV genotypes, its highlights and findings of this present study. There is a wide variation in HCV prevalence pattern in different regions of the world with higher incidence in developing countries. The most common genotype in Iran's neighboring countries; including Kuwait, Iraq, and Saudi Arabia is type 4 (Simmonds et al., 1993). Genotypes 1b and 3a are the most prevalent genotypes in Turkey and Pakistan, respectively (Abacioglu et al., 1995; Shah et al., 1997).

The prevalence of HCV in the general population of Iran is less than one percent (Alavian, 2006). Based on this findings (a study on HCV RNA positive patients from some regions of Iran), genotypes 1a, 3a, and 1b were the predominant genotypes with an overall prevalence rate of 61.2, 25 and 13.8%, respectively (Amini et al., 2009). In a

study, in Fars province (Iran), during 2004 to 2005, the most common genotypes were 1a, 42.0%; 3a, 44.1%; and 1b, 13.8% with an overall prevalence rate of 61.2, 25 and 13.8%, respectively (Davarpanah et al., 2009). Genotypes 1a (53.3%) and 3a (37.7%) were reported from a study in a southern city of Iran, Ahwaz (Farshadpour et al., 2010). Genotypes 1 and 3 were the most common isolates from Azarbayjan province (Mohammad et al., 2008), and in Isfahan, genotype 3a was the most prevalent subtype (Zarkesh-Esfahani et al., 2010). In the present study, the most prevalent genotype in Charmahal-O-Bkhtiari Province and its center, Shahre-Kord, was HCV 3a (65%) subtype with almost 2/3th of all isolated from patients of this region, followed by genotypes 1a, 1b, and 2. Mixed genotypes of 1a and 3a were found in one of the patients studied. The genotypes found in this study were nearly the same as those found in our nearby province, Isfahan, as 3a (61.2%), 1a (29.5%), 1b (5.1%), 2 (2%) and mixed genotypes of 1a and 3a (2%) were reported from this province (Zarkesh-Esfahani et al., 2010).

The same kit was used in these two studies although the kit used was able to detect only genotypes 1a, 1b, 2 and 3a, all the samples were identified suggesting that the common genotypes are those reported here. However, there is a possibility that the kit is not designed to detect all of the HCV genotypes. Genotypes 1a and 3a are the most prevalent genotypes in the most regions of Iran (Alavian, 2006; Zali et al., 2000). Despite the diverse distribution and predominant pattern of HCV genotypes in Isfahan and Shahre-Kord than in other regions of Iran, we think that acquisition methods in our area is nearly the same as in other regions of our country. The minor difference showed could be, either due to use of different detection procedure or different rate of communication of these cities with nearby countries in this region of the world. In conclusion, this study shows that the most frequent genotype in Shahre-Kord is 3a, followed by 1a. Our HCV genotype distribution is different from that of other Iranian regions, but similar to Isfahan Province, a neighboring Province of Iran.

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