Original article

Prevalence of hepatitis C virus genotypes in chronic infected patients, southern Iran

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Abstract

Introduction and objective: Chronic infection with hepatitis C virus (HCV) is a major cause of cirrhosis and hepato-cellular carcinoma. Since response to anti-viral therapy in sufferers depends on HCV genotypes, determination of such genotype is of great significance to the treatment. This study seeks to estimate the HCV genotype prevalence in Shiraz, southern Iran and help specify the treatment course.

Materials and methods: A RT-PCR available kit with genotypes specific primer sets for major HCV genotypes (1a, 1b, 2 and 3a) was used. These primers amplified different parts of 5' un-translating region-core region of HCV genome. Genotyping test was performed for 634 patients with positive qualitative RT-PCR results.

Results: Of the 634 studied participants, 550(86.8%) were male and 84(13.2%) were female. Two hundred fifty nine (40.9%) of them were infected with 3a, 137(26.2%) with 1a, 55(8.7%) with 1b and 15(2.4%) with genotype 2 of hepatitis C. Mixed infection was found in 12 patients [1a+3a in 5(0.8%), 1a+1b in 4(0.6%) and 1a+2 in 3(0.5%)]. The extracted nucleic acid from 156(24.6%) samples did not react to the primer sets. This might be due to the presence of a genotype other than the above, or no sufficient copy of the virus.

Conclusion: The results revealed the highest level of infection belonging to 3a followed by 1a. Since a considerable proportion of chronic hepatic C infected patients were intravenous drug abusers in the region; genotype 3a appears to be more prevalent among this group.

Significance and impact of the study: The present study determined some critical information about the distribution of HCV genotypes in southern Iran.

Keywords: Hepatitis C virus (HCV); Genotyping; RT-PCR; Prevalence

Introduction

Hepatitis C virus (HCV) is an enveloped positive single stranded RNA virus which is the major cause of chronic hepatitis worldwide. More than 200 million people are infected with HCV; following the primary infection, 60-88% of the cases become chronic [1]. The virus is among the important causes of hepatocellular carcinoma and cirrhosis. HCV has six genetic groups, so-called genotypes and a number of subtypes. The six known genotypes differ by more than 30% of the nucleotide (nt) sequence and have unequal geographic distributions [2].

Identification of the causative virus genotype is of significance to both clinical practices and epidemiological studies. Regarding the former, once the genotype is identified, the prognosis of anti-virus treatment and determination of its duration are facilitated. Current treatment courses for the cases infected with the genotypes 1, 4 and those with 2, 3 are one year and six months, respectively [3-5]. In general, HCV type two and three isolates have higher rates of response to therapy than type one isolates [1,3,4].

Epidemiologically, the prevalence rates of different virus genotypes vary from region to region and there are different risk groups exposed to different genotypes of the virus [1]. The present study is an attempt to determine the prevalence rates of HCV genotypes among the chronic HCV infected patients in southern Iran, where no sufficient data are available.

Materials and methods

A total of 634 HCV- RNA positive patients with chronic hepatitis were enrolled in this study. Genotype identification was performed for the patients referred to Professor Alborzi Clinical Microbiology Research Center at Namazi hospital, a major tertiary teaching hospital with 1700 beds and affiliated with Shiraz University of Medical Sciences, Shiraz, southern Iran. The risk factors in the patients were divided sequentially as follows: drug injection abusers with or without prison records, thalassemia sufferers, hemodialysis patients, those with unsafe sexual contacts, hemophiliac patients and finally those with unknown risk factors. All the enrolled patients were informed of the procedures and their written consents were obtained and approved by the ethics committee of Shiraz University of Medical Sciences.

The diagnosis of chronic HCV infection was confirmed by the presence of anti-HCV antibodies and HCV-RNA in the collected sera. Anti-HCV detection was carried out by a third generation enzyme immunoassay according to the manufacturer's instructions (DIA. PRO. Diagnostic Bioprobes Srl, Italy) and for HCV RNA, by a commercial available RT-nPCR kit with primers directed to the conserved region of 5´ UTR of the viral genome, according to the manufacturer's directions (Cinnagen, Iran).

RNA extraction

RNAs were recovered from 100µl sera or plasma by a modification of the guanidinium thiocyanate-phenol extraction, iso-propanol precipitation method reported previously [6]. The extracted RNAs were pelleted and dissolved in 20µl RNAse DNAse free water. The extracted RNA was stored at -70°C until the PCR reaction was carried out.

Genotyping

HCV genotypes were determined by a commercially available kit (Sacace Biotechnologies Srl, Caserta, Italy). The test was carried out with regard to the manufacture's instructions. Briefly, the assay procedure is a tree-stage test; in the first step the extracted RNA was converted to cDNA, in the second step the cDNA was

amplified by genotype specific primers in two separated ready- to- use single dose test tubes, each containing specific primers for the genotypes 1a/1b and genotypes 2/3a, respectively. Finally, the analysis of results was done on the basis of the presence or absence of specific bands of amplified DNA in the 2.5% agarose gel. Positive and negative controls were included in every PCR experiment (Fig. 1).

Statistical analysis

Statistical analysis was performed using the SPSS version 15.0 software package (Chicago, IL, USA). Results are expressed in means ± SD or percentages. The Chi-squared test and Student's t-test were used for data analysis. The significance level was set at a p-value of ≤ 0.05.

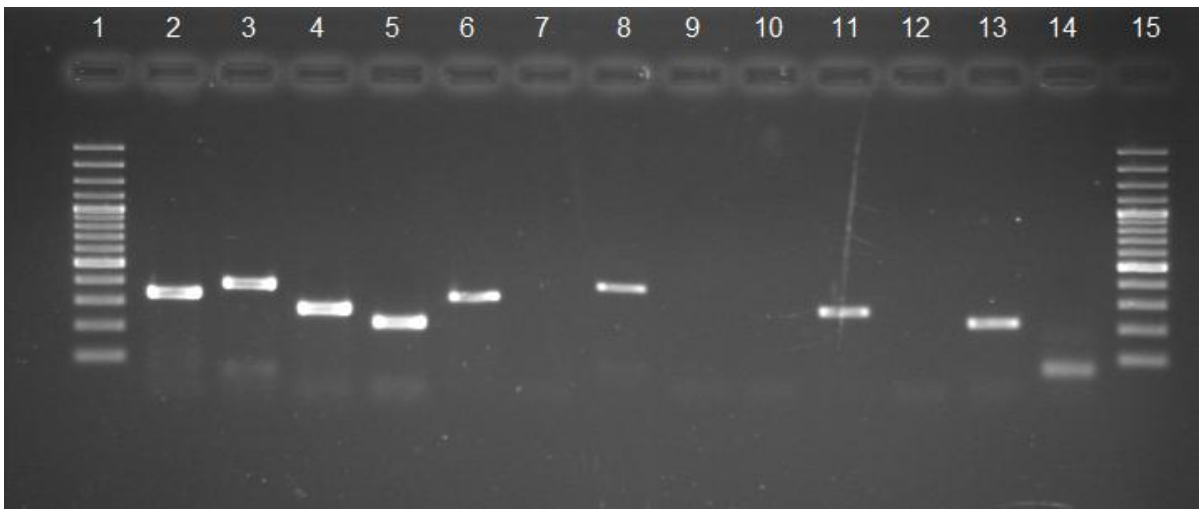

Fig. 1: Typical electrophoresis patterns of PCR products from different HCV genotypes. Patterns of reaction tube one and two are shown for four serum samples containing HCV genotypes 1a (lanes 6 and 7), 1b (lanes 8 and 9), 2 (lanes 10 and 11), 3a (lanes12 and 13).The migration patterns of molecular size markers (GeneRuler™ 100 bp Plus DNA Ladder, ready-to-use; Fermentas UAB, Vilnius, Lithuania) are indicated on lanes 1 and 15. The lengths of PCR products were 338 bp for genotype 1a (lane 2), 398 bp for 1b (lane 3), 286 bp for 2 (lane 4) and 227 bp for 3a (lane 5). Lane 14 was negative control

Results

The studied 634 participants comprised 550 males (86.8%) and 84 females (13.2%). The age range was 13-79 years with the mean of 34.3 and SD=12.01. Among them, there were sequentially 246(38.8%) drug-injection addicts consisting of 165(26%) drug-injection addicts without prison records and 81(12.8%) drug-injection addicts with prison records, 45(7.1%) thalassemia sufferers, 30(4.1%) hemophiliacs, 29(4.6%) with multiple sexual partners, and 27(4.3%) hemodialysis patients. It is worth noting that 257(40.5%) either refused to respond to the questions or mentioned other minor risk factors like injuries, surgeries, or minor outpatient surgeries.

There were 259(40.9%) cases affected by the genotype 3a, 137(26.2%) by 1a, 55(8.7%) by 1b and 15(2.4%) by the genotype 2. Furthermore, 12 patients were affected simultaneously by two genotypes; 5 (0.8%) by the genotypes 1a, 3a; 4(0.6%) by 1a, 1b and 3 patients (0.5%) by the genotypes 1a, 2. Having performed the

same procedures at least twice with the use of the same kit, we detected no specific genotype for 156 (24.6%) patients.

Discussion

HCV genotyping plays an important role in epidemiological studies of HCV infection. Furthermore, it can help efficient clinical management and prognosis in chronic cases and ultimately in vaccine development [7]. The geographical distribution of HCV genotypes varies globally, with 1a genotype more frequent in the US and Europe, 1b in the US, Europe and Japan, [8-10] genotype 3 as prevalent in south-eastern Asia and India, [11] type 4 most prevalent in north Africa and the Middle East, [12,13] and finally the types five and six as the most frequent in South Africa and the Middle East, respectively [14-16].

Iran, located in the Middle East, serves as a connection between Far East and Near East and there have been regional reports on the hepatitis genotyping among special high risk groups within Iran. However, very few reports are available about the situation in southern Iran. In a study by Keyvani *et al*. [17] carried out with 2231 patients in Tehran Hepatitis clinic, it was revealed that the most frequent genotypes were 1a with 39,7% prevalence, followed by 3a and 1b with 27.5% and 12.1%, respectively. In 18% of the cases, no specific genotype was identified.

In the present study, the highest prevalence belonged to 3a (40.9%), followed by 1a (26%), and 1b (8.7%). It is worth mentioning that 38.8% of the total 59.5% of the cases who maintained their risk factors similar to those predetermined in the study, were drug-injection addicts with or without prison records. The prevalence rate of the genotype 3a in this group was found to be significantly higher than that in the other groups (47.6% Vs 28.2%), P=0.001. These finding are in agreement with other studies [10,18].

In a study conducted on 66 HCV RNA positive hemodialysis patients in Tehran, genotyping demonstrated the subtypes 3a and 1a as the predominant ones, accounting for 30.3% and 28.8%, respectively followed by 1b (18.2%), 4(16.7%), mixed genotypes 1a and 1b (3%) and the genotype 3b (3%). Genotype two was not detected in that study [19]. In the present study genotyping of 27 HCV-RNA positive hemodialysis patient serum samples demonstrated that the genotype 1a was predominant (33.4%) followed by 1b and 3a (14.8%) and finally 2(7.4%). 6(22.2%) samples were indeterminate.

The prevalence of the genotype 3a among the group, who didn't respond to the questions or claimed other minor risk factors, was 40.85% which is remarkably greater than the rate in other groups ($P \leq 0.05$). This finding indicates that there have been some drug-injection cases among them who, for some reasons, concealed the injection. Also, previous studies have reported greater prevalence rate of type 3a among the drug injection groups, compared to others. Similarly, in the current study, it was shown that simultaneous infections with the two genotypes among the drug injection groups with or without prison record, is significantly more common ($P \leq 0.05$).

Conclusion

As demonstrated in the current study, the highest prevalence rate belongs to genotype 3a, followed by 1a and 1b. Taking into account the previous studies which have revealed correlation between infection with 3a and hepatocellular carcinoma [20] and the present findings, it seems advisable to distribute free disposable syringes among the drug-injection addicts both in prison and in their local reservations in the community.

In so doing, the spread of hepatitis C among them and their sexual partners who are potentially at the risk of contracting the infection is reduced

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Conflict of interest statement: All authors declare that they have no conflict of interest.

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