

PREVALENCE OF COMMON TYPES OF HEPATITIS C VIRUS GENOTYPES AND THE RELATION BETWEEN AGE, SEX AND TRANSMISSION RISK FACTORS IN KERMAN , IRAN

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Abstract: Hepatitis C virus (HCV) has 6 known genotypes which are different in clinical features and are related with hepatic disease. The viral types and subtypes differ in their geographical distribution and antigenic features. This study aimed to determine the prevalence of different HCV genotypes and their relation with age, sex and transmission factors in the city of Kerman.

A total of eighty five subjects were chosen for this study from patients referred to Blood Transfusion Organization and gastroenterology clinic of Afzalipour general hospital. All of the subjects had a positive anti-HCV antibody and a secondary positive RIBA test for HCV. Genotyping was done by the RFLP method in 5'NCR region of HCV genome.

The most common types of genotype in our study were 1a (38.8%), 3a (29.4%), 1b (8.2%), however some genotypes of the cases could not be determined (23.5%). 37.6% of the cases were thalassemic patients and 11.8% of the cases were heroin abusers and/or had tattoo. No significant difference was observed with regard to age or sex, however some patterns could be seen. In females, youth, thalassemic and hemodialysis patients genotype 1a was dominant, however among Heroin abusers and individuals with Tattoo genotype 3a was more prevalent.

Distribution of HCV genotype depends on geographical location. In Iran the most common genotypes are 1a and 3a, which is not consistent when compared to the findings reported from developed countries. The relationship among different genotypes and sex, and age, remains controversial.

Keywords: • Hepatitis C virus • genotype • RFLP

Introduction

Based on WHO reports more than 3% (170 million) of world population are infected with HCV and about 85% of HCV cases progress toward chronic infection. HCV is a mysterious disease which results in cellular damage in 20 to 40 years of chronic infection [1,2]. Chronic infections could lead to primary hepatocellular cancer (PHC) [1,4] and liver cirrhosis. The most common way of acquiring hepatitis C infection is through contaminated blood and blood products, HCV is the major cause of

post transfusion- hepatitis (PTH) [1,5]

One recent study cited a 64% rate of infection among IV drug abusers, the overall rate of infection among individuals who injected drugs for more than 6 years is about 76.5%.[1]. Most of the cases of hepatitis in IRAN used to be due to HBV infection, however due to mass vaccination programs implemented in our country against HBV, Hepatitis C virus is progressively replacing HBV as the major cause of hepatitis in IRAN. The overall prevalence of HCV infection in IRAN is about 0.12% in blood donors, however in general population the prevalence rate is less than 1%. According to recent data 0.12%-0.89% of general population have antibody against HCV, which corresponds to 0.5 million chronic carries. [1,2,6]

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Since Iran is in the route of drug smuggling, our country has a large population of drug addicts of which about 2 million are IV drug abusers with high risk of acquiring blood born diseases such as HCV, HBV and HIV.

Hepatitis C virus (HCV) is an RNA virus and a member of Flaviviridae, the genome size is about 10 kilobase (Plagemann, 1991). The genomic organization of several prototypes has been completely determined. HCV is characterized with a high degree of nucleotide sequence variability. Overall the heterogeneity of the viral genome ranges from 30% to 35% among different genotypes. HCV has 6 major genotypes (1-6) and each of them has other subtypes (e.g. a,b,c etc). These subtypes are different in mode of transmission, biological, and clinical features, however all of them are associated with hepatocellular carcinoma and advanced hepatic disease. These subtypes also have different geographical distribution and antigenic features. Types 1, 2, and 3 are distributed worldwide, however types 4, 5, and 6 have been found in distinct geographical areas.

HCV has a high genetical variation and is able to hide from cellular and humoral immune system of the host. Serological tests for detection of anti-HCV antibody are very sensitive, and Recombinant Immunoblastic Assay (RIBA) and reverse transcriptase (RT) PCR for detection of HCV RNA are very good supportive tests. RIBA and RT-PCR are very sensitive tests to help the physician to diagnose the infection in a patient which is anti-HCV antibody negative (e.g. immunodeficient patients, cryptogenic liver disease). Determination of HCV RNA load is a useful tool to evaluate the efficiency of the course of the treatment.

Since there was no prior study to recognize different genotypes of HCV in Kerman, this study was designed to determine common genotypes of HCV in Kerman and the relation to age, sex and factors affecting transmission of the virus. We hope that our findings help future studies in determining clinical pattern of different HCV genotypes.

Material and Methods

In this cross sectional study a total of 85 sera were collected from patients by the method of convenient sampling during 20 months from 2004 to 2006. All of the patients who were referred to the Blood Transfusion Organization and Gastroen-

terology clinic of Afzalipoor general hospital were anti-HCV positive. Five ml of venous blood was taken from the patients, after clotting samples were frozen at -20° and were sent weekly to reference lab in Tehran in order to determine the genotypes.

All of the data were entered to SPSS software to perform the needed descriptive and chi square test to determine the relation between variables whereas $\alpha=0.05$ considered as the significance level.

Laboratory Methods

In hepatitis C genome, there is a region named Non Coding 5' region (5'NCR) which is preserved among different genotypes of HCV and used for classifying the virus to different genotypes. Since there is a strong relation between the special nucleotides sequence of this part of the genome and different genotypes of HCV, Restriction Fragment Length Polymorphism (RFLP) method in 5'NCR can be used in recognition of different genotypes of HCV. In this method, the viral RNA was isolated from serum of patients using Phenol-Chloroform method and then by Reverse Transcriptase-PCR (RT-PCR) method using RNA as a model, the complementary DNA (cDNA) was made. In the next stage, cDNA is copied by Nested PCR method which one pair is external primer:

5' -TACTGGTGCAACGGTCTACGAGACCT- 3'
5' -CTGTGAGGAACTACTGTCTT- 3'

And the other one is internal primer :

5' -CACTCGCAAGCACCCCTATCAGGCAGT- 3'
5' -TTCACGCAGAAAGCGTCTAG-3'

The product of nested PCR is cut to different lengths using the restrictive enzymes HaeIII / RsaI and MvaI/Hinfi in 37° c during 4 to 16 hours period. These pieces of DNA were visible on 4% Gel agarose after staining by Ethidium bromide under a transilluminator. Based on this technique, all six genotypes of HCV can be differentiated from each other; however some of genotypes can also be divided into different sub types. The 1a/c and 1b subtypes can be differentiated with the BclI enzyme and 2a/c,2b and 3b can be differentiated from each other with the SrfI enzyme.

Results

In this study, 85 patients aged between 9 to 78 years (mean 32.22±16.8) participated. Table 1 shows some of the criteria about important variables. As shown in this table, the education

level was not determined in about half of the subjects; the ratio of male to female was almost 2 to one. The group was relatively young (about 75% below 40) and the type of narcotics used among drug addict subjects was based on self declaration.. Considering the risk factors, 32(37.6%) of patients were thalassemic patients and in 22(25.9%) cases we could not find any specific risk factor. Fourteen (16.5%) were IV heroin addicts and tattooing was seen in 10(11.8%) of the cases, prior history of blood transfusion except in thalassemic patients was positive in 3(3.5%) and 4 of the subjects had been going under hemodialysis (2.5%).

Table 2 shows the comparing ratio of genotypes according to sex and age. No significant difference was observed in different age groups and between males and females; however a non-significant pattern was noticeable. Genotype 1a was the most frequent in young females, thalassemic patients, and hemodialysis cases. The only genotype detected in 3 cases that had transfusion and the most frequent type in IV heroin abusers and people with tattoo was 3a. We did not detect any other genotype in this group and some of the genotypes reported (23.5%) could not be determined.

Table 1 The frequency of certain variable in patients

| Variable | Frequency | Percentage |
|----------------------------|-----------|------------|
| Sex | | |
| Male | 57 | 67.1 |
| Female | 28 | 32.9 |
| Age | | |
| <=25 yrs | 36 | 42.4 |
| 26-40yrs | 21 | 24.7 |
| >=41yrs | 22 | 25.9 |
| Undetermined | 6 | 7.1 |
| Mean±SD | 32.2±16.8 | |
| Educational Level | | |
| Below Diploma | 11 | 13.0 |
| Diploma and higher | 29 | 34.1 |
| Undetermined | 45 | 52.9 |
| Addiction condition | | |
| No addiction | 19 | 22.4 |
| Oral Opium | 8 | 9.4 |
| Inhaler Opium | 9 | 10.6 |
| Injective Heroin | 9 | 10.6 |
| Undetermined | 40 | 47.1 |
| Type of genotypes | | |
| 1a | 33 | 38.8 |
| 1b | 7 | 8.2 |
| 3a | 25 | 29.4 |
| NT(Non-Typable) | 20 | 23.5 |

Table 2 The Frequency of each genotype in sex and age groups

| Variable | 1a-N(%) | 1b-n(%) | 3a-N(%) | NT ^b -N(%) | P-value |
|---------------------|----------|---------|----------|-----------------------|---------|
| Sex | | | | | |
| Male | 19(33.3) | 5(8.8) | 16(28.1) | 17(29.8) | 0.219 |
| Female | 14(50.0) | 2(7.1) | 9(32.1) | 3(10.7) | |
| Age | | | | | |
| <=25 yrs | 16(44.4) | 4(11.1) | 10(27.8) | 6(16.7) | 0.705 |
| 26-40yrs | 7(33.3) | 2(9.5) | 7(33.3) | 5(23.8) | |
| >=41yrs | 7(31.8) | 1(4.5) | 6(27.3) | 8(36.4) | |
| Risk factors | | | | | |
| Thalassemia | 14(43.8) | 4(12.5) | 8(25.0) | 6(18.8) | 0.715 |
| Transfusion | 3(100) | 0(0.0) | 0(0.0) | 0(0.0) | |
| Hemodialysis | 2(50.0) | 0(0.0) | 1(25.0) | 1(25.0) | |
| Tattoo | 3(30.0) | 1(10.0) | 4(40.0) | 2(20.0) | |
| IV Heroin Abuse | 3(21.4) | 0(0.0) | 6(42.9) | 5(35.7) | |
| Unknown | 8(36.4) | 2(9.1) | 6(27.3) | 6(27.3) | |

a, Row Percentage is considered ; b, NT=Non Typable; P-value>0.05= Non significant

Discussion

Epidemiological and clinical differences have been observed in Hepatitis C virus infection in several areas of the world [13, 14]. The prevalence and distribution of HCV genotypes depend on geographical location [4, 8, 15, 16] and has relation with age and the transmission risk factors and also affects the type of treatment, and the response to the specific treatment.

There is higher probability of liver failure, cirrhosis and hepatocellular carcinoma following infection with genotypes 1 and 4 [19], however infections with types 2, and 3 is less severe with minimal consequences. Hence chronic liver problems due to hepatitis C were more common in region with more prevalence of genotypes 1 and 4, the cost of patient management and liver transplantation are very high in these regions [19]. Central Italy and Brazil are countries that have high prevalence of hepatitis C infection with genotype 1. In Myanmar the most frequent genotype is 3b [21], in Egypt the most frequent type is 4a [10] and the genotype 4 with 12 subtypes is relatively widespread in south-west France [22]. In Iran a study done on 156 cases reported the 1a and 3a subtypes as the most common genotypes overall, but less frequent among IV drug abusers [2]. This result can be compared with that reported from Belgium which none of the patients with genotype 5, were IV drug abusers [23]. Also in another study on 125 cases in Iran, 3a and 1a genotypes were reported as the most common [17]. In south of Iran 1a genotype (70%) and in north of Iran the 3a genotype (83%) are the most common genotypes [17]. In the present study the most common genotype is 1a which is similar with the findings from Samimi-Rad's study, but obviously different with the neighboring countries like Turkey with 1b and Pakistan with 3a and Arabic countries with 4 as the most common genotypes [24]. Although genotype 4 is found almost exclusively in Middle East and western countries, this genotype is uncommon in our country [2, 17, 24] and not seen in the present study.

The duration of treatment is also directly dependent on the genotype. With genotypes 2 and 3, the 6 months treatment is sufficient but for other genotypes at least one year of treatment is recommended [12].

The relation of age and genotypes is mentioned in certain studies. In Italian children, genotype 2 was

seen more in older patients [25]. In current study there was no difference in genotypes in terms of age and sex of the patients and these results are very similar to Kabir's study but different when compared to that reported from developed countries, where lifestyles among young adults seem to have influenced the molecular epidemiology of HCV [2]. In Southeast France, the difference between sex in some of the subtypes is significant and the patients infected with types 2 and 1b are older than those who are infected with types 1a, 3a and 4d [4].

No significant relationship was found between risk factors of HCV infection and the prevalence of genotypes in this study, but genotypes 1a and 3a were more frequent in patients who had blood transfusion and IV heroin abusers respectively. The frequency of Heroin IV drug usage is about 10.6%, but most of the patients (47.1%) have refused to explain about the way they use other narcotic material. Genotypes 3a and 1a are more prevalent in IV drug abusers in Europe and USA [2] which is similar to our results.

The annual risk of infection for acute HCV in dialysis patients is estimated by CDC at about 0.15% among hemodialysis patients and 0.03% in peritoneal dialysis patients [11]. In other studies, longer duration of hemodialysis, more weekly dialysis sessions, history of blood transfusion and history of previous renal transplantation were found to be associated with a higher rate of HCV infection [5, 26].

Genotypes 3a and 1a in Kerman are relatively prevalent in IV heroin abusers when compared with Europe and USA [2].

It seems that more studies are needed to determine the prevalence of HCV infection and its genotypes and related risk factors in different regions of Iran and the neighboring countries to be able to plan future health services.

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