The Relationship between Hepatitis C Genotypes and Viral Load: a study on hepatitis C patients in Kerman, Iran using Real-time PCR

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Abstract

Background: Distribution of hepatitis C virus genotypes varies geographically and can be associated with clinical aspects. The virus has six major genotypes and eleven sub-genotypes. According to the recent studies, in hepatitis C patients, treatment duration and ribavirin dosage should be determined based on the type of virus genotype. Because of contradictory results reported in different countries and races, the present study aimed to determine the genotype of hepatitis C and its relationship with viral load in HCV patients in Kerman, Iran.

Methods: The study included 106 patients with hepatitis C referred to Dr. Bazrafshani Medical Genetic Lab. in Kerman. Patients' blood plasma was collected and used for virus genotyping and viral load determination by Real-Time PCR technique.

Results: From 106 studied patients, 82 ones were male (77.4%) and 24 ones were female (22.6%). Most frequent genotypes were respectively genotype 3 (55.7%) and genotype 1(44.3%). Viral load in genotype1 was greater than that in genotype 3 (P=0.011).

Conclusions: In this study, the predominant genotype was genotype 3. Therefore, treatment strategies should go towards type 3 virus. On the other hand, copy number of genotype 1virus was higher than that of type 3 that should be considered very important in determining the treatment duration in HCV patients.

Introduction

Hepatitis C (HCV) virus is one of the members of Flaviviridae virus family and one of the six common viral hepatitis viruses (G, E, D, C, B, A). The main rout of virus transmission is contact with contaminated blood and blood products. Contaminated blood transfusion, intravenous drug abuse, sexual contact outside the family framework and the use of contaminated personal items like razors, are important causes of disease transmission. Hepatitis C virus is not easily cleared by the host immune defense, so recurrent infection occurs in at least 70% to 85% of patients. Chronic hepatitis C can be progressed gradually and slowly and led to cirrhosis

and liver failure after a long time (1). Due to mutations that occur during viral replication, the genome of HCV, which is an RNA molecule, shows heterogeneity (creating sub-species) and it seems that this change is due to the persistence of infection (2). HCV nucleotide sequences vary very much from an isolated sample to other samples. Accordingly, at least 6 HCV genotypes and more than 150 subtypes have been identified (3). HCV genetic components are protected in all isolates, but in some cases, 45% of nucleotides sequences may be different. Iran, as a country located in the Middle East, has a high prevalence of this disease (4). For the first time, the prevalence of different genotypes in Iranian patients was investigated by Zali and colleagues on 15 samples. They reported type I / 1a in 7 cases, type II / 1b in 3 cases, type V / 3a in 4 patients and type 4 in one case (5). In Samimi Rad et al study, 96 Ab positive Anti-HCV patients were examined. According to their results, genotype 1a was dominant (47%) and the prevalence of 3a, 1b and 4 were, 36%, 8% and 7%, respectively (6).The prevalence of genotypes in our country is the same as that in UK (7) and is different from other countries in the Middle East, including Yemen, Kuwait, Iraq and Saudi Arabia, where the most common genotype is genotype 4 (8). Currently, the standard treatment for chronic hepatitis C is injection of Pegylated Interferon plus Ribavirin once a week (9). Before starting antiviral treatment against hepatitis C virus, genotype should be determined in order to decide about the duration of therapy, dose of ribavirin and method of treatment monitoring. In other words, genotyping can predict treatment success and duration. HCV genotypes are mainly determined based on the analysis of the virus genome sequence (usually genome un translated 5 UTR region) (10).In addition to the genotyping, knowing the viral load before treatment is useful for predicting response to antiviral therapy. Increased viral load would decrease the chance of response to interferon. Studies showed that patients with lower viral load before the treatment were more likely to respond positively to the treatment (11). In a research in Spain, 281 patients with hepatitis C were investigated in terms of viral

load and genotypes and genotypes 1a (38.4%) and 3a (23.1%) were reported as the most common ones. Also, patients with genotype 3 had a higher viral load (12). In other study in this country, the highest viral load has been reported for 1b, 1a and 3a genotypes (13). Another study on 379 patients with hepatitis C in Germany showed that viral load in patients with genotype 3 was significantly lower than patients with genotype 1 and 2 (14).

Until now, no study has been done to determine the predominant genotypes in Kerman region and the relationship between genotypes and viral load. Therefore, it seems that the present study is essential for designing effective therapeutic strategies.

Methods

This cross-sectional study was done on 106 individuals infected with hepatitis C who were referred to Doctor Bazrafshani Genetics lab. In Kerman, Iran. Positivity for HCV had been previously approved by ELISA kit (CAT N: BP-214) and patients were not under any medication. First, 5 ml Peripheral blood was collected into EDTA tubes and plasma kept at -70°C immediately after separation.

RNA was extracted from plasma using ROCH HCV RNA extraction Kit (Germany) and according to the manufacturer's instructions. Genotyping of HCV through Real time method was performed using HCV Genotype RGkit. In order to determine the prevalence of hepatitis C, Kosar Kavosh Fanavar company kit was used. Each sample was repeated twice and the average value was determined as the level of virus RNA. SPSS 18 (PASW Statistics) software was used for statistical analysis of the obtained data.

Cross tab command and Chi-square test were used for comparing qualitative variables (grouping) and the dependency or independency of two quantities was determined. Finally, t-test or ANOVA was used for comparison of two or more groups.

Results

In this study, from all genotypes, only two types of 1 and 3 were observed. From the total of 106 patients, 47 patients (44.3%) had been infected with genotype 1 and 59 patients (55.70%) with genotype 3. This difference was not significant (P value = 0.24). The results can be seen in table 1.

Table 1. Distribution of hepatitis C virus genotypes

Genotype	Frequency (Percent)
1	44.30
3	55.70

The frequency of genotypes 1 and 3 based on patients' sex

From 106 studied patients, 82 ones (77.4%) were male and 24 ones (22.6%) were female. As it is seen in table 2, from 82 male patients, 37 patients (45.1%) had been infected with genotype 1 and 45 patients (41.7 %) with genotype 3. Among 24 female patients, 10 patients (54.9 %) had been affected with genotype 1 and 14 patients (58.3%) with genotype 3 (P = 0.47).

Table 2. The frequency of hepatitis C virus genotypes based on patients' sex

Sex	Genotype 1 (Percent)	Genotype 3 (Percent)
Male	45.10	41.70
Female	54.90	58.30

The frequency of hepatitis C virus genotypes based on patients' age

From 106 patients, 13 patients (12.3%) were under 20 years, 58 patients (54.7%) were 20-40 years and 35 patients (33%) were over 40 years old. As it has been shown in table 3, in the age group of below 20 years, 9 patients (69.2%) had genotype 1 and 4 patients (30.8%) had genotype 3. In the group of 20-40 years, 24 patients (41.4%) had genotype 1 and

34 patients (58.6%) had genotype 3. In the age group over 40 years, 21 patients (40%) had genotype 1 and 14 patients (60%) had genotype 3. In none of the age groups, the frequency of two genotypes showed significant difference.

Table 3. The frequency of hepatitis C virus genotypes based onage groups

Genotype	Age group (years)		
	<20	20-40	>40
1	69.20%	41.40%	40%
3	30.80%	58.60%	60%

The results of Real-time PCR
The relationship between genotypes and viral load

As it has been shown in table 4, there is a significant difference (on the assumption of the variance equality) in viral load based on the virus genotype (P = 0.011).

Table 4. The frequency distribution of hepatitis C virus genotypes and number of virus copies

Genotypes	Number	Virus copies
1	47 (44.3%)	$2.1 \times 10^8 \pm 1.2 \times 10^8$
3	59 (55.7%)	$7 \times 10^7 \pm 2.1 \times 10^7$
total	106 (100%)	

The relationship between genotype and mean number of virus in the two sex groups

Mean viral load based on genotype and sex has been shown in Table 5 and as it is seen, there were71, 114, 70 and 356, 825, 677 copies of virus per ml in men and women respectively and by assuming variance equality, viral load showed a significant difference based on genotype (P = 0.00). Mean viral load in women and men, without considering genotypes and by assuming variance equality, showed a significant difference too (P = 0.00).

Table 5. The mean number of virus copies based on genotype and sex

Mean in each sex	Genotype 3	Genotype 1	Sex
71, 114, 70	43, 744, 218	104, 403, 234	**Male**
356, 825, 677	155, 703, 949	638, 396, 097	**Female**

Chance of infection with genotype 3 is 2.62 times greater in women than men, although this difference is not significant.

The relationship between mean number of virus and genotype in different age groups

Mean viral load based on the two genotypes and different age groups has been presented in table 6. As it is seen, the viral load in the age group 20-40 years was higher compared to other age groups, although this difference was not significant.

Table 6. Mean number of HBVcopies based on genotypes and age group

Mean of each age group	Genotype 3	Genotype 1	Age groups (year)
4, 050, 180	4, 325, 480	3, 927, 824	**<20**
181, 960, 196	33, 110, 643	220, 968, 465	**20-40**
108, 253, 772	101, 050, 578	206, 582, 154	**>40**
135, 804, 016	70, 310, 934	218, 018, 737	**Mean of each genotype**
P=0.581	P=0.42	P=0.66	**p-value**

Evaluation of quantitative and qualitative factors affecting genotypes involved in infected individuals showed that:

Quantitatively, the age does not play a role in the development of 1or 3 genotypes.

Discussion

Based on the latest studies, in the treatment of hepatitis C, the exact dose of ribavirin and treatment duration should be determined according to the virus genotype. In addition to genotyping, estimating the number of virus in HCV patients is very important for designing therapeutic strategies. In other words, genotype and viral load are two important prognostic factors in this disease and have main roles in making decision about the treatment strategy. In fact, patients with high viral load give poor response to interferon therapy than those with low viral load. The risk of relapse after discontinuation of therapy in patients with higher virus load is higher. In addition, factors that can predict poor response to treatment include virus genotype 1 and 4 (over 2 million copies per ml), cirrhosis and old age; in contrast virus genotypes 2 and 3 (HCV RNA less than one million copies per ml), absence of cirrhosis and age are factors that are predictors of better

response to treatment (15).Virus genotype is one of the most important criteria for predicting treatment response and as the distribution of viral genotypes varies in different populations, determining the dominant genotype of the virus in each region is required (16).

In the present study, among 106 patients, the majority were men and in the age group 20-40 years. According to the results of genotyping, the most frequent genotype was type 3, and was reported more than genotype 1 among men and women. Although this difference was not statistically significant, but it is better to select therapeutic strategies based on type 3 virus. Studies carried out about the frequency of involved genotypes in other cities of Iran, show similar results. In the study performed by Sarvghad and his colleagues in Mashhad between the years 2003-2004, the dominant HCV genotype was type 3 (17). In Molaabedin and his colleagues study on 92 patients with chronic infection in Yazd, genotype

3a was the predominant type (16). From doctors' view, the most important difference is between patients with genotype 1 and patients with genotype 3 in responding to treatment; in the combined therapy with pegylated alpha interferon and ribavirin, SVR is between 40 and 50 in patients with genotype 1 and between 70 to 80 percent in patients with genotype 3. Also at present, based on the virus genotype, length of treatment should be different and usually patients infected with genotype 1 and patients infected with genotype 3 should be treated with pegylated alpha interferon and ribavirin compound for 48 and 24 weeks, respectively (18).

In this study, among 47 patients with genotype 1, 37 patients were male (45.1%) and 10 patients were female (41.7%) that shows higher frequency of genotype 1 in men than in women. Of 59 patients with genotype 3, 45 patients were male (54.9%) and 14 patients were female (58.3%), but this difference was not statistically significant. Also, 34 cases (58.6%) of 59 patients with genotype 3 were 20 to 40 years old and among 47 patients with genotype 1, 41.4% were in the age group of 20-40 years old, but this difference was not statistically significant too.

In this study, virus number of genotype 1 was higher than genotype 3 so that genotype 1 mean viral load was three times higher than type 3, which shows a significant difference (P=0.011).The reason of this difference is that the viral replication with this genotype is more efficient than most of the rest genotypes (19) In a study in India by Anita Chakravartiet al (2011) on HCV genotype distribution and its relation with the number of virus, 63% had been infected with genotype 3, 30.98% with genotype 1, and 5.63% with genotype 2. The highest virus frequency was of Genotype 1 (20). But in a study in Spain by Rodriguez et al (2003) performed on 281 patients with hepatitis C, the most frequent genotypes were type 1 and 3 and patients with genotype 3 had a higher number of viruses (21).In another study by Singh (2013) conducted in India on 298 samples, most patients had been infected with genotype 3 (50.33%) followed by genotype 1 (27.8%),genotype 4 (12.4%) and genotype 5 (43%).Three samples with mixed genotypes of 3 and 4, and four samples with mixed genotypes of 1 and 3 were found. Viral load in genotype 1 was significantly higher than viral load in genotype 2 and 3 (22). In a study in Yazd Province (2013), frequency of genotypes 3, 1a, 1b, 2 and mixed genotypes were 50.3%, 38.7%, 6.8%. 1.6% and 2.6%, respectively. No significant difference was found in mean viral load between genotypes 3 and 1 (23).

In this study, viral load in women was about 5 times higher than that in men. Also, after dividing genotypes, women showed significantly higher numbers in both genotypes. Viral load in 20-40 years age group was higher compared to the other two age groups, but the difference was not significant. Mean viral load in each genotype showed no significant difference according to age. In a study in Tehran (2003-2005) conducted on the relationship between age and number of virus, the results showed that with age increase to the 40-50 years, the prevalence of people with viral load of higher than 106increased and then decreased, but there was not any statistical significant correlation between age and the virus number (24).

Despite the shocking statistical reports and large number of studies in the past 20 years, no vaccine has been approved for prevention and treatment of this disease so far and the most effective available treatment protocol, ribavirin and alpha interferon complex, is effective only in 50% of cases. The mentioned problems show the necessity of comprehensive researches to find solutions for the treatment and prevention of this disease.

Hepatitis B and C are the leading cause of liver disease that might be led to liver cancer and hepatocellular carcinoma. Virus genotypes are important in the process of disease and all studies done in Iran and abroad, have been emphasized on the regional differences of genotypes and their relation with

treatment progress. Therefore, the management of hepatitis should be done based on genotype frequency in order to decrease the treatment duration and costs. Also, the results of

viral load can be used in confirming active HCV infection and predicting response to therapy before, during and after the treatment.

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