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The Relationship of Chronic Hepatitis C Virus Genotypes and Ribonucleic Acid Viral Load in Central China

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Abstract

There are seven genetically divergent genotypes of hepatitis C virus (HCV). This study aimed to investigate the gender differences of chronic HCV genotypes and the correlation between genotype and HCV RNA viral load. HCV RNA was detected by Roche Fluorescence Quantitative PCR Analyzer in 52 serum samples. Genotype 3500 DNA sequencer was used to evaluate the gender differences in genotypes and their correlation with HCV RNA viral load. The HCV genotypes were determined as: type 1a in 1.9%, type 1b in 63.5%, type 2a in 19.3%, type 3 in 11.5%, type 6 in 1.9%, type 1/6 mixed in 1.9% cases. There was no statistically significant gender difference (X_2=0.000, P>0.05) in the HCV genotypes. But there were significant differences in serum HCV RNA viral load between different genotypes (type 1b, type 2 and 6 higher than type 3) (F=36.08, P<0.001). In central China, chronic hepatitis C virus was predominantly genotype 1b with no gender difference. The genotype 1b, 2 and 6 HCV RNA viral loads were higher than genotype 3 in central China.

Keywords: HCV; Genotype; RNA; Viral load; China

Introduction

Chronic hepatitis C (HCV) is an infectious liver disease caused by the hepatitis C virus infection that has previously been referred to as parenteral non-A, non-B hepatitis. Currently, it has become one of the world's major health problems. HCV is a single-stranded RNA virus of the Flaviviridae family that has a high genetic diversity due to the high mutation rate of the viral polymerase and the high turnover rate of the virus. Variants of the virus are divided into seven genotypes (following Arabic numerals 1 to 7) and at least 67 subtypes (labeled with a, b, c) [1]. HCV is mainly transmitted by blood or blood products. According to the World Health Organization (WHO), about 3% of people worldwide are infected with HCV [2,3]. Genotypes 1, 2 and 3 have global distribution, while genotypes 4, 5 and 6 are more concentrated in specific regions, of which genotype 4 and 5a subtype are mainly distributed in the Middle East countries and northern South Africa. Genotype 6 is mainly distributed in China and Southeast Asia, especially Hong Kong, Thailand and Vietnam [4,5]. The pathogenicity of different HCV genotypes is different from that of IFN [6]. HCV RNA is the direct evidence of infection, especially for the diagnosis before the development of HCV antibodies at early stage of infection. It is also valuable for the evaluation of efficacy of treatment. Therefore, HCV genotype and RNA load have significant clinical relevance. This study aims to define genotypes of HCV epidemic strains in recent years and to explore the relationship between HCV genotype and HCV RNA load in patients from the central region of China.

Materials and Methods

Fifty-two serum samples of HCV RNA-positive patients from the Department of Gastroenterology of the First and Fifth Affiliated Hospital of Zhengzhou University from May 2016 to July 2017 were selected, including 26 males and 26 females, age 21-75 years, with an average age of 50. All samples were frozen and stored at -70°C. The specimens were collected for laboratory testing within 1 week. The study was approved by the hospital Institution Review Board.

The reverse transcription sequencing of HCV 5'-UTR region was performed by nested PCR. The sequencer was 3500 DNA sequencer provided by AB Company, United States. HCV Genotyping Assay kit provided by Shanghai Shenyou Biotechnology Co., Ltd. was used. The tests were performed strictly according to the reagent manual. HCV genotypes and subtypes were determined by analysis of the sequencing results using the National Center for Biotechnology Information (NCB1) genome database.

The statistical analysis was performed on the proportions of males and females in 34 patients of genotype 1 and 17 patients of non-genotype 1 (type 2 and type 3). A total of 49 HCV genotypes (except Type 1a and 6 due to too few numbers of cases) were analyzed for gender difference.

Real-time fluorescent quantitative PCR (RT-PCR) was used to measure HCV RNA load in patients' sera. The kit was provided by Da'an Gene Co., Ltd. of Sun Yat-sen University and determined by Roche Fluorescence Quantitative PCR Analyzer.

R software was used for statistical analysis. HCV genotype distribution difference in gender was determined with four grid table X2 test. HCV RNA load in different genotypes was analyzed with logarithmic univariate analysis of variance and q test. Type 1 and non-

type 1 HCV RNA load were compared with t test. P<0.05 for the difference was statistically significant.

Results

In 52 samples, there were 1 genotype 1a (1.9%), 33 genotype 1b (63.5%), 10 genotype 2a (19.3%), 3 genotype 6 (11.5%), 1 genotype 6 (1.9%) and 1 mixed genotype 1/6 (1.9%).

There were 18 males and 16 females in 34 genotype 1 patients, 9 males and 8 females in 17 non-genotype 1 (types 2, 3 and 6). There was no significant difference in gender distribution (X_2=0.000, P>0.05).

The serum HCV RNA loads of 49 HCV genotypes (1b, 2 and 3) were analyzed. The results showed that the HCV RNA load among different genotypes was statistically different (F=36.08, P<0.001). There was significant difference in the HCV RNA load between genotype 3 and genotype 1 and genotype 2 (t=10.462 and 7.92, respectively, P all<0.001). The results are shown in Tables 1 and 2. Where, p=probability of sample, t=t-test performed on the sample population, $\bar{X}$=mean of samples, S=standard deviation of samples.

Genotype (kIU/L)	Number of cases (lg)	HCV RNA ($\bar{X} \pm S$)
1b	33	6.35 ± 0.27
2	10	6.31 ± 0.27
3	6	5.38 ± 0.20
F		36.08
P		<0.001

Table 1: Comparison of HCV RNA loads among patients with different HCV genotypes.

Genotype	Number of cases	HCV RNA
1b	33	t=10.462
3	6	p<0.001
2	10	t=7.92
3	6	p<0.001

Table 2: Test of HCV RNA loads in patients with different HCV genotypes.

Fifty-one HCV genotypes were divided into two types: genotype 1 (34 cases) and non-genotype 1 (17 cases). There was no statistically significant difference in HCV RNA viral load between the two groups (Table 3).

Genotype (kIU/L)	Number of cases (lg)	HCV RNA ($\bar{X} \pm S$)
1	34	6.34 ± 0.27
Non-1	17	5.96 ± 0.52
T		2.743
P		<0.05

Table 3: HCV genotype 1 and non-genotype 1 HCV RNA load comparison.

Discussion

Most of the HCV infection is occult with progression to chronic phase. The viral replication in the body causes sustained damage of liver cells, with subsequent compensatory proliferative repair. The repeated cycle of injury and repair eventually leads to cirrhosis, and development of liver cancer in a subset of patients. Hepatitis C is prevalent in the world and different sexes, ages and ethnic groups are susceptible to HCV. Due to resistance to previous anti-HCV drugs caused by HCV mutations, prevention and treatment of hepatitis C face new challenges. Currently, HCV has diverse genotypes. According to the latest "Chinese Guide to Prevention and Treatment of Hepatitis C", the prevention and treatment of HCV should be based on the virus genotype to achieve a cost-effective effect. HCV genotypes show geographical and ethnic differences in distribution [7-12]. The most prevalent of HCV genotypes in China is 1b [13]. The HCV genotypes in this study were also predominantly type 1b (63.5%) and type 2 (19.3%). This study detected a rare domestic genotype 1a. It has been reported that HCV genotype 6a has replaced 2a to become the second most popular genotype in the Pearl River Delta region [14]. A new genotype 6 subtype has also been detected in this region. In our study, the detection of 1 case of genotype 6 (1.9%) was likely related to population mobility. In recent years, as the population migrates in large numbers, the distribution of HCV genotypes may also gradually change.

This study detected 1 case of mixed 1/6 genotype. This patient was a female with severe anemia and multiple blood transfusions. The mixed genotype is most likely due to infection through blood from different persons. Mixed infection through blood transfusion has some impact on HCV immune response and treatment response [15]. It has been reported in the literature that HCV major genotypes/subtypes (1b, 3b, 1, 2a, 3a, 6a) can be co-infected with HBV, and HCV and HBV co-infection is more likely to lead to the occurrence of cirrhosis so such patients should be closely monitored [16].

This study found no difference in gender distribution of genotypes. The correlation analysis of genotype and HCV RNA load showed that the HCV RNA loads of type 1b and type 2 were significantly higher than those of type 3, suggesting that HCV genotypes were closely related to the pretreatment viral load *in vivo*. This may indicate the necessity to personalize the treatment of patients of difference HCV genotypes. The RNA viral load of patients with genotype 1b was slightly higher than that of genotype 2, but the difference was not statistically significant. A study of 51 patients with HCV genotype showed that there was significant difference in serum HCV RNA viral load between genotype 1 (34 cases) and non-genotype 1 (17 cases). A similar study in other countries has also showed higher HCV RNA load in patients with HCV genotype 1 than in patients with non-type 1 HCV infection [17], but the difference was higher than our study. This is likely due to the differences in the composition of the study cases, sample processing, or different HCV RNA quantitative detection system, ethnic differences may also contribute to the result.

HCV genotype 1b is mainly transmitted through surgical and transfusion infection. HCV type 1b is closed related to severe hepatitis, cirrhosis, and liver cancer. The prognosis is usually poor. The efficiency of interferon therapy in patients with HCV genotype 1b is only 40%. Conventional interferon plus ribavirin, has limited efficacy. Currently Telaprevir and Boceprevir and other direct-acting antiviral drugs, combined with interferon and ribavirin can achieve efficacy of over 90% in patients with HCV genotype 1b.

Although the 52 patients were from two hospitals in Zhengzhou, China, the patients' families originated throughout the northeast, north, south and southeast provinces of China. Therefore, our results Therefore, our results were representative of the overall national genotype distribution.

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