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ORIGINAL ARTICLE

Medicine Science International Medical Journal

Medicine Science 2021;10(2):605-8

Genotype distribution in chronic hepatitis C, relationship of genotype with HCV RNA, alpha fetoprotein, alanine aminotransferase and liver fibrosis

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Received 04 February 2021; Accepted 09 March 2021
Available online 20.05.2021 with doi: 10.5455/medscience.2021.02.034

Abstract

In this study, the genotype distribution in chronic hepatitis C (CHC) and its relationship with alanine aminotransferase (ALT), alpha fetoprotein (AFP), HCV RNA and liver fibrosis were compared. HCV RNA was negative in 63 and positive in 69 CHC cases who applied to the infectious diseases outpatient clinic between May 2016 and June 2018 were included in the study. Demographic data, laboratory and pathology results of the cases were evaluated retrospectively. 76 of the cases were male, 56 of them were female. In HCV RNA positive cases, Serum ALT and AFP level were statistically significantly higher than negative ones (p: 0.04, p: 0.001). Of HCV RNA positive cases, genotype 1b in 42 (60.9%), 3a in 10 (14.5%), 1a in 8 (11.6%), 4d in 5 (7.2%), and 2b in 4 (5.8%) were found. There was no statistically significant difference between genotypes in terms of HCV RNA, ALT and fibrosis. AFP level was statistically significantly higher in genotype 1b than other genotypes. Consistent with studies, the most common genotype was 1b. One of the main results is that there is no significant relationship between genotype difference and ALT, AFP and HCV RNA and fibrosis in CHC cases. Therefore, although these parameters may play a role in chronicity and inflammation, they may not be sufficient in the evaluation of fibrosis. High level of AFP in genotype 1b compared to other genopes may be useful in determining prognosis. Such studies may be important in epidemiological evaluation of HCI's and follow-up of genotype profile changes.

Keywords: Chronic hepatitis C, alanine aminotransferase, alpha feto protein, HCV RNA, genotype

Introduction

Hepatitis C virus infection (HCI) is mainly a parenterally transmitted disease with slow progression, but it can progress as an acute infection. While it resolves spontaneously in approximately 20% of the cases, it becomes chronic in 80% of the cases and may result in chronic HCI, liver cirrhosis (LC) and hepatocellular carcinoma (HCC) [1, 2]. The mechanism of liver damage due to HCI has not been fully explained and it is thought that the virus escapes from the immune system as a result of high chronicality rate and high genetic heterogeneity [3]. Hepatitis C virus (HCV) is a single-stranded RNA virus and consists of seven major genotypes (HCV 1-7) with 67 confirmed and 20 transient subtypes, and genotypes 1, 2 and 3 show a wide distribution, 4, 5 and 6 are only seen in certain regions [4].

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Genotyping of HCV is important for obtaining epidemiological data, determining prognosis and choosing antiviral therapy. Patients infected with genotype 1b have a higher risk of developing LC and HCC, and this genotype is more resistant to antiviral therapy than other genotypes [5]. The most frequently detected genotype in Turkey are genotype 1 and genotype 3 [6, 7]. In cases with chronic hepatitis C (CHC), HCV RNA and serum alanine aminotransferase (ALT) levels are important markers in showing the degree of viral replication and liver damage, initiating treatment [4].

Prevention of disease transmission and treatment of infected cases are the main strategies for the management of CHC due to the complications that may occur in the future due to the disease and its contagiousness. The disease is almost curable with the introduction of many new and effective drugs in treatment; besides, survival times have significantly increased [8, 9].

In this study, it was aimed to evaluate the genotype distribution and its relationship with ALT, alpha fetoprotein (AFP), viral load (HCV RNA) and liver fibrosis in patients with CHC and to contribute to the diagnosis, determination of prognosis in demonstrating liver damage.

Materials and Methods

132 anti-HCV (+), HCV RNA positive and negative cases for at least 6 months who admitted to the Infectious Diseases outpatient clinic of Fethi Sekin City Hospital between May 2016 and June 2018 were included in the study. Demographic data of the cases, anti-HCV, HCV RNA and HCV genotype, and liver fine needle aspiration biopsy results were evaluated retrospectively. Anti-HCV enzyme "immunoassay" (ELISA), HCV RNA level Cobas AmpliPrep / Cobas Taqman 48 (Roche Diagnostics, USA), genotype determination was determined by quantitative "real time" HCV Genotype II system (Abbott Laboratories, USA).

Demographic data, ALT, AFP values of HCV RNA positive and negative cases were recorded and comparison was made. In HCV RNA positive cases, liver fine needle aspiration biopsy results were recorded together with the genotype determination. Liver biopsy specimens were scored using the Ishak histological scoring system (fibrosis was evaluated out of 6) (10). Genotype 1a, 1b, 3a were included in the study and a comparison was made between these three genotypes in terms of ALT, AFP, HCV RNA levels and fibrosis.

Patients with a history of hepatotoxic drug and alcohol use, autoimmune diseases, acute hepatitis, LC and HCC, and those aged 18 years or younger were excluded from the study. This study was in accordance with the international ethics committee (Helsinki Declaration) principles and was approved by Fırat University Non-Interventional Research Ethics Committee (11.04.2019/06).

Statistical Analysis

Data analysis procedures were carried out using SPSS 22.0 (Chicago, USA) package statistics software. Paired-samples t test was used for the analysis of continuous variables conforming to normal distribution. The analysis of categorical data was made using the chi-square test. One-way test (tukey) was used in multiple comparisons. Numerical data were expressed as mean ± standard deviation, and categorical data as %. P <0.05 value was accepted as significant in statistical comparisons.

Results

76 of the cases were male (mean age 53.6 ± 3.29), 56 of the cases were women (mean age 62.9. ± 1.46). In 63 of 132 cases, HCV RNA was negative, 69 of them were HCV RNA positive and HCV RNA levels were between 3553-34.243.424. 990 IU / mL. The demographic characteristics of HCV RNA negative and positive cases are summarized in Table 1.

Table 1. Demographic characteristics of HCV RNA negative and positive chronic hepatitis C cases

	HCV RNA negatif	HCVRNA pozitif	p value
	Avarage±SD	Avarage±SD	
Age	52.7±2.40	58.3±2.04	0.48
Gender (F/M)	27/30	21/43	0.42
AST(IU/L)	23.28±3.96	32.77±3.67	0.05
ALT(IU/L)	22.86±1.93	38.40±4.41	0.04
AFP	2.67±0.20	7.03±1.54	0.001

Alanine aminotransferase; AFP: Alpha feto protein; AST: Aspartate aminotransferase; M: Male; F: Female

No statistically significant difference was found between HCV RNA negative and positive cases in terms of age and gender (p> 0.05). However, ALT and AFP levels were higher in HCV positive group than negatives and this was statistically significant (38.40 ± 4.41IU / ML, 7.03 ± 1.54 µg / L, respectively, p: 0.04, p: 0.001). There was no statistically significant difference between serum ALT, AFP and HCV RNA levels and fibrosis values in HCV RNA positive cases (p: 0.383, p: 0.211, p: 0.324, respectively).

Of the cases, genotype 1b in 42 (60.9%), genotype 3a in 10 (14.5%), genotype 1a in 8 (11.6%), genotype 4d in 5 (7.2%), genotype 2b in 4 (5.8%) were detected. The mean age of cases with genotype 1b was higher than 1a and 3a and this was statistically significant (p: 0.001, p: 0.003, respectively). There was no significant relationship between genotypes in terms of gender (p: 0.218). In Table 2, ALT, AFP, HCV RNA and fibrosis values are given according to genotypes. There was no statistically significant difference between genotypes in terms of HCV RNA, ALT and fibrosis. Alpha feto protein level was higher than 1a and 3a in genotype 1b and this was statistically significant (p: 0.03, p: 0.04). Table 3 shows p values of ALT, AFP, HCV RNA and fibrosis distribution according to genotypes.

Table 2. ALT, AFP, HCV RNA and fibrosis values by genotypes

Genotipe	Age	ALT	AFP	HCVRNA	Fibrosis
1a	35.3± 4.62	32.14±12.9	3.5±2.8	5602700±2947510	2.5±1.7
1b	62.1±2.26	32.42±5.54	6.9±11.6	929950.10^3±5628840.10^3	3.4±2.0
3a	41.6±7.93	44.62±12.3	2.3±1.6	5035900±7907680	1.7±0.6

Alanine amino transferase; AFP: Alfa feto protein

Table 3. P values between genotypes and ALT, AFP, HCV RNA and fibrosis

p values	ALT	AFP	HCV RNA	Fibrosis
p1	0.23	0.03	0.24	0.26
p2	0.24	0.24	0.24	0.66
p3	0.24	0.04	0.22	0.14

p1: Genotipe 1a-1b; p2: Genotipe 1a-3a; p3: Genotipe 1b-3a; ALT: Alanine amino transferase; AFP: Alfa feto protein

Genotypes 2b and 4d were not evaluated because they were seen in a small number of cases.

Discussion

Hepatitis C virus infection has an important place among liver diseases due to its high rate of chronicity and transmission routes [11]. In this study, genotype 1b in 42 (60.9%) of the HCV RNA positive cases, and genotype 3a in 10 (14.5%) and genotype 1a in 8 (11.6%) were found. There was no significant difference between genotypes in terms of HCV RNA, ALT and fibrosis (p>0.05). Alpha feto protein level was statistically significantly higher in genotype 1b than 1a and 3a (p: 0.03, p: 0.04, respectively).

The frequency of HCV genotypes in a population varies depending on the age of infection and the route of transmission [5]. In one study, it was observed that the majority of CHC cases admitted to the hospital were among 50 years or older [12]. In another study, Aydemir et al. [13] evaluated 97,527 patients retrospectively and found that the highest positivity value of anti-HCV result was among those of 50 years and above.

Harman et al. [12] found that 75% of the cases were in those of 50 years old and above. 104 (65%) of the cases were female and 56 (35%) were male. Similarly, in a study assessing the epidemiology of HCI, HCI prevalence in all age groups were seen to be higher among women than men in Turkey [14, 15]. Küçüköztaş et al. [16] reported that the average age of patients infected with genotype 1 was statistically significantly higher than those infected with genotype 2 and 3, but there was no significant relationship between genotypes in terms of gender (p: 0.218).

In another study, according to genotypes, no statistically significant difference was found in terms of age (p = 0.431) and gender (p = 0.057) distributions [17]. In this study, also in accordance with the others, 52.3% of CHC cases were among 50 years and older. The mean age of the cases with genotype 1b was statistically significantly higher than 1a and 3a (p: 0.001, p: 0.003, respectively). However, there was no significant relationship between genotypes in terms of gender (p: 0.218).

There are studies stating that serum ALT levels are important in determining liver damage and may be a guide in liver biopsy and treatment decisions [18]. Al Swaff [19] found that there is a relationship between grade 1 and grade 4 liver fibrosis and ALT levels, and higher HCV RNA levels are found in grade 3 liver fibrosis in patients with CHC. In another study, no clinically appropriate relationship was found between ALT levels and liver fibrosis [3]. In the studies of Küçüköztaş et al. [16] including 52 cases, genotype 1b in 76.9% of the cases, genotype 3a in 9.6%, genotype 4e in 5.7%, genotype 2a / 2c in 3.8%, genotype 4 and 1a in 1.9% were found. Again, in one study, no statistically significant difference was found between genotypes in terms of HCV RNA levels and ALT levels (p> 0.05) [17]. In this study, there was no significant difference between genotypes between HCV RNA and serum ALT levels.

Due to the genetic diversity of the HCV, it can escape from the immune system and this may cause resistance in treatment and therefore, determination of the genotype can guide the determination of the prognosis and the appropriate treatment regimen [5]. Genotype 1b is associated with poor prognosis and is more resistant than other genotypes [20]. In various studies [3], the relationship between the degree of liver damage and serum ALT, HCV RNA levels and HCV genotype in cases with CHC has been investigated, but inconsistent results have been reported [3]. Shahid et al. [2] found that HCV RNA titers are associated with the degree of fibrosis in CHC genotype 3a.

In their study including 500 HCV RNA positive patients, Kabakçı et al. [21] showed that the most frequent genotype was 1b (93.5%) and the second most common genotype was 1a (6.7%). In the study carried out by Öztürk et al in Antakya [22], they reported the frequency of type 1a as (0.31%), 1b as (86.73%), 2 as (9.26%), 3 as (0.93%) and 4 as (2.78%). Of 308 patients with CHC, genotype 1 in 271 (88%), genotype 3 in 15 (4.9%), mix genotype in 9 (2.9%), genotype 2 (2.6%) 8 and genotype 4 (1.6%) in 5 were detected by Uzun et al. [23]. Chakravarti et al. [24] found 63.39% type 3, 30.98% type 1, 5.63% type 2 in their study on 71 cases. Gower et al. [25], analyzing 2,320 studies from 98 countries, examined the HCV genotype distribution and genotype 1 constitutes 46% of HCV infections in adults (22% type 1b) and others are distributed as genotype 3 (22%), genotype 2 (13%), genotype 4 (13%), genotype 6 (2%) and genotype 5 (1%).

In this study, in accordance with the studies, the most common genotype was genotype 1b with a rate of 60 9%. No statistically significant difference was found between genotypes in terms of fibrosis scores. However, AFP level was statistically significantly higher in genotype 1b than 1a and 3a (p: 0.03, p: 0.04, respectively).

The exclusion of patients with hepatocellular carcinoma and advanced stage fibrosis and the relatively small sample size were the limitations of the study.

In conclusion, determination of genotype distribution can be important in determining prognosis and selection of antiviral therapy. In this study, consistent with the studies, the most common genotype was found to be 1b. One of the main results of this study is that there is no significant relationship between genotype difference and ALT, AFP and HCV RNA and fibrosis in CHC patients. Therefore, although these parameters may play a role in chronicity and inflammation, they may not be sufficient in the evaluation of fibrosis. High level of AFP in genotype 1b compared to other genotypes may be useful in determining prognosis. Such studies may be important in epidemiological evaluation of HCIs and follow-up of genotype profile changes. For the evaluation of treatment results and the relationship with fibrosis, there is a need to study larger patient populations, including cases of advanced fibrosis.

Conflict of interests
The authors declare that they have no competing interests.

Financial Disclosure
All authors declare no financial support.

Ethical approval
This study was in accordance with the international ethics committee (Helsinki Declaration) principles and was approved by Fırat University Non-Interventional Research Ethics Committee (11.04.2019/06).

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