UPDATED HEPATITIS C VIRUS GENOTYPE DISTRIBUTION IN ADANA, TURKEY AND AN INVESTIGATION OF THE ASSOCIATION BETWEEN GENOTYPE AND VIRAL RNA LOAD

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ABSTRACT

Introduction*: The management and treatment approach of chronic hepatitis C infection are closely related with HCV genotypes. In this study, we aimed to examine the HCV genotype distribution in Adana.*

Case Presentation*: A total of 1002 patients who were HCV RNA positive and were admitted to Adana City Training and Research Hospital for HCV genotyping between 2011 and 2017 were included in this study. The distribution of HCV genotypes, subtypes, gender, viral RNA loads, AST and ALT levels were recorded. Genotype 1 was observed in 548 of the 1002 patients (54.7%), followed by genotype 3 (28.6%), genotype 2 (13.6%), and genotype 4 (2.7%), respectively. Subtype 1b was the most prevalent subtype (43.4%) followed by subtype 1a (7.8%), and subtype 3a (4.7%). Age and ALT levels were closely related with genotype distribution. No significant differences were found in median HCV RNA values of the patients infected with different genotypes.*

Conclusion*: We describe the most recent data on genotype distribution of hepatitis C virus in Adana province. The present study confirms that genotype distributions vary with age, gender, and ALT levels.*

Keywords: *HCV (hepatitis C virus), HCV RNA, genotyping, Adana, Turkey.*

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Introduction

The hepatitis C virus is an enveloped RNA virus within the Flaviviridae family. It has been estimated that there are approximately 170 million HCV infected persons worldwide[1, 2]. Persistent HCV infection is a leading cause of cirrhosis, hepatocellular carcinoma (HCC), and liver transplantation[3, 4]. Hepatitis C virus (HCV), a blood-borne virus transmitted mainly via medical equipment, sharing drug injection equipment, and transfusion of unscreened blood/blood products[5]. Screening HCV is necessary for early diagnosis of infection. A one-time HCV test is recommended for asymptomatic persons born between the years of 1945 and 1965 and other persons who have conditions that increase the risk for HCV infection[6]. The risk of transfusion-associated HCV infection has decreased since antibody detection-based screening assays started to be used routinely. Anti-HCV tests have been added for screening purposes before blood transfusions since 1996[7,8].

The HCV genome has revealed both highly conserved and highly variable regions. The 5'UTR, C, E1, and NS5B regions are the highly conserved, and the 5' untranslated region (UTR) of HCV RNA is used as the basis for classification (9). The envelope 2 (E2) glycoprotein region of HCV is the most hypervariable region. HCV is classified into seven major genotypes and more than 100 subtypes have been identified based on its genetic diversity[9,10].

Subtypes 1a and 1b are the most common worldwide. They predominate in Northern Europe and North America. In countries of South Asia, including Afghanistan, Bangladesh, India, and Pakistan, genotype 3 is the most common genotype. Genotype 4 is common in the Middle East Countries(11). Genotype 5 mostly occurs in South Africa, and genotype 6 mostly occurs in South China and Southeast Asia (12). Genotype 7 has been isolated from central African immigrants in Canada (13). In Turkey, genotype 1b has been found to be the predominant genotype, followed by genotype 3, 2, and 4, respectively[1, 14-16].

In this study, the recent HCV genotype distribution in Adana province located in central-southern of Turkey was examined[17]. Association between genotype and age, gender, liver transaminase levels, and viral RNA loads were also determined.

Material and method

Study population and design

A total of 1002 patients who were HCV RNA positive (706 males, 296 females; age range: 1-94 years, median age: 46.7 years) and were admitted to Adana City Training and Research Hospital for HCV genotyping between 2011 and 2017 were included in the study. The laboratory data of the patients were obtained from the hospital automation system. The distribution of HCV genotypes and subtypes, age, gender, viral RNA loads, aspartate transaminase (AST) and alanine transaminase (ALT) levels were recorded. The study was approved by Adana City Training and Research Hospital Clinical Research Ethics Committee.

Molecular Analysis

A COBAS TaqMan HCV quantitative, version 2 assay was used to determine HCV RNA load before initiation of antiviral therapy (Cobas TaqMan HCV, Roche Diagnostics). Blood was collected in ethylenediaminetetraacetic acid (EDTA) anticoagulated tubes. The EDTA tubes were centrifuged at 800-1600 x g for 20 minutes for plasma separation from whole blood[18, 19].

HCV Real-TM Genotype Plus was used for the determination of HCV-RNA genotypes 1a, 1b, 2, 3, 4, 5a, and 6 (Sacace Biotechnologies) in accordance with the manufacturer's instructions[20]. Whole blood was collected in EDTA and separated into plasma and cellular components using centrifugation at 800-1600 x g for 20 minutes. RNA was converted to complementary cDNA using a reverse transcriptase reaction. cDNA was amplified using real- time PCR with a Rotor-Gene™ 3000/6000/Q (Corbett Research, Qiagen) and fluorescence curves were analyzed with the software of Real Time PCR instruments for genotype determination (Sacace™ HCV Genotype Plus Real-TM).

Statistical analysis

All statistical analyses were performed using the IBM SPSS V23 software package. The normality of distribution was assessed using the Mann-Whitney U-test and the Kruskal-Wallis test. A p-value of <0.05 was considered statistically significant.

Results

Age and gender distribution

In this study, 70.5% and 29.5% of patients were males and females, respectively. The gender distribution was similar in patients infected with genotype 4; however, male sex was predominant in patients infected with the other genotypes (see Table 1). Differences were observed in median age values of patients infected with different genotypes ($p<0.005$). The median age per genotype was 60 years for genotype 1, 29.5 years for genotype 2, 28 years for genotype 3, and 58 years for genotype 4. No significant difference was observed in the median age between genotype 1 and genotype 4. The median ages of patients with genotypes 2 and 3 were lower than other genotypes (Table 2).

	Total population n, %	Median Age	Females n, %	Median Age	Males n, %	Median Age
	1002	46 (1-94)	296 (29.5)	59.5 (17-94)	706 (70.5)	35 (1-87)
Genotype 1	548 (54.7)	60 (1-94)	243 (82.09)	61 (17-94)	305 (43)	59 (1-87)
Genotype 2	136 (13.69)	29.5 (17-85)	16 (5.4)	43 (20-81)	120 (17)	29 (17-85)
Genotype 3	287 (28.6)	28 (5-81)	23 (7.7)	27.5 (5-81)	264 (37)	27.5 (5-81)
Genotype 4	27 (2.7)	58 (23-80)	13 (4.3)	58 (43-80)	14 (1.9)	50 (23-72)
Genotype 5	4 (0.4)	-	1	-	3	-
Mixed genotype	10 (1)	-	2	-	8	

Table 1: Distribution of the main HCV genotypes, gender distribution, and median age values in different genotypes.

Genotype distribution

Genotype 1 was observed in 548 of the 1002 patients (54.7%), followed by genotype 3 (28.6%), genotype 2 (13.6%), and genotype 4 (2.7%). Subtype 1b was the most prevalent subtype (43.4%), followed by subtype 1a (7.8%), and subtype 3a (4.7%). Age and ALT levels were closely related to genotype distribution. The prevalence of mixed-genotype infections was 1%. Genotype 7 was not detected in the present study.

	HCV RNA	ALT	AST	AGE
Genotype 1	528.845,5 (41 - 46544223)	40.5 (7 - 1373)[a]	40 (10 - 873)	60 (1 - 94)[b]
Genotype 2	565.921,5 (223 - 26543590)	61.5 (5 - 1497)[b]	36 (7 - 640)	29.5 (17 - 85)[a]
Genotype 3	323.572 (44 - 36446120)	71 (0 - 1269)[b]	41 (6 - 1063)	28 (5 - 81)[a]
Genotype 4	115.139 (1257 - 3303859)	50 (13 - 150)[ab]	60 (18 - 151)	58 (23 - 80)[b]
p	0.050	<0.001	0.343	<0.001

Table 2: HCV RNA, ALT, and AST concentrations in different genotypes. Similar symbols were used for results with statistically significant differences (a, b, ab).

Viral Load

The median HCV RNA values did not change in the different genotypes, they only changed according to gender in genotype 2 (Table 2 and 3).

		HCV RNA	ALT	AST	AGE
Genotype 1	Female	434892 (41 - 31670057)	37 (7 - 824)	40 (13 - 873)	61 (17 - 94)
	Male	639231 (84 - 46544223)	44 (7 - 1373)	39 (10 - 806)	59 (1 - 87)
	p	0.228	**0.006**	0.600	**0.027**
Genotype 2	Female	103964.5 (502 - 8389732)	52.5 (12 - 311)	31.5 (20 - 194)	43 (20 - 81)
	Male	719954 (223 - 26543590)	66 (5 - 1497)	36.5 (7 - 640)	29 (17 - 85)
	p	**0.015**	0.255	0.738	0.269
Genotype 3	Female	229657 (3871 - 13459291)	46 (11 - 258)	35 (18 - 94)	31 (17 - 80)
	Male	325701.5 (44 - 36446120)	72 (0 - 1269)	41 (6 - 1063)	27,5 (5 - 81)
	p	0.757	0.104	0.271	0.125
Genotype 4	Female	29815 (1257 - 3303859)	56 (20 - 82)	66 (23 - 151)	58 (43 - 80)
	Male	152431 (5089 - 2680521)	48 (13 - 150)	29 (18 - 131)	50 (23 - 72)
	p	0.528	0.962	**0.029**	0.253

Table 3: HCV RNA, ALT and AST levels according to gender in different genotypes.

Liver transaminases

The ALT concentrations of the patients were between 0 - 14978 IU/L (median value: 48.5 IU/L) and AST concentrations were between 6 and 1063 IU/L (median value: 40 IU/L) (normal blood range of AST and ALT: 10-40 IU/L and 7-56 IU/L, respectively). ALT concentrations were closely related to genotype distribution; no relationship was found between genotype and AST levels. The median value of ALT levels was found lower in genotype 1 than in genotype 2, 3, and 4 (Table 2). ALT concentrations only changed according to sex in genotype 1, and AST concentrations only changed according to sex in genotype 4 (Table 3).

Discussion

Serum HCV RNA concentrations and HCV genotype are the main and independent factors associated with accurate treatment of HCV infection[21]. The development of HCV vaccines requires an understanding of relative HCV genotype distribution[22, 23]. Infection with subtype 1b and genotype 4 shows poor response to interferon (IFN) therapy[24].

Genotype 1 is reportedly associated with a more severe liver disease and a more aggressive course than the other HCV genotypes[25, 26]. In addition, subtype 1b has been reported to carry a greater risk for developing hepatocellular carcinoma[27, 28].

Genotype 1 is usually seen as the result of unsafe medical practices and DAA-based and interferon-free regimens have high efficacy with sustained virologic response (SVR) rates ≥95%. against genotype 1. It is the most prevalent genotype worldwide, especially in high-income and upper-middle income countries[14, 29, 30]. Genotype distribution is changing significantly in different parts of Turkey. Previous studies reported that subtype 1b was the most prevalent subtype in Turkey, followed by subtype 1a, genotype 2, and genotype 3[17, 31-34]. When we examine the change in genotype 1 ratio in Adana province, Yarkın et al. reported 96.7% in 2000, Kuscu et al. reported 78.3% in 2013, and most recently, Duran et al. reported 71.4% in 2017. In the present study, genotype 1 was observed in 548 of the 1002 patients (54.7%). The decrease in genotype 1 was attributed to safer medical applications. Regarding subtypes in genotype 1, subtype 1b was most prevalent subtype (43.4%), followed by subtype 1a (7.8%) (35-37). The 5' UTR is a target region for most diagnostic HCV RNA PCR, but it is not able to differentiate the HCV genotype 6c-1, which can be mistyped as HCV genotype 1/1b because of sequence homology. Therefore, these methods give sufficient information for clinical purposes and epidemiologic studies. NS5B genotyping methods are more reliable for subtype determination, so it is noteworthy that sequencing is the gold standard for determining HCV genotype. Unfortunately, nucleotide sequencing could not be performed in the present study. Some 2.6% of undetermined subtype was observed within genotype 1[38, 39].

Increasing genotype 2 and 3 rates have been reported from Adana in recent years. The rate of patients with genotype 2 and 3 was 2.7% between 1996 and 2008, and increased to 44% in 2012-2013[36]. In the present study, the rate of genotype 2 and 3 was 42%, similar to the last report from Adana[40]. The inability to document transmission routes of HCV infection in patients is a limitation of the present study. However, current studies show that intravenous drug use (IDU) is the main transmission route of HCV infection. Genotype 3 has been reported as the most prevalent genotype in IDUs from the Cukurova region of Turkey[41]. Kuscu et al. reported higher genotype 2 and 3 rates in young male patients who were

using intravenous drugs[36]. In this study, the median ages of patients with genotype 2 and 3 were lower than in the other genotypes. Accordingly, these findings suggest that drug abuse is increasing among young people in Southern Turkey[35].

Unsafe medical instructions, especially blood transfusion, are the main risk factor for genotype 4 HCV infection. It is the most common type in North Africa and Middle Eastern Countries: Egypt, Kuwait, Lebanon, Jordan, Saudi Arabia, and Syria[11]. Previous studies from different regions of Turkey reported different genotype 4 rates. Although the genotype 4 rates in our country were lower, Gokahmetoglu et al. and Kayman et al. reported higher genotype 4 rates as 35.6% and 32%, respectively[42, 43]. In this study, the frequency of genotype 4 in Adana was higher with a rate of 2.7% than the rate of 0.68% that was reported in 2014[17, 40]. In the last few years, migration rates from outside the country have increased. Although genotype 4 is prevalent in Syria, in the present study, only three of 32 patients with genotype 4 HCV infections were Syrian.

HCV sex and age distribution among patients with HCV infections shows the variation by years and regions. HCV infection is seen among males more than females[44]. In this study, 70.5% and 29.5% of patients were males and females, respectively. Male sex was predominant in patients infected with all genotypes, in contrast to some studies form Turkey and around the world (Table 1)[44]. Altuglu et al. reported no significant difference in sex distribution in different genotypes in 2014. Karabulut et al. reported higher GT1 and GT2 rates in females in 2018[31]. In the present study, the median ages of patients were higher in females than in males. Also, the median ages of patients with genotype 2 and 3 were lower than with the other genotypes (Table 2).

In conclusion, genotype 1 is the most common genotype (54.7%), followed by genotype 3 (28.6%) in Adana provinence. The current study also confirms that genotype distributions vary with age, gender, and ALT concentrations.

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