

# Genotype distribution in Hepatitis C patients admitted to Erzincan Mengücek Gazi Training and Research Hospital

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## Abstract

**Aim:** We aimed to determine the genotype distribution in Hepatitis C patients who Parasitology applied to Erzincan Binali Yildirim University, Mengücek Gazi Training and Research Hospital. Hepatitis C virus, the only member of the genus Hepacivirus of Flaviviridae family, is responsible for 25%-40% of all liver diseases. Hepatitis C virus causes acute hepatitis C and chronic hepatitis C infections. Chronic hepatitis C infection causes cirrhosis, liver failure, hepatocellular cancer and liver diseases in terminal periods.

**Material and Methods:** A total of 77 patients, 41 male and 36 female, who were admitted to Erzincan Binali Yildirim University, Mengücek Gazi Training and Research Hospital in the period of January 2013-June 2019, were included in our study. RT-PCR and DNA sequencing for the 5'UTR region of the HCV genome for HCV genotyping was performed using the ABI Prism 3130 × 1 DNA Sequencer device.

**Results:** Sex of a total of 77 patients included in the study; 41 of them are men (53.2%) and 36 are women (46.8%). Average age of the patients; It was  $59.9 \pm 16.6$  (minimum 20 and maximum 94). HCV G1b (80.5%) in 62 patients, HCV G1a (7.8%) in 6, HCV G3a (3.9%) in 3, HCV G3 (2.6%) in 2 and HCV G2 (2.6%) was found in 2 patients. HCV G1 (1.3%) was detected in 1, HCV G4 (1.3%) in 1 patient.

**Conclusion:** The dominant genotype in Hepatitis C patients who applied to Mengücek Gazi Training and Research Hospital was determined as "HCV Genotype 1b".

**Keywords:** Erzincan; genotype; Hepatitis C virus

## INTRODUCTION

It was noticed in the 1970s that another virus other than hepatitis A and Hepatitis B viruses were transferred to human during blood transfusion. This virus was called as non-A non-B but could not be identified in those years. Then, in 1989, hepatitis C virus was proved as one of the factors causing hepatitis by Choo et al., which was called "non-A and non-B" using recombinant DNA technology (1). Hepatitis C virus belongs to Hepacivirus genus of Flaviviridae family. It is spherical, enveloped and single-stranded, positively directed, containing about 9,600 nucleotides, which mostly non-symptomatic or show acute or chronic course (1). It is a very serious and insidious disease that causes liver failure, cirrhosis and hepatocellular cancer, resulting in death when untreated (2,3).

The International Committee on taxonomy of viruses of Flaviviridae study group reported that the number of

Hepatitis C genotypes was 8 (on the basis of the viral genome sequence), 90 were certain, 44 were uncertain, and 13 were temporary (4). World Health Organization stated that 399,000 people died in the world due to cirrhosis and hepatocellular carcinoma caused by the Hepatitis C virus in 2016 and today approximately 71 million people in the world suffer from chronic hepatitis C (5).

Transmission of hepatitis C virus; it occurs primarily through the transfusion of blood and blood products (6,7). Today, in developed countries, other infectious materials, especially infected needles used by people using intravenous agents, are responsible for unsafe therapeutic injections and blood transfusion in developing countries (8-10). The most commonly used methods in the diagnosis of hepatitis C infection were serologically detection of antibodies (anti-HCV) against the hepatitis C virus with the enzyme immunoassay and molecular procedures. This molecular diagnostic method called polymerase

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chain reaction (PCR), in which the presence and quantity of RNA are determined. 7-14 days after the person's exposure to the virus, HCV RNA becomes positive while the person has not yet developed anti-HCV antibodies (11). Central Asia, South Asia, North Africa and the Middle East are geographies in the world where the prevalence of Hepatitis C virus infection is high (12). Genotypes with the highest prevalence; It is HCV G1 (49.1%) and HCV G3 (17.9%) and then HCV G4 (16.8%), HCV G2 (11%), HCV G5 (2%) and HCV G6 (1.4%) (13). Hepatitis C virus G5, HCV G6 occurs in a small geography and few people are infected with HCV G7, HCV G8 in the world. In Turkey, the dominant genotype is HCV G1 with 91.8-93.3%, followed by HCV G3 (3.7-4.9%), HCV G2 (1.5-2.2%), HCV G4 (1.1-2.5%) (14-16). When the studies conducted in Turkey examined, 80% of Hepatitis C virus G1 cases consist of HCV G1b cases (14-16).

## MATERIAL and METHODS

Serum samples of patients who previously admitted to Erzincan Binali Yıldırım University, Mengücek Gazi Training and Research Hospital, Department of Infectious Diseases and Clinical Microbiology with various complaints from January, 2013 to June, 2019 were examined. Of these, those with anti-HCV antibodies were identified and 77 patients with HCV RNA levels were included in this study. Presence of anti-HCV antibodies in these patients; the chemiluminescent microparticle enzyme immunoassay (EIA) was determined using the ABOTT Architect (i2000) sr device (Abbot Diagnostics, Chicago, IL, USA), which operates on the principle of the microparticle enzyme immunoassay (EIA) and qualitatively detects antibodies formed in the body against the Hepatitis C virus. Then, AmpliPrep/COBAS TaqMan 48 device (CAP / CTM, Roche, Diagnostics, Pleasanton, USA) was used in the 3-stage hybridization study to determine HCV RNA levels. In step 1, reverse transcription of the target was performed. In step 2, cDNA synthesis was performed. In step 3, million copies of complementary target DNA from cDNA were synthesized by PCR amplification. Finally, using the patient sera to determine the genotypes of the patients, the device ABI Prism 3130 × 1 DNA Sequencer (Applied Biosystems, ThermoFisher Scientific, USA.) was used for RT-PCR and DNA sequencing for the 5'UTR region of the HCV genome. This device produced capillary electrophoresis and generated fluorescent signals and converted them into digital data.

### Statistical analysis

The results were presented for categorical variables as n (%) and for continuous variables as mean ± standard deviation, median (minimum-maximum) value. Normality assumption for continuous variables was checked with the Kolmogorov-Smirnov normality test and hypothesis tests were selected according to the type of distribution. For variables with normal distribution, t-test was used while comparing groups (COPD-ACOS), and Mann-Whitney U test was used for variables that did not show

normal distribution. For all tests  $p < 0.05$  were considered statistically significant. Statistical analyses were performed by using IBM SPSS 20 (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.).

## RESULTS

The total of 77 patients [41 (53.2%) males and 36 (46.8%) females] was applied to the hospital infected with hepatitis C virus (Table 1). There is no significant difference between male and female patients. The average age of male patients was  $55.8 \pm 18.6$  (minimum 20 and maximum 87), the average age of female patients was  $64.5 \pm 12.7$  (minimum 31 and maximum 94) and the average age of all patients was  $59.9 \pm 16.6$  (minimum 20 and maximum 94), regardless of gender. According to this; the average age of women is more than men ( $p = 0.021$ ) (Table 1).

**Table 1. Gender distribution of patients infected with HCV**

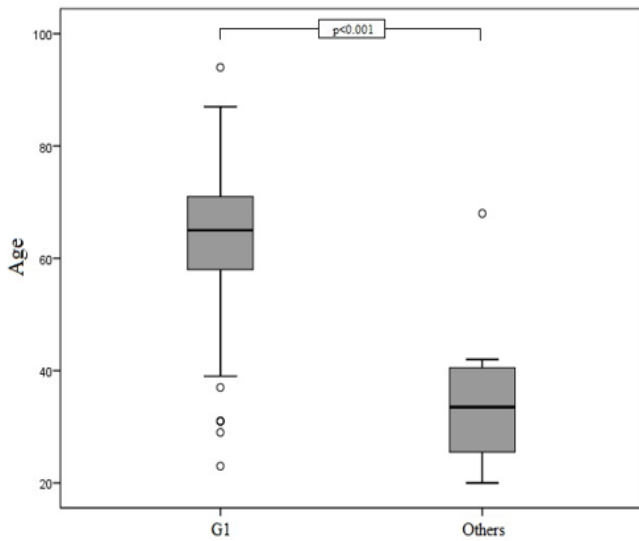
	n	%	
Gender	Male	41	53.2
	Female	36	46.8
	Total	77	100.0

In these 77 patients with hepatitis C, HCV G1b in 62 patients (80.5%), HCV G1a in 6 (7.8%), HCV G3a in 3 (3.9%), HCV G2 in 2 (2.6%), HCV G3 in 2 (2.6%), HCV G1 was detected in 1 (1.3%), and HCV G4 in 1 patient (1.3%) were identified (Table 2).

**Table 2. The genotype distribution of infected people with HCV**

Genotype	numbers	%
HCV G1	1	1.3
HCV G1a	6	7.8
HCV G1b	62	80.5
HCV G2	2	2.6
HCV G3	2	2.6
HCV G3a	3	3.9
HCV G4	1	1.3
Total	77	100.0

The average age of infected people with hepatitis C virus G1 is  $62.7 \pm 14.5$  and the average age of other genotype patients is  $35.9 \pm 15.2$  (Figure 1). According to this the average age of infected people because of hepatitis C virus G1 is more than the average age of other genotype patients ( $p < 0.001$ ) (Table 3).



**Figure 1.** The average age graphic of patients infected with hepatitis C virus G1 and other genotypes

The HCV RNA level of infected people with hepatitis C virus is found minimum 5104 IU/ml, maximum 11.430.000 IU/ml, mean 1.163.143 IU/ml.

### DISCUSSION

Today, the presence of Hepatitis C virus, which has 8 genotypes and many subtypes, was proved in 1989 by Choo et al using recombinant DNA technology (1, 4). Hepatitis C virus is one of the important health problems in the world as in our country (17-20). According to World Health Organization 2015 world report; 2.3 million people were infected with the Hepatitis C virus (21), 399,000 people died due to cirrhosis in the World in 2016 and today approximately 71 million people worldwide were infected with chronic hepatitis C (4). The World Health Council decided in 2016 to destroy viral hepatitis from the world until 2030 (22). The World Health Organization has published "World Health Sector Strategy" to achieve this goal and is carrying out the necessary studies (21). In addition, the American Liver Diseases Working Association

**Table 3.** HCV genotype and / or subtype studies conducted in Turkey

Researchers	Year	Province	Patient Numbers	G1 (%)	G1a (%)	G1b (%)	G2 (%)	G2a (%)	G2b (%)	G3 (%)	G3a (%)	G4 (%)	Mixed (%)	Others (%)
Abacioglu et al. (24)	1995	Izmir	89	-	19.1	75.3	3.4	-	-	-	-	2.2	-	-
Kucukoztas et al. (25)	2010	Istanbul	115	-	5.2	81.7	1.7	-	-	6.1	-	3.5	-	-
Buruk et al. (26)	2012	Six Eastern Black Sea Provinces	304	92.8	5.3	87.5	1.6	-	-	4.9	-	0.7	-	-
Us et al. (27)	2014	Eskisehir	203	74.4	2.4	17.7	1.4	-	-	1.9	-	1.9	-	-
Aktas et al. (17)	2014	Seven provinces in Eastern Anatolia	108	-	8.3	87	-	-	-	-	3.7	1 (G4d)	-	-
Balin et al. (28)	2016	Elazig	71	87.3	-	-	2.8	-	-	9.9	-	-	-	-
Karabulut et al. (29)	2016	Istanbul	412	82.5	-	-	4.6	-	-	10.7	-	2.2	-	-
Zeytinli et al. (30)	2017	Istanbul	554	-	23.1	56.5	-	-	-	-	17.3	-	1	-
Oz et al. (31)	2017	Sakarya	235	2.1	5.5	77.4	0.9	-	-	8.5	-	3	2.6	-
Our Study	2019	Erzincan	77	1.3	7.8	80.5	2.6	-	-	2.6	3.9	1.3	-	-

AASLD and the American Infectious Diseases Association IDSA share posts for the diagnosis, management and current treatment of Hepatitis C virus infection with the HCV Guidance website "www.HCV Guidelines.org" (23-31).

In our study, HCV G1b, which was detected in 62 (80.5%) of 77 patients, it was 81.7% in the study of Küçüköztaş et al. (25), 87.5% in the study of Buruk et al. (26) and it was 82.6% in the study of Tüzüner et al. (32).

In our study, HCV G1a was found as 2nd most frequent with 6 patients (7.8%), it was 23.1% in İstanbul province by Zeytinli et al. (30), and HCV G3a (3.9%) which was found in the 3rd place in our study, it was also 17.3% in Zeytinli et al. (30).

In our study, HCV G2 and HCV G3, which were 2.6%, were found to be 0.4% and 3.9%, respectively, in the studies of Ağca et al. (33). The study conducted by Üçbilek et al. in Çukurova Region with those who use intravenous substance, HCV G1 and HCV G3 were found to be 29.9% and 58.6%, respectively and HCV G1 was found to be 11.5% and HCV G1b was not detected (18).

In our study, HCV G4, which was detected in the lowest prevalence with 1.3% in 1 male patient using intravenous substance, it was 32% in the study conducted in Kayseri by Kayman et al. (34) and the highest rate in our country was in the study of Gökahmetoğlu et al. (35). While it was determined in prevalence, HCV G4 was not detected in the study of Üçbilek et al. (18) with those who used intravenous substances in Çukurova Region. In our study, HCV G1, which had the lowest prevalence in 1 patients with a rate of 1.3%, it was detected in the rate of 2.9% in the study of Tüzüner et al. (32). The genotype and subtype distribution of the patients infected with the hepatitis C virus were detected in our study. The ages of HCV G1b patients were older than the other genotypes patients. Our study was compatible with the studies of Abacıoğlu et al. (24), Küçüköztaş et al. (25), Gökahmetoğlu et al. (35), Kirişçi et al. (36). When our study was compared to the study results of Abacıoğlu et al. (24), Küçüköztaş et al. (25), Balın et al. (28), Gökahmetoğlu et al. (35), Sağlık et al. (37), Borcak et al. (38), Lee et al. (39), It could be seen that there was accordance with our study. No statistically significant correlation was found between HCV RNA quantitative levels of patients with different genotypes.

## CONCLUSION

Nowadays, HCV infection and HCV-related deaths have been largely prevented by taking wide-ranging preventive measures, especially the screening of blood and blood products. However, blood and blood products are still not screened in more than half of the world countries, intravenous substance use in both high-income and low-income countries is the primary route of transmission of the Hepatitis C virus, with a high incidence of HCV in the African and Asian continents. For these reasons, each country needs to pay attention to the distribution of HCV genotypes and create its own unique diagnosis and

treatment strategies.

With this study, genotype and subtype determination was performed for the first time in HCV patients who applied to Erzincan Mengücek Gazi Training and Research Hospital. According to the results of the study; In HCV patients who applied to the Hospital, HCV G1b was detected as the dominant genotype in 80.5%. Detection rates as follows; HCV G1a was in the 2nd place, HCV G3a was in the 3rd place, HCV G2 and HCV G3 at the same proportion was in the 4th place, and HCV G1 and HCV G4 at the same proportion was in the 5th place. This genotype distribution is compatible with our country in general. No statistically significant relationship was found between the HCV RNA quantitative levels of patients in different genotypes.

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