

Original Article

Hepatitis C virus genotypes and viremia in a tertiary hospital in Istanbul, TurkeySema Alacam¹, Ayfer Bakir², Aysel Karatas¹¹ *University of Health Sciences, Istanbul Training and Research Hospital, Department of Microbiology, Istanbul, Turkey*² *University of Health Sciences, Gulhane Training and Research Hospital, Department of Microbiology, Ankara, Turkey***Abstract**

Introduction: The World Health Organization estimates that 71 million people with chronic HCV infection lived worldwide in 2015. HCV is a globally prevalent pathogen, that genotype1 is the most common. In this study, the prevalence of anti-HCV, distributions of HCV genotype, and viremia rates in patients with chronic hepatitis C were evaluated.

Methodology: In this retrospective single-center study, anti-HCV results of 197,081 patients were evaluated between 2017 and 2020. Quantitative HCV-RNA PCR tests were performed on the Rotor-Gene Q real-time PCR instrument. HCV genotypes determination of 546 samples was carried out with the Gen-C 2.0 Reverse Hybridization strip and HCV Genotype Plus Real-TM kit.

Results: The prevalence of anti-HCV was 0.95% and viremic HCV infection was 0.3% (610/197,081). HCV viremia rate was 33.17%. HCV viremia rate was highest in 2017 (52.36%) and the lowest in 2020 (18.3%) ($p < 0.001$). Genotype1 (72%) was the most common genotype, followed by genotype3 (14.1%), and genotype4 (8.8%). The most common subtypes were determined as genotype1b (56.2%) and genotype1a (13.2%). The viral load was higher in patients infected with genotype5.

Conclusions: In this study, the rate of viremic HCV infection was found to be 0.3%. This rate was lower than the worldwide rate of HCV viremia. The distribution of HCV genotypes was like the global data. The identification of circulating genotypes and subtypes is essential for epidemiological purposes and remains important in the choice of treatment in patients with chronic HCV.

Key words: Hepatitis C virus; HCV genotype; HCV subtype; HCV viremia.

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Introduction

The hepatitis C virus (HCV), first identified in 1989, is a single-stranded an enveloped RNA virus belonging to the genus *Hepacivirus* within the family *Flaviviridae*. HCV is an important health problem affecting about 3% of the population in the world by causing chronic liver disease [1]. In a population infected with HCV, the infection clears up spontaneously within six months in 15-45% of them, whereas in 55-85% the infection becomes chronic. Liver fibrosis, cirrhosis, and chronic infection progressing to hepatocellular carcinoma may develop in 75-85% of infected individuals [2,3]. The World Health Organization (WHO) estimates that 71 million people with chronic HCV infection lived worldwide in 2015 [2]. Studies have shown that the prevalence of chronic HCV is highest in North Africa and the Middle East (> 2%) and lowest in Western Europe (< 1%). In addition, regional differences within the same country and

different distribution rates, especially in risk groups, have been reported [4].

The advent of direct-acting antiviral agents (DAAs) has significantly improved the field of hepatitis C treatment over the past few years. This recent development provided a cure rate of over 95% in HCV with a shorter treatment time and a very good safety profile [5]. Before starting treatment with these new DAAs, the clinical status of the patients should be evaluated, as well as the HCV genotype (GT) and viral load [6,7].

HCV, which has a high degree of genetic diversity, has been classified into 8 main GT and 86 subtypes [8]. GT1 is the most common in the world. It is estimated that more than one-third of GT1 cases are in East Asia and three-quarters of GT3 cases are in South Asia. GT2 and GT6-infected patients were reported to be mostly from East Asia, GT4 from North Africa and the Middle

East, and GT5 from South Africa and sub-Saharan East Africa [9].

HCV GT can be investigated by various laboratory methods. Sequencing of highly conserved regions such as NS5, Core, E, and 5' untranslated region (5'-UTR) is the gold standard method used for HCV genotyping [10,11]. Alternative HCV genotyping methods are amplification methods using type-specific primers or probes, restriction fragment length polymorphism (RFLP) analysis, line-probe assay and heteroduplex mobility analysis [12].

This study presented here had three objectives. The first was to document HCV GT distribution in patients in a tertiary hospital in Istanbul, Turkey's most heavily populated city. The second was to learn about the relationship between GTs and viral loads. The final was to evaluate the differences in anti-HCV, HCV viremia prevalence, and HCV viremia rates by years.

The study presented here had three aims. The first was to document the HCV GT distribution of patients in a tertiary hospital in Istanbul, Turkey's most densely populated city. Next was to learn about the relationship between GTs and viral loads. Finally, it was to evaluate the differences in anti-HCV, HCV viremia prevalence, and HCV viremia rates by years.

Methodology

Study design

This retrospective, single-center, cross-sectional study included 197,081 patients of all ages in whom anti-HCV testing serum samples were studied between January 2017 and November 2020. Demographic information of the patients was got from the hospital's information system and patient files. The first samples of the patients who were studied over one sample were evaluated. Since the study was designed retrospectively, informed consent was not got from the patients. Chronic HCV patients with reactive HCV test, positive HCV RNA and GT detected were included in the evaluation. To understand the changes in HCV prevalence according to the age of the patients, their patients were analyzed into five groups: < 30, 30-49, 50-69, and ≥ 70 years. The first test results of the patients with over one anti-HCV, HCV RNA, and HCV GT test were included in the study. This study was conducted with the approval of the Istanbul Training and Research Hospital Clinical Research Ethics Committee (Reference number: 2021/02/2721).

Anti-HCV analysis

Anti-HCV analyzes in patient serum samples were performed using Diasorin-Murex Anti HCV v4.0

(Diasorin, Italy) and Enzygnost Anti HCV 4.0 (Siemens, Germany) microELISA kits. Considering the cut-off (Co) value calculated automatically by the device, the results of the samples (S) were determined as S / Co.

Quantitative HCV RNA analysis

HCV-RNA in plasma samples was determined by quantitative real-time RT (reverse transcriptase) PCR. Viral nucleic acid isolation was performed using "The QIASymphony DSP Virus/pathogen midi kit" (Qiagen, Catalog No: 937055, Hilden, Germany) and QIASymphony SP/AS (Qiagen, Catalog No: 9001297, Hilden, Germany) instrument. Quantitative HCV-RNA PCR tests were performed on the Rotor-Gene Q real-time PCR instrument (Qiagen, Catalog No: 9001580, Hilden, Germany) using the Arthus HCV QS-RGQ PCR (Qiagen, Catalog No: 4518366, Hilden, Germany) kit. The dynamic range of the test was 50 IU/mL - 1×10^7 IU/mL, and the linear range was between 1.77×10^7 IU/mL - 2.50×10^7 IU/mL.

HCV Genotype Plus Real-TM

In this study, two different kits were used for the determination of HCV GT over 4 years. HCV Genotype Plus Real-TM (Sacace Biotechnologies, Como, Italy) kit is a real-time PCR test that simultaneously uses Internal Control to detect HCV RNA 1a, 1b, 2, 3, 4, 5a, and 6 GT in human plasma. For the determination of GT, a Rotor-Gene Q real-time PCR instrument (Qiagen, Catalog No: 9001580, Hilden, Germany) was used, and the results were evaluated in accordance with the kit manufacturer's recommendations. The analytical sensitivity of the HCV Genotype Plus Real-TM kit was 1000 IU / mL. Both the diagnostic specificity and sensitivity of the HCV Genotype Plus Real-TM kit tested were 100%.

Gen-C 2.0 Reverse Hybridization Strip Assay

Gen-C 2.0 Reverse Hybridization Strip Assay (Nuclear Laser Medicine, Italy) is an in vitro line probe assay for genotyping of HCV. The assay distinguishes between HCV GT based on variations in the 5'-UTR and core regions (GT1, 2, 3, 4 and 6 and subtypes; 1a, 1b, 2a/2c, 2b, 3a, 3b, 3c, 3k, 4a, 4b, 4c/4d, 4e, 4f, 4h, 5a, 6a/6b, 6g, f, q, m and 7a).

Amplification products were hybridized with oligonucleotide primers specific to different HCV GT on nitrocellulose strips. Hybridized sequences were marked with specific probes. According to the manufacturer's instructions, the bands got were compared with guides, and GTs were determined.

Statistical Analysis

SPSS version 22 (IBM Corp.) package program was used for the statistical evaluation of the data got in the study. Continuous data were given as median, categorical data were given as numbers and percentages. The compliance of the variables to normal distribution was examined using visual methods (histogram etc.) and the Kolmogorov-Smirnov test. Quantitative variables were compared using the Mann-Whitney U test and Kruskal-Wallis tests. Pearson’s Chi-Square or Fisher’s exact tests were used to comparing qualitative variables. The relationship between GTs and HCV RNA levels was compared with the Kruskal-Wallis test. The results were evaluated at the 95% confidence interval. For all statistical tests, results with a p-value below 0.05 were significant.

Results

Anti-HCV and viremic HCV infection prevalence

In our study, anti-HCV tests of 197,081 patients studied between 2017 and 2020 were examined. The age range of the patients was 2- 91 years. Anti-HCV test was determined as reactive in the serum of 1,878 patients, and the prevalence of anti-HCV was found to be 0.95%. [95% CI: 0.91-1.0]. HCV RNA was detected in 610 of 1,878 patients and HCV viremia rate was 33.17% [95% CI: 31.02-35.37]. The prevalence of viremic HCV infection was 0.3% (610/197,081) [95% CI: 0.29-0.33]. HCV GT was detected in 546 of 610 patients who were positive for HCV RNA by real-time PCR. The rate of HCV viremia was the highest in 2017

(52.36%) and the lowest in 2020 (18.30%) ($p < 0.001$) (Table 1).

HCV GT prevalence

A total of 563 GT and subtypes were detected in 546 patients with chronic hepatitis C. GT1 (72%) was the most common GT, followed by GT3 (14.1%) and GT4 (8.8%). According to the results, GT1b is the most common subtype, with 56.2% [95% CI: 51.9-60.4], followed by GT 1a 13.2% [95% CI: 10.5-16.3]. GT6 was detected in only one patient in the study population. Subtypes of GT could not be determined in 20% of patients. Demographic parameters of patients and distribution of HCV GTs are presented in Table 2.

In this study, foreign national patients comprised 20.5% of all patients. According to the ethnicity of the study population, GT1b was detected most frequently in both Turkish (59.9%) and foreign national (42.0%) patients ($p = 0.001$). Subsequently, GT1a and GT3 were the most common GT in Turkish and GT1a and GT4 in foreign nationals. When ethnic origins were compared, GT1b was significantly higher in the Turkish population, GT4 and GT4c/4d were significantly higher in foreign nationals. Infection with mixed HCV GT was detected in 8.6% (47/546) of the study population. Mixed infection rate was higher in foreign nationals ($p = 0.02$) (Table 2).

Distribution of HCV GT by age groups

In this study, the median age of 546 patients was 57.2 years. The median ages of the male and female patients were 54.2 [IQR (interquartile range) = 38.7-

Table 1. Anti-HCV, HCV viremia prevalence and viremia rates between 2017-2020.

Years	Anti-HCV		Anti-HCV prevalence		p-value
	Reactive, n	Total, n	%	95% CI	
2017	609	58,468	1.04	0.96-1.13	0.009
2018	468	54,267	0.86	0.79-0.94	
2019	455	45,784	0.99	0.90-1.09	
2020	346	38,562	0.89	0.81-1.0	
Total	1878	197,081	0.95	0.91-1.0	
	HCV RNA		Viremia prevalence		< 0.001
	Positive, n	Total, n	%	95% CI	
2017	122	58,468	0.21	0.17-0.25	
2018	272	54,267	0.50	0.44-0.56	
2019	158	45,784	0.34	0.29-0.40	
2020	58	38,562	0.15	0.11-0.19	
Total	610	197,081	0.30	0.29-0.33	
	HCV RNA		Viremia rate		< 0.001
	Positive, n	Total*, n	%	95% CI	
2017	122	233	52.36	45.74-58.92	
2018	272	751	36.22	32.77-39.77	
2019	158	538	29.37	25.55-33.42	
2020	58	317	18.30	14.20-23.0	
Total	610	1839	33.17	31.02-35.37	

HCV: hepatitis C virus; CI: confidence interval; Number of patients whose HCV RNA test was performed.

Table 2. Demographic parameters and HCV genotypes distribution of patients with HCV infection.

	Total	Female	Male	p-value	Turkish	Foreign nationals	p-value
Number of patient	546 (100)	297 (54.4)	249 (45.6)		434 (79.5)	112 (20.5)	
Age (median, IQR)	57.2 (42.2-68.2)	60.5 (45.9-70.7)	54.2 (38.7-64.2)	< 0.001	58 (44.3-69.0)	52.2 (37-65.7)	0.01
Single infection	499 (91.4)	270 (90.9)	229 (92.0)	0.66	403 (92.9)	96 (85.7)	0.02
Mixed infection	47 (8.6)	27(9.1)	20 (8.0)		31 (7.1)	16 (14.3)	
GT 1	14 (2.6)	7 (2.4)	7 (2.8)	0.74	14 (3.2)	0	0.05
GT 1a	72 (13.2)	33 (11.1)	39 (15.7)	0.12	57 (13.1)	15 (13.4)	0.94
GT 1b	307 (56.2)	168 (56.6)	139 (55.8)	0.86	260 (59.9)	47 (42.0)	0.001
GT 2	22 (4.0)	11 (3.7)	11 (4.4)	0.67	18 (4.1)	4 (3.6)	0.78
GT 2a/2c	15 (2.7)	9 (3.0)	6 (2.4)	0.66	12 (2.8)	3 (2.7)	0.96
GT 3	41 (7.5)	22 (7.4)	19 (7.6)	0.92	30 (6.9)	11 (9.8)	0.30
GT 3a	35 (6.4)	19 (6.4)	16 (6.4)	0.86	25 (5.8)	10 (8.9)	0.22
GT 3b	1 (0.2)	1 (0.3)	0	1.00	1 (0.2)	0	1.00
GT 4	31 (5.7)	15 (5.1)	16 (6.4)	0.49	18 (4.1)	13 (11.6)	0.002
GT 4a	1 (0.2)	1 (0.3)	0	1.00	0	1 (0.9)	0.21
GT 4c/4d	16 (2.9)	11 (3.7)	5 (2.0)	0.24	7 (1.6)	9 (8.0)	< 0.001
GT 5a	7 (1.3)	7 (2.4)	0	0.02	4 (0.9)	3 (2.7)	0.14
GT 6	1 (0.2)	1 (0.3)	0	1.00	1 (0.2)	0	1.00

HCV: hepatitis C virus; IQR: interquartile range; GT: genotype. *Data are expressed as n (%).

64.2] and 60.5 (IQR = 45.9-70.7) years, respectively ($p < 0.001$). GT1b was the most prevalent in all age groups. A significant difference in GT distribution by age groups was determined only for GT3. GT3 was highest in the 30-49 age group (22.8%) and lowest in the < 30 age groups (7.7%) ($p = 0.002$).

Distribution of HCV GT by gender

In this study, 54.4% of 546 patients were female. GT1b was the most common subtype in female (56.6%) [95% CI: 50.7-62.3] and male patients (55.8%) [95% CI: 49.4-62.1] ($p = 0.86$). Although GT5a was detected in 2.4% of females, it was not detected in any of the male patients ($p = 0.02$) (Table 2). GT6 was found in only one female.

Viral load relationship with HCV GT

GT5 was associated with higher viral loads and GT4 was associated with lower viral loads, and this was statistically significant ($p = 0.04$). The distribution of viral load according to GTs is shown in Table 3.

Discussion

HCV is an important cause of chronic liver disease, cirrhosis, and liver transplantations in Turkey [13]. It is estimated that there are approximately 200 to 500,000 viremic HCV-infected patients in Turkey [14]. In this study, we investigated HCV prevalence and GT distribution in our hospital over a 4-year period.

HCV is a globally prevalent pathogen. According to WHO data, the regions most affected by HCV infection are the Eastern Mediterranean Region (2.3%) and WHO European Region (1.5%) in 2015. The prevalence of HCV infection in other WHO regions range from 0.5% to 1.0%. In high-income countries, HCV prevalence is below 2%, but rises to over 5% in low- or middle-income countries [2,14]. In our study, the prevalence of HCV was 0.95%.

The global prevalence of viremic HCV infection was estimated to be 1.0%. Egypt has the highest prevalence of viremic HCV (10%) in the world, probably because of improper vaccination practices. It is also significantly higher in Pakistan (5.8%), Uzbekistan (4.4%) and Thailand (1.7%), while it is low in Austria (0.4%), Sweden (0.7%) and Canada (0.8%) and Iran (0.4%) [15]. In our study, the prevalence of

Table 3. Distribution of HCV RNA viral loads by genotypes.

GT	HCV RNA viral load (IU/mL)		p-value
	Median	IQR (Interquartil range)	
GT1	2.7×10 ⁶	8.5×10 ⁵ -1.0×10 ⁷	0.05
GT2	1.6×10 ⁶	4.3×10 ⁵ -5.5×10 ⁶	
GT3	2.7×10 ⁶	4.5×10 ⁵ -8.1×10 ⁶	
GT4	4.5×10 ⁵	1.5×10 ⁵ -4.2×10 ⁶	
GT5	1.9×10 ⁷	1.5×10 ⁶ -3.4×10 ⁷	
GT6*	4.5×10 ⁶	-	
Mixed GTs	2.9×10 ⁶	9.6×10 ⁵ -1.0×10 ⁷	

HCV: hepatitis C virus; GT: genotype; IQR: Interquartile range; * One patient.

viremic HCV infection was 0.3%. This prevalence rate was consistent with the results of studies conducted both in our country and in countries in our geographical region (the Middle East and Europe).

HCV caused the classification of different GT sequences, as its genome showed significant sequence diversity. These GT series have different geographic distributions around the world [16]. GTs are clinically relevant as current treatment regimens are tailored to GT type [17]. DAAs offer an unprecedented opportunity to reduce the disease burden, which has been used for HCV infection treatment since 2014 [18]. When planning the treatment of a patient with HCV, the HCV GT, previous treatment history of the patient and the stage of the liver disease are the information that should be known. HCV GT is important as it will determine the specific DAAs selection and the duration of treatment [19]. In this study, we retrospectively analyzed HCV GTs in patients with chronic HCV diagnosis in the last four years to support clinical management and contribute to the HCV GT epidemiology.

In this study, GT1 (72%) was the most common GT (subtype GT1b 56.2% and GT1a 13.2%) followed by GT3 (14.1%), GT4 (8.8%) and GT2 (6.7%), respectively. Globally, HCV GT1 and GT3 are more common than all other GTs [10,20]. In a multicenter study conducted to evaluate the distribution of HCV GT in Turkey in the period 2009-2014, GT1b was the most common (67.7%), followed by the indeterminate subtype GT1 (7.7%), GT4 (7.3%), and GT3 (6.7%). In 2014, GT3 (11.3%) was the second most common and GT4 (9.8%) was the third most common [21]. Turkey is a country with territory in both Europe and Asia. In addition, Turkey's geographical location is in the Middle East. GT distribution in Iran, Bulgaria, and Greece were like GT distribution in our country while in Syria and Iraq, Turkey's southern neighboring countries, the most common GTs are GT1 and GT4 [15]. This study may reflect the regional GT distribution, as it was carried out in Istanbul, one of Turkey's most immigrant-receiving cities.

HCV transmission occurs mainly through contact with blood products. Hemodialysis, blood transfusion, unsafe medical practices, and other parenteral exposures are important modes of transmission and are associated with GT [22,23]. In previous studies, it has been determined that there is a significant relationship between the routes of acquiring HCV infection and GT. GT1a, 3, and 4 have been associated with intravenous drug users, 1b and 2 with transfusion-related infection [24]. The distribution of HCV GTs and subtypes may

vary in large metropolitan areas with travel centers where intravenous drug use trends are increasing [25,26].

Considering the 4-year data in this study, there was no significant difference between the detection rates of GTs by years. The GT5 was less detected than the others. GT6 was detected only in one case in 2017. Since this study is retrospective, the risk factors of the patients in terms of GT distribution could not be questioned. Similar to recent years, GT1b was found to be the most frequent in studies conducted in different centers in our country in the last 20 years. However, it was observed that the distribution order of GT2, 3, and 4 varied according to years and regions [27].

In our study, the mixed infection rate was 8.6%. HCV infections with mixed GT have been reported previously in the literature, and numerous studies have been conducted to evaluate their prevalence in many geographic regions. A large difference is observed in the prevalence rates of mixed infections. It has been reported with estimates of 1.0-25.3% in various studies [28,29]. The natural course of HCV mixed infection, whether it is transient during the life of the host, its effects on disease progression, and treatment outcome are not fully understood [30].

In this study, subtypes could not be determined in 20% of the patients. This high rate suggests that the specificity of the probe and primer used in the PCR test should be improved. Improving primer and probe sets will increase the efficiency of each GT-specific amplification in the real-time PCR method [31].

HCV has high genetic variability. Different genotypes have been associated with response to treatment, disease progression, and comorbidities of patients [32,33]. In addition, patients with a higher HCV viral load have lower response rates to treatment based on interferon and DAAs [31,34]. In this study, the viral load of patients with HCV GT5 was significantly higher than those with other GT. This suggests that it may affect treatment options and the duration of treatment in patients with GT5.

This study has some limitations. Since the study was retrospective, transmission routes and risk groups could not be evaluated in patients. Because the designs of the kits used to identify GT were different, their subtype identification capabilities were not the same. According to the test procedures of the kits, 20% of the samples could not be subtyped because of its limited success in subtyping based on 5'-UTR.

Conclusions

In this study, the rate of viremic HCV infection was found to be 0.3%. This rate was lower than the worldwide rate of HCV viremia. The distribution of HCV GTs was like the global data. The viral load was higher in patients infected with GT5. Identification of circulating GTs and subtypes is essential for epidemiological purposes and remains important in the selection of treatment for patients with chronic HCV.

Authors' Contributions

Concept - S.A., A.B., A.K.; Design - S.A., A.B.; Supervision - S.A., A.B., A.K.; Data Collection and/or Processing - S.A., A.K.; Analysis and/or Interpretation - S.A., A.B.; Literature Search - S.A., A.B., A.K.; Writing - S.A., A.B., A.K.; Critical Reviews - S.A., A.B., A.K.

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