



The Predominance of Genotype 3 in Hepatitis C Virus in the Province of Kahramanmaras, Turkey/Genotype Distribution of Syrian Refugee Patients with Hepatitis C in Kahramanmaras Province

Özlem Kirişçi ^{1,*} and Ahmet Çalışkan ²

¹Medical Microbiology Department, Necip Fazil City Hospital, Kahramanmaras, Turkey

²Department of Medical Microbiology, Faculty of Medicine, Pamukkale University, Denizli, Turkey

*Corresponding author: Medical Microbiology Department, Necip Fazil City Hospital, Kahramanmaras, Turkey. Tel: +90-3442282800, E-mail: dr_ozlemgitmisoglu@hotmail.com

Received 2018 June 14; Revised 2019 February 26; Accepted 2019 March 01.

Abstract

Background: The genotype determination is of importance in the clinical management of hepatitis C virus (HCV) infection.

Objectives: The aim of this study was to evaluate the distribution of genotypes in HCV patients in the Kahramanmaras region, Turkey, and determine if the genotype rates of Syrian refugee patients with hepatitis C are different from the population they migrated to.

Methods: Blood samples sent to the microbiology laboratory between September 2014 and February 2018 were examined with genotyping using a real-time PCR reagent kit.

Results: The results of 274 patients who were HCV genotype-assigned were obtained from the hospital electronic information system. Of 230 Turkish HCV patients, 121 (52.8%) were identified as genotype 3, 100 (43.3%) as genotype 1, five (2.2%) as genotype 2, and four (1.7%) as genotype 4. Of the 44 Syrian refugee patients, 24 (54.5%) were identified as genotype 1, 18 (40.9%) as genotype 4, one (2.3%) as genotype 2, and one (2.3%) as genotype 3.

Conclusions: Predominant genotype 3 with the prevalence of 52.8% was seen to be more frequent in the current study region than in other regions of Turkey, demonstrating the highest rate of genotype 3 infection in Turkey to date. In Syria, the predominant HCV genotype is genotype 4 with a 57% prevalence, followed by genotype 1 with 29%. In the Syrian refugees, however, the current study showed genotype 1 was more frequent than genotype 4 (54.5% vs. 40.9%). This suggests that HCV genotypes may be affected by situations such as war and migration.

Keywords: Hepatitis C Virus, Genotype, Refugees, Turkey

1. Background

Following developments in molecular diagnosis methods, hepatitis C virus (HCV) has been determined as the main source of hepatitis that is non A, non B, which occurs following blood transfusion (1). It is estimated that between 170 and 200 million people worldwide have contracted chronic HCV and approximately 3% of the global population is affected by the HCV infection (2). The prevalence in Turkey has been reported in the range of 0.3-1.8% (3). The HCV genotype prevalence in different regions shows differences according to the population tested, the infection route, and virus mutation. It has been reported that throughout the world, genotype 1 (G1) is most common, accounting for 46% of all adult anti-HCV infections, followed by G3 (22%), G2 (13%), G4 (13%), G6 (2%), and G5 (1%).

Of the total HCV infections, 3% are associated with undefined or combination genotypes (4).

Genotype 1 is known to be predominant in Australia, China, Taiwan, Japan, Korea, Iran, and most countries in North Asia, Europe, Latin America, and North America (53-71% of all cases) while in Vietnam and other Southeast Asian countries, genotype 6 is more frequent. At lower rates than genotype 1, genotype 2 is seen at a considerable rate in Japan, Korea, and Taiwan. However, it is dominant in Mediterranean Countries and other areas of the Far East (4, 5).

In India, Pakistan, and Thailand predominates genotype 3. Middle Eastern countries such as Egypt, Saudi Arabia, Syria, Lebanon and North Africa report the genotype 4 infections to be predominant although genotype 3 is also seen in other Middle Eastern countries such as Iraq and

Iran, which can be possibly attributed to the patterns of migration in these regions (4, 5). Genotype 5 has been seen in a relatively small number of Syrian patients, and there have been few reports from Asia. Genotype 6 is known to be predominant in Asia, with the highest numbers reported from Vietnam (4, 5). In Turkey, HCV genotype 1 is predominant, with the frequency of genotypes 2, 3, and 4 varying according to the region (6).

2. Objectives

The aim of this study was to determine the HCV genotypes among Syrian refugee patients with hepatitis C in the Kahramanmaraş region and investigate if there is any difference in genotype compared to the population they have migrated to. Therefore, we also aimed to determine the genotype distribution in the Kahramanmaraş region, Turkey.

3. Methods

3.1. Sample Collection

Genotype analysis was applied to the samples of 274 patients with determined HCV RNA positive plasma samples from among different patients, which had been sent to the microbiology laboratory between September 2014 and February 2018. Hepatitis C Virus RNA and HCV genotypes are regularly tested in the setting hospital in samples from HCV-positive patients from the general population and from legal cases both in the city center and in the surrounding provincial areas.

3.2. Hepatitis C Virus RNA Analysis

Viral DNA was extracted using a commercial extraction kit (QIA-symphony DSP Virus/Pathogen Midi Kit, QIAGEN, Hilden, Germany). The quantification of HCV RNA was performed using Artus HCV QS-RG Q Kit (QIAGEN, Hilden, Germany) and the real-time polymerase chain reaction (PCR) method in the Rotor-Gene RG-Q (QIAGEN, Hilden, Germany) device.

3.3. Genotype Analysis of HCV RNA Positive Serum Samples

The HCV genotype was tested using the real-time PCR method as per the manufacturer's instructions. Genotypes 1, 2, 3, and 4 were analyzed. Genotyping was performed in serum samples obtained from 274 patients that were found to be positive for HCV-RNA. The sera were stored at -80°C until the day of analysis when they were warmed to room temperature before assay. The HCV genotype analysis was applied using the GenoSen HCV Genotyping 1/2/3/4 RT-PCR Reagent Kits (Genome Diagnostics, New Delhi, India).

For each reaction, $7.5\ \mu\text{L}$ of HCV Genotyping Super Mix (R1) was mixed with $2.5\ \mu\text{L}$ of HCV Genotyping Mg solution (R2) and $15\ \mu\text{L}$ of patient's serum. After 10 minutes of a single cycle at 95°C in the Rotor-RG-Q (QIAGEN, Hilden, Germany), 45 cycles were completed (15 seconds at 95°C , 20 seconds at 55°C , and 15 seconds at 72°C).

3.4. Statistical Analysis

All statistical analyses were done using SPSS V.24.0 software. Continuous variables were reported as means \pm standard deviation (SD) and categorical data as numbers (n) and percentages (%). The conformity of the data was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Kruskal-Wallis variance analysis and Mann-Whitney U test were applied in the comparisons of independent groups. For categorical variables, the chi-square test was used. A value of $P < 0.05$ was accepted as statistically significant.

4. Results

In this study, the HCV genotypes were examined in a total of 274 patients living in the province of Kahramanmaraş between September 2014 and February 2018. The total samples were obtained from 80 females (29%) and 194 males (70%) with a mean age of 42.8 ± 21.17 years. The age of patients showed a statistically significant difference between the four groups according to their genotype status ($P = 0.0001$). The age of patients with genotype 3 was found to be statistically significantly lower than that of individuals with genotype 1 and genotype 4 ($P = 0.0001$ and $P = 0.02$, respectively). A statistically significant difference was determined in terms of gender between the four genotype groups ($P = 0.0001$). The highest rate of GT1 was seen in women while and the rate of GT1 was 0.6 in men. GT3 was the most common genotype in men while it was only 4% in women.

The predominant genotype in hepatitis C patients living in Kahramanmaraş province was GT3 (52.8%) while in the Syrian refugees, GT1 was predominant with 54.5%. The genotype distributions, mean age, and gender of the patients are summarized in Tables 1 and 2.

In Syrian refugees, there was no statistically significant difference between gender groups in terms of age ($P = 0.791$).

5. Discussion

It is essential for an appropriate treatment algorithm to determine the general genotype distribution of the

Table 1. Genotype Distribution of HCV Patients in the Region of Kahramanmaraş, Turkey (n = 230)^a

| Genotype | No. (%) | Female | Male | Mean Age of Female Patients | Mean Age of Male Patients | Mean Age of Total Patients |
|----------|------------|---------|------------|-----------------------------|---------------------------|----------------------------|
| GT1 | 100 (43.5) | 45 (90) | 55 (30.6) | 65.5 ± 13.5 | 57.3 ± 19.4 | 61 ± 17.4 |
| GT2 | 5 (2.2) | 1 (2) | 4 (2.2) | 67 ^b | 24.3 ± 4.8 | 32.8 ± 19.6 |
| GT3 | 121 (52.6) | 2 (4) | 119 (66.1) | 19.5 ± 3.5 | 25.2 ± 6.7 | 25.1 ± 6.7 |
| GT4 | 4 (1.7) | 2 (4) | 2 (1.1) | 65 ± 2.8 | 50 ± 17 | 57.5 ± 13.2 |

Abbreviation: GT, Genotype.

^aValues are expressed as mean ± SD or No. (%).^bBecause of there was only one person, mean and standard deviation could not be calculated.**Table 2.** Genotype Distribution of Syrian Refugee Patients with HCV in the Region of Kahramanmaraş, Turkey (n = 44)^a

| Genotype | No. (%) | Female | Male | Mean Age of Female Patients | Mean Age of Male Patients | Mean Age of Total Patients |
|----------|-----------|-----------|----------|-----------------------------|---------------------------|----------------------------|
| GT1 | 24 (54.5) | 17 (56.7) | 7 (50) | 49.7 ± 16.5 | 45.4 ± 16.9 | 48.5 ± 16.4 |
| GT2 | 1 (2.3) | 1 (3.3) | - | 32 ^b | - | 32 ^b |
| GT3 | 1 (2.3) | - | 1 (7.1) | - | 42 ^b | 42 ^b |
| GT4 | 18 (40.9) | 12 (40) | 6 (42.9) | 53 ± 8.2 | 54.8 ± 21.8 | 53.6 ± 13.6 |

Abbreviation: GT, Genotype.

^aValues are expressed as mean ± SD or No. (%).^bBecause of there was only one person, mean and standard deviation could not be calculated.

country (7, 8). The HCV genotype distribution in a geographical area can be altered by increasing cultural diversity. War, migration, and tourism can affect the epidemiology of infections. In recent years, the change in HCV epidemiology has been noticeable in Turkey (9). Whether this change is regional or is reflected in the country, in general, could be revealed by studies conducted at specific intervals.

The most frequently seen genotype in Turkey has been reported to be 1b with a frequency of 60 - 100% (10). Genotypes 1a, 2, 3, and 4 infections are also seen at lower rates (10, 11). Generally, the rate of genotype 1 is high in internal regions (except in Kayseri) but in large cities and areas of immigration, it has been reported to be lower (10, 12-24). The lowest rate of genotype 1 (52.8 - 57.6%) has been reported in Kayseri (10). In a 2013 study, Kirişci et al. (25) reported a 60% frequency for type 1. In a study conducted by Caliskan et al. in 2015 (26), genotype 1 was determined at the rate of 51.7%. Thus, it can be seen that there was a decrease in genotype 1 in the region during that period. Compared to previous studies reporting genotype rates in Turkey, the current study showed the lowest rate to date for genotype 1 as 43.3%.

The prevalence of genotype 2 has been reported as 0 - 3.8% with regional differences (14, 16, 18). In the current study, the prevalence of genotype 2 was determined at 2.2%, which was consistent with other studies in Turkey (15, 16, 18). Given the prevalence of HCV genotype 3 in previous studies, it is observed that the rates of this genotype are low in Turkey (0 - 5.4%) (12-14, 23). However, Kucukoztas et al. re-

ported a rate as high as 9.6% (16).

In the current study, we observed a different prevalence rate for genotype 3 in the province of Kahramanmaraş so that it was highest, 52.8%, among the other rates reported to date in Turkey. In a 2013 study, Kirişci et al. reported a prevalence of 40% for genotype 3 (25). In a study conducted by Caliskan et al. in 2015 (26), genotype 3 was determined at a rate of 46%. Thus, an increase in the genotype 3 prevalence can be seen in the region during that period. The high prevalence rate in this study, which is different from other studies, indicates a difference, which could be explained by further analytical studies of the roots of population (refugees, prisoners, drug addicts, etc.) to determine whether this is a regional difference or a sign of a change in the genotype prevalence within the region as a result of the patients' distribution in the study area because of war and migration.

Studies in Turkey have reported the rate of HCV genotype 4 in the range of 0.6 - 35.6%. Hepatitis C Virus genotype 4 has a high prevalence in the provinces of Kayseri and Afyon in Turkey. In a study in Kayseri by Gokahmetoglu et al. (21), genotype 4 was found at a rate of 35.6%. In another study in Kayseri by Kayman et al. (10), Hepatitis C Virus genotype 4 was found to be 32%. In the current study, genotype 4 was detected at a rate of 1.7%, which was consistent with other studies in Turkey (9, 16). In Syria, the predominant genotype is genotype 4 (4c/4d) (59%), followed by genotype 1 (28.5%), genotype 5 (10.1%), genotype 3 (1.8%), and genotype 2 (0.8%) (4, 27). With the start of the

Syrian civil war in 2011, a massive migration wave began from Syria to Turkey. The results of studies carried out after this date in the regions of Turkey neighboring Syria are striking and will emerge through epidemiological studies on genotypes that are changing over time. In the current study, genotype 1 was determined at a higher rate (54.5%) than genotype 4 (40.9%) in the Syrian refugee patients. Although genotype 4 is predominant in Syria, genotype 1 was determined to be more dominant in the refugees who had migrated to Turkey.

5.1. Conclusions

This study suggests that genotype rates could be affected by migration. Moreover, this genotype profile is not similar to a regional genotype distribution (genotype 1, 54.5%) of refugee patients, but is close to the genotype profile of Turkey. This could be further explained by future studies conducted on larger patient populations.

A limitation of this study was the low number of Syrian refugee patients and therefore, the results may not reflect the entire population of Syrian refugee patients.

Footnotes

Authors' Contribution: Özlem Kirişci and Ahmet Çalışkan performed the experimental work, designed the study work, and provided samples. Özlem Kirişci prepared manuscript and reviewed the work.

Conflict of Interests: The authors had no conflict of interests.

Ethical Approval: The Ethics Committee of the Faculty of Medicine, Sıtcu Imam University, Kahramanmaraş, Turkey, approved this study with an issue number 28, dated August 29, 2018.

Financial Disclosure: The authors had no financial disclosure.

Funding/Support: The authors had no funding/support.

References

- Alter HJ, Purcell RH, Shih JW, Melpolder JC, Houghton M, Choo QL, et al. Detection of antibody to hepatitis C virus in prospectively followed transfusion recipients with acute and chronic non-A, non-B hepatitis. *N Engl J Med.* 1989;**321**(22):1494-500. doi: [10.1056/NEJM198911303212202](https://doi.org/10.1056/NEJM198911303212202). [PubMed: 2509915].
- Khan N, Akmal M, Hayat M, Umar M, Ullah A, Ahmed I, et al. Geographic distribution of hepatitis C virus genotypes in Pakistan. *Hepat Mon.* 2014;**14**(10). e20299. doi: [10.5812/hepatmon.20299](https://doi.org/10.5812/hepatmon.20299). [PubMed: 25477975]. [PubMed Central: [PMC4250967](https://pubmed.ncbi.nlm.nih.gov/PMC4250967/)].
- Sunbul M. [Epidemiology of HCV infection and prevention]. *J Viral Hepat.* 2007;**1**:208-19. Turkish.
- Gower E, Estes C, Blach S, Razavi-Shearer K, Razavi H. Global epidemiology and genotype distribution of the hepatitis C virus infection. *J Hepatol.* 2014;**61**(1 Suppl):S45-57. doi: [10.1016/j.jhep.2014.07.027](https://doi.org/10.1016/j.jhep.2014.07.027). [PubMed: 25086286].
- Sievert W, Altraif I, Razavi HA, Abdo A, Ahmed EA, Alomair A, et al. A systematic review of hepatitis C virus epidemiology in Asia, Australia and Egypt. *Liver Int.* 2011;**31** Suppl 2:61-80. doi: [10.1111/j.1478-3231.2011.02540.x](https://doi.org/10.1111/j.1478-3231.2011.02540.x). [PubMed: 21651703].
- Buruk CK, Bayramoglu G, Reis A, Kaklikkaya N, Tosun I, Aydin F. [Determination of hepatitis C virus genotypes among hepatitis C patients in Eastern Black Sea Region, Turkey]. *Mikrobiyol Bul.* 2013;**47**(4):650-7. Turkish. [PubMed: 24237433].
- European Association for Study of Liver. EASL Recommendations on Treatment of Hepatitis C 2015. *J Hepatol.* 2015;**63**(1):199-236. doi: [10.1016/j.jhep.2015.03.025](https://doi.org/10.1016/j.jhep.2015.03.025). [PubMed: 25911336].
- AASLD-IDS A HCV Guidance Panel. Hepatitis C guidance: AASLD-IDS recommendations for testing, managing, and treating adults infected with hepatitis C virus. *Hepatology.* 2015;**62**(3):932-54. doi: [10.1002/hep.27950](https://doi.org/10.1002/hep.27950). [PubMed: 26111063].
- Cetin Duran A, Kibar F, Cetiner S, Yaman A. Determination of Hepatitis C virus genotype and HCV infection transmission routes in Cukurova University Medical Faculty Hospital. *Turkish Bull Hyg Exp Biol.* 2017;**74**(3):201-10. doi: [10.5505/TurkHijyen.2017.24471](https://doi.org/10.5505/TurkHijyen.2017.24471).
- Kayman T, Karakucuk C, Karaman A, Gozutok F. [Genotype distribution of hepatitis C virus infection in Kayseri Region]. *Turk Mikrobiyol Cem Derg.* 2012;**42**(1):21-6. Turkish. doi: [10.5222/tmcd.2012.021](https://doi.org/10.5222/tmcd.2012.021).
- Ciccozzi M, Ciccaglione AR, Lo Presti A, Yalcinkaya T, Taskan ZP, Equestre M, et al. Reconstruction of the evolutionary dynamics of the hepatitis C virus 1b epidemic in Turkey. *Infect Genet Evol.* 2011;**11**(5):863-8. doi: [10.1016/j.meegid.2011.02.006](https://doi.org/10.1016/j.meegid.2011.02.006). [PubMed: 21324382].
- Abacioglu YH, Davidson F, Tuncer S, Yap PL, Ustacelebi S, Yulug N, et al. The distribution of hepatitis C virus genotypes in Turkish patients. *J Viral Hepat.* 1995;**2**(6):297-301. doi: [10.1111/j.1365-2893.1995.tb00045.x](https://doi.org/10.1111/j.1365-2893.1995.tb00045.x).
- Ozacar T, Altuglu I, Zeytinoglu A, Sayiner A, Akarca U, Erensoy S, et al. [Distribution of HCV genotypes in chronic hepatitis C]. *Mikrobiyol Bul.* 2001;**35**:451-8. Turkish.
- Kalayci R, Altindis M, Gulamber G, Demirturk N, Akcan Y, Demirdal T. [Genotype distribution of chronic hepatitis B and hepatitis C patients and investigation of the resistance patterns in hepatitis B cases]. *Mikrobiyol Bul.* 2010;**44**(2):237-43. Turkish. [PubMed: 20549958].
- Sanlidag T, Akcali S, Ozbakkaloglu B, Ertekin D, Akduman E. [Distribution of hepatitis C virus genotypes in Manisa region, Turkey]. *Mikrobiyol Bul.* 2009;**43**(4):613-8. Turkish. [PubMed: 20084914].
- Kuckoztas MF, Ozgunes N, Yazici S. [Investigation of the relationship between hepatitis C virus (HCV) genotypes with HCV-RNA and alanine aminotransferase levels in chronic hepatitis C patients]. *Mikrobiyol Bul.* 2010;**44**(1):111-5. Turkish. [PubMed: 20455406].
- Aktas E, Ogedey ED, Kulah C, Begendik Comert F. [Hepatitis C virus genotypes in a province of western Black-Sea region, Turkey]. *Mikrobiyol Bul.* 2010;**44**(4):647-50. Turkish. [PubMed: 21063977].
- Bozdayi G, Rota S, Verdi H, Derici U, Sindel S, Bali M, et al. [The presence of hepatitis C virus (HCV) infection in hemodialysis patients and determination of HCV genotype distribution]. *Mikrobiyol Bul.* 2002;**36**(3-4):291-300. Turkish. [PubMed: 12838663].
- Ural O, Arslan U, Findik D. [The distribution of hepatitis C virus genotype in the Konya region, Turkey]. *Turkish J Infect.* 2007;**21**:175-81. Turkish.
- Celik C, Bakici MZ, Kaygusuz R, Erturk R. [Investigation of HCV genotype distributions in Sivas region]. *J Viral Hepat.* 2010;**16**(3):106-10. Turkish.
- Gokahmetoglu S, Atalay MA, Kylync A. [Determination of the hepatitis C virus genotypes with 'Pyrosequencing' method]. *Erciyes Med J.* 2011;**33**(2):99-102. Turkish.
- Altuglu I, Soyler I, Ozacar T, Erensoy S. Distribution of hepatitis C virus genotypes in patients with chronic hepatitis C infection in Western Turkey. *Int J Infect Dis.* 2008;**12**(3):239-44. doi: [10.1016/j.ijid.2007.07.003](https://doi.org/10.1016/j.ijid.2007.07.003).

23. Cil T, Oezekinci T, Goeral V, Altintas A. [Hepatitis C virus genotypes in the southeast region of Turkey]. *Turkey Clin J Med Sci*. Turkish.
24. Tezcan S, Ulger M, Aslan G, Yaras S, Altintas E, Sezgin O, et al. [Determination of hepatitis C virus genotype distribution in Mersin province, Turkey]. *Mikrobiyol Bul*. 2013;**47**(2):332-8. Turkish. [PubMed: [23621733](#)].
25. Kirişci O, Caliskan A, Alkis Koçturk S, Erdogmus P, Gul M. [Genotype distribution in infected individuals with hepatitis C virus in Kahramanmaraş and the relationship between HCV-RNA load and ALT-AST in genotype]. *J Viral Hepat*. 2013;**19**(2):67-70. Turkish. doi: [10.4274/Vhd.57966](#).
26. Caliskan A, Kirişci O, Ozkaya E, Ozden S, Tumer S, Caglar S, et al. Distribution and predominance of genotype 3 in hepatitis C virus carriers in the province of kahramanmaraş, Turkey. *Hepat Mon*. 2015;**15**(4). e25142. doi: [10.5812/hepatmon.15\(4\)2015.25142](#). [PubMed: [25972903](#)]. [PubMed Central: [PMC4426333](#)].
27. Ramia S, Eid-Fares J. Distribution of hepatitis C virus genotypes in the Middle East. *Int J Infect Dis*. 2006;**10**(4):272-7. doi: [10.1016/j.ijid.2005.07.008](#). [PubMed: [16564719](#)].