TÜBİTAK

Turkish Journal of Medical Sciences

http://journals.tubitak.gov.tr/medical/

Research Article

Turk J Med Sci
(2014) 44: 661-665

doi:10.3906/sag-1308-35

Hepatitis C virus genotypes in Adana and Antakya regions of Turkey*

Agah Bahadır ÖZTÜRK[1], Ümit Bilge DOĞAN[2,], Nevin AKÇAER ÖZTÜRK[2], Gonca ÖZYAZICI[3], Mehmet DEMİR[4], Mustafa Salih AKIN[2], Ali Suha BÖNGÖL[5]**

[1]Department of Family Medicine, Adana Numune Training and Research Hospital, Adana, Turkey
[2]Department of Gastroenterology, Adana Numune Training and Research Hospital, Adana, Turkey
[3]Department of Microbiology, Adana Numune Training and Research Hospital, Adana, Turkey
[4]Department of Gastroenterology, Faculty of Medicine, Mustafa Kemal University, Hatay, Turkey
[5]Çukurova Dr Aşkım Tüfekçi State Hospital, Adana, Turkey

Received: 09.08.2013 • **Accepted:** 27.11.2013 • **Published Online:** 27.05.2014 • **Printed:** 26.06.2014

Background/aim: Hepatitis C virus (HCV) genotype 1 was found to be dominant in Turkey. In this study, HCV genotypes were examined in the Adana and Antakya regions of Turkey.

Materials and methods: The study consisted of 639 HCV-RNA–positive patients with chronic HCV infection in Adana (214 males and 101 females) and Antakya (139 males and 185 females) in Turkey. Real time-polymerase chain reaction was used for genotype determination.

Results: In Antakya, it was determined that the percentages of genotypes of type 1a (0.31%), 1b (86.73%), 2 (9.26%), 3 (0.93%), and 4 (2.78%) were compatible with the nationwide results seen in Turkey. In Adana, the percentages of genotypes of type 1a (3.49%), 1b (55.24%), 2 (14.60%), 3 (26.03%), and 4 (0.63%) were found to be different. This difference was mainly due to the infection rates in males: genotype 1b was significantly lower (42.5% versus 82.2%, $P < 0.001$) in men in Adana, but genotype 2 (17.8% versus 7.9%, $P = 0.021$) and genotype 3 (34.6% versus 7.9%, $P < 0.001$) were significantly higher in men than in women in Adana.

Conclusion: Rates of genotypes 2 and 3 were unexpectedly high in Adana compared to other parts of Turkey.

Key words: Hepatitis C, genotypes, Adana, Antakya, Turkey

1. Introduction

Hepatitis C virus (HCV) infection is a serious global public health problem, with approximately 180 million people chronically infected with hepatitis C worldwide (1). HCV is a frequent cause of chronic liver disease, including cirrhosis and hepatocellular carcinoma (2). RNA transcription errors that occur during replication of the virus play an important role in chronicity. Differences in the genetic sequence resulting from these errors create genotypes of HCV. HCV is classified into 6 genotypes and a large number of subtypes based on their genomic sequences (3). Genotypes 1–3 have worldwide distribution. Genotype 1 is predominant in northern Europe and North America, and in southern and eastern Europe and Japan. Genotype 2 is less frequently represented than type 1. Genotype 3 is endemic in Southeast Asia and is variably distributed in different countries. Genotype 4 is principally found in the Middle East, Egypt, and central Africa (4). In Turkey, genotype 1b has been found to be dominant.

Determination of the genotype of HCV is an important parameter that determines the duration and response of antiviral therapy in patients with chronic hepatitis C. Thus, determination of genotype is considered to be cost-effective (5). In this study, HCV genotypes were examined in the Adana and Antakya regions of Turkey.

2. Materials and methods

2.1. Patients

Between December 2010 and December 2012, 639 HCV-RNA–positive patients, who were admitted to Adana Numune Training and Research Hospital, Adana Çukurova State Hospital, or the Faculty of Medicine of Hatay Mustafa Kemal University (all within southern Turkey), were included in this study. The study was approved by

* This manuscript was presented at the 23rd Conference of the Asian Pacific Association for the Study of the Liver, 7–10 March 2013, Singapore, as a poster, and was published as a poster presentation in Hepatol Int 2013; 7 (Suppl. 1): 426.

** Correspondence: ubdogan@ hotmail.com

the institutional review board and was conducted in accordance with the provisions of the Declaration of Helsinki and good clinical practice guidelines.

2.2. Viral RNA extraction

Viral RNA was extracted from serum samples using a QIAGEN Viral RNA Kit (Milan, Italy) according to the manufacturer's instructions.

2.3. Real-time quantitative PCR

HCV-RNA load was determined by quantitative real-time PCR. Quantification of HCV-RNA load in serum samples was performed using the QIAGEN Artus HCV RG RT-PCR Kit (QIAGEN). An aliquot of 20 µL of purified sample isolated from the serum was used for amplification in a total reaction volume of 50 µL. The amplification reaction for each sample and standard was performed in duplicate. Amplification cycling was performed using the Rotor-Gene 6000 device (QIAGEN). Data analysis was performed with the Rotor-Gene software according to the manufacturer's instructions.

2.4. Pyrosequencing

Two PCRs were generated for each sample by amplifying 2 different regions of the HCV genome using a One-Step RT-PCR Kit (QIAGEN). PCR-1 and PCR-2 products of approximately 240 bp and 180 bp in length were obtained. PCR products were immobilized on Streptavidin Sepharose (GE) beads. HCV genotypes were determined by pyrosequencing assay using HCV genotype sequencing primers (PyroMark Q24, QIAGEN). Four sequencing primers were used. Aliquots of 2.5 µL of each sequencing primer were added to the immobilized PCR products. For pyrosequencing, the single-stranded PCR amplicon that served as a template was hybridized with sequencing primers and the reaction was started by addition of one of the nucleotides as standard pyrosequencing. Analysis of sequences was performed using the PyroMark Q24 software (QIAGEN). Genotypes were classified according to Simmonds et al. (6).

2.5. Statistics

Analyses were calculated with SPSS 18.0.0 for Windows. Student's t-test was used to assess the significance of genotype rates.

3. Results

Location, sex, and age characteristics of 639 HCV-RNA–positive patients are shown in Table 1. Of the patients, 214 were male and 101 were female in Adana; 139 were male and 185 were female in Antakya. The mean ages were 47.2 ± 19 years in Adana and 57.1 ± 13.2 years in Antakya.

Genotype distributions of the patients in Adana and Antakya are shown in Table 2. Distributions of genotypes were different in Antakya and Adana. Genotype 1b was found to be dominant and genotype 2 the second most common type in Antakya. In Adana, genotype 1b was also the most common type, but the incidence was very low. Surprisingly, genotype 3 was the second most common type in Adana, and genotype 2 was ranked third.

In order to understand the reason for these differences between the regions of Antakya and Adana, the genotypes of the patients were also compared by sex. Genotypes 1 and 2 were seen in men and women at the same rate in Antakya. Only genotypes 3 and 4 were found just slightly more often in males ($P < 0.05$) (Table 3).

In contrast, distributions of genotypes of the patients in Adana according to sex were different. In Adana, the rate of genotype 1b was found to be lower (42.52%) in males than in females ($P < 0.001$). The rate of genotype 3 was also very high (34.58%) in males ($P < 0.001$). Genotype 2 was ranked third, and again it occurred at a higher rate in males (Table 4).

The rates of genotypes 1a, 1b, 2, and 4 in the females of Adana were similar to those in the females of Antakya. The rate of genotype 3 was found to be higher in women in Adana than in women in Antakya ($P < 0.001$) (Table 5).

4. Discussion

HCV genotypes are important determinants in the treatment protocols in the treatment of chronic hepatitis C. In addition, changes in the distribution of genotypes are important because they may show interregional transmission according to migration or drug use. In the previous studies within Turkey, genotype 1b has been found to be dominant (Table 6). However, the rates of genotypes other than genotype 1b vary compared to those of Turkey overall. Genotype 1a was the most common in İzmir (7,8), Ankara (9–11), Diyarbakır (12), and Afyon (13). Genotypes 2 and 3 were the least commonly seen genotypes in Turkey. Genotype 2 was found to be relatively common in Gaziantep (14), and genotype 3 was found to be relatively common in İstanbul (15). Genotype 4 has been reported at low rates in many Turkish cities, but very high rates of genotype 4 were observed in Kayseri in recent studies (16,17).

Table 1. Region, sex, and age of 639 HCV-RNA–positive patients.

Region	n	Male, n (%)	Female, n (%)	Age, mean ± SD (min–max)
Adana	315	214 (67.9)	101 (32.1)	47.2 ± 19.0 (18–82)
Antakya	324	139 (42.9)	185 (57.1)	57.1 ± 13.2 (20–86)
Total	639	353 (55.2)	286 (44.8)	52.2 ± 17.0 (18–86)

Table 2. Genotype distributions of the patients in Adana and Antakya.

	Total (n = 639)	Antakya (n = 324)	Adana (n = 315)
Genotype 1a, n (%)	12 (1.9)	1 (0.31)	11 (3.49)
Genotype 1b, n (%)	455 (71.2)	281 (86.73)	174 (55.24)
Genotype 2, n (%)	76 (11.9)	30 (9.26)	46 (14.60)
Genotype 3, n (%)	85 (13.3)	3 (0.93)	82 (26.03)
Genotype 4, n (%)	11 (1.7)	9 (2.78)	2 (0.63)

Table 3. Genotype distributions of the patients in Antakya according to sex.

	Male (n = 139)	Female (n = 185)
Genotype 1a, n (%)	1 (0.72)	0 (0.00)
Genotype 1b, n (%)	117 (84.17)	164 (88.65)
Genotype 2, n (%)	10 (7.19)	20 (10.81)
Genotype 3, n (%)	3 (2.16)	0 (0.00)
Genotype 4, n (%)	8 (5.76)	1 (0.54)

Our study was conducted in 2 major cities. Distributions of genotypes in Antakya were consistent with the overall distributions in Turkey. The only exception is that the rate of genotype 2 was found to be higher compared to the overall national rate, but it was similar to the rate in Gaziantep (14). This is likely because Antakya and Gaziantep are geographically close to each other. On the other hand, rates of genotypes 2 and 3 were found to be significantly higher in Adana. These results are very different from the results of Yarkın and Hafta (18), who reported the rates of genotypes 1a, 1b, and 2 as 14.5%, 82.2%, and 3.3% in Adana, respectively. They found no relationship between genotype and sex.

Table 4. Genotype distributions of the patients in Adana according to sex.

	Male (n = 214)	Female (n = 101)
Genotype 1a, n (%)	9 (4.21)	2 (1.98)
Genotype 1b, n (%)	91 (42.52)	83 (82.18)
Genotype 2, n (%)	38 (17.76)	8 (7.92)
Genotype 3, n (%)	74 (34.58)	8 (7.92)
Genotype 4, n (%)	2 (0.93)	0 (0.00)

In our study, in the females of Adana, the rate of genotype 3 was significantly higher than in Antakya, even though the rate of genotype 2 was similar to the rate found in Antakya.

The rate of genotype 1b, which was 42.52% in males in Adana, was the lowest rate detected so far in Turkey. Moreover, the rates of genotypes 2 and 3, which were 17.76% and 34.58% in males in Adana, respectively, were the highest rates detected so far in Turkey.

In conclusion, the distribution of HCV genotypes differed in neighboring cities. In Adana, the rate of genotype 1 was found to be low, and the rates of genotypes 2 and 3 were unexpectedly high in men compared to other parts of Turkey. Further analytical studies are needed to uncover the origin of HCV genotypes 2 and 3 as seen in these patients.

Table 5. Genotype comparison of males and females in Adana and Antakya.

	Adana, female (n = 101)	Antakya, female (n = 185)	P*	Adana, male (n = 214)	Antakya, male (n = 139)	P*
Genotype 1a, n (%)	2 (1.98)	0 (0.00)	0.055	9 (4.21)	1 (0.72)	0.054
Genotype 1b, n (%)	83 (82.18)	164 (88.65)	0.128	91 (42.52)	117 (84.17)	0.000**
Genotype 2, n (%)	8 (7.92)	20 (10.81)	0.434	38 (17.76)	10 (7.19)	0.005
Genotype 3, n (%)	8 (7.92)	0 (0.00)	0.000**	74 (34.58)	3 (2.16)	0.000**
Genotype 4, n (%)	0 (0.00)	1 (0.54)	0.461	2 (0.93)	8 (5.76)	0.008

*: Student's t-test, **: $P < 0.001$.

Table 6. HCV genotype determinations made at different centers in Turkey.

Study	Year	Centers	n	Genotype %							
				1 (%)	1a (%)	1b (%)	2 (%)	3 (%)	4 (%)	Mixed (%)	X** (%)
Abacioglu et al. (7)	1995	İzmir	89	-	19.1	75.3	3.4	-	2.2	-	-
Erensoy et al. (8)	2002	İzmir	50	-	30	60	-	-	-	-	10
Bozdayı et al. (9)	2002	Ankara	36	-	22.2	77.8	-	-	-	-	-
Bozdayi et al. (10)	2004	Ankara	365	-	11	84	3	1	1	-	-
Selcuk et al. (11)	2006	Ankara	130	-	24.6	68.5	-	-	7	-	-
Çil et al. (12)	2007	Diyarbakır	22	-	22.7	72.7	-	4.5	-	-	-
Kalaycı et al. (13)	2010	Afyon	30	3.3	20	63.3	-	-	13.3	-	-
Karslıgil et al. (14)	2011	Gaziantep	51	-	9.8	78.4	7.8	2	2	-	-
Küçüköztaş et al. (15)	2010	İstanbul	115	-	1.9	76.9	3.8	9.6	7.6	-	-
Gökahmetoğlu et al. (16)	2011	Kayseri	146	5.5	3.4	52.8	2.7	-	35.6	-	-
Kayman et al. (17)	2012	Kayseri	375	2.4	2.4	57.6	3.2	1	32	1.4	-
Yarkın and Hafta (18)	2000	Adana	62	-	14.5	82.2	3.3	-	-	-	-
Sönmez et al. (19)	1996	Malatya	59	-	-	69.5	-	-	-	5.1	25.4
Yalçın et al. (20)	1999	Diyarbakır	28	-	-	100	-	-	-	-	-
Özacar et al. (21)	2001	İzmir	170	-	10	81.2	2.4	0.6	1.2	4.7	-
Altindis et al. (22)	2006	N. Cyprus	53	-	5.7	92.4	1.9	-	-	-	-
Gökahmetoğlu et al. (23)	2007	Kayseri	57	-	3.5	96.5	-	-	-	-	-
Ural et al. (24)	2007	Konya	80	-	-	100	-	-	-	-	-
Altuglu et al. (25)	2008	İzmir	345	-	9.9	87.2	0.9	1.4	0.6	-	-
Şanlıdağ et al. (26)	2009	Manisa	100	-	2	90	2	-	5	-	1
Çiftçi et al. (27)	2009	Afyon	34	91.2	-	-	-	-	8.8	-	-
Ozbek et al. (28)	2009	Diyarbakır	74	4.1	-	87.8	2.7	5.4	-	-	-
Aktaş et al. (29)	2010	Zonguldak	39	-	2.6	97.4	-	-	-	-	-
Çelik et al. (30)	2010	Sivas	178	-	9	88.2	1.1	1.7	-	-	-
This study	2013	Antakya	324	-	0.3	86.7	9.3	0.9	2.8	-	-
		Adana	315	-	3.5	55.2	14.6	26.0	0.6	-	-

**X = Genotype could not be determined.

References

1. World Health Organization. Hepatitis C fact sheet. Geneva, Switzerland: WHO; 2014. Available at http://www.who.int/mediacentre/factsheets/fs164/en/.
2. Perz JF, Armstrong GL, Farrington LA, Hutin YJ, Bell BP. The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. J Hepatol 2006; 45: 529–538.
3. Forns X, Bukh J. The molecular biology of hepatitis C virus. Genotypes and quasispecies. Clin Liver Dis 1999; 3: 693–716.
4. Shepard CW, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. Lancet Infect Dis 2005; 5: 558–567.
5. Hadziyannis SJ, Sette H Jr, Morgan TR, Balan V, Diago M, Marcellin P, Ramadori G, Bodenheimer H Jr, Bernstein D, Rizzetto M et al. Peginterferon-α2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. Ann Intern Med 2004; 140: 346–355.
6. Simmonds P, Bukh J, Combet C, Deléage G, Enomoto N, Feinstone S, Halfon P, Inchauspé G, Kuiken C, Maertens G et al. Consensus proposals for a unified system of nomenclature of hepatitis C virus genotypes. Hepatology 2005; 42: 962–973.
7. Abacioglu YH, Davidson F, Tuncer S, Yap PL, Ustacelebi S, Yulug N, Simmonds P. The distribution of hepatitis C virus genotypes in Turkish patients. Viral Hepatit J 1995; 2: 297–301.
8. Erensoy S, Goksel S, Akarca US, Ozkahya M, Canatan D. Genotyping of hepatitis C virus by direct sequence analysis of polymerase chain reaction products. Flora 2002; 7: 104–111.
9. Bozdayı G, Rota S, Verdi H, Derici Ü, Sindel Ş, Bali M, Başay T. Investigation of the presence of hepatitis C virus (HCV) infection in hemodialysis patients and determination of HCV genotypes. Mikrobiyol Bul 2002; 36: 291–300 (article in Turkish with an abstract in English).
10. Bozdayi AM, Aslan N, Bozdayi G, Türkyilmaz AR, Sengezer T, Wend U, Erkan O, Aydemir F, Zakirhodjaev S, Orucov S et al. Molecular epidemiology of hepatitis B, C and D viruses in Turkish patients. Arch Virol 2004; 149: 2115–2129.
11. Selcuk H, Kanbay M, Korkmaz M, Gur G, Akcay A, Arslan H, Ozdemir N, Yilmaz U, Boyacioglu S. Distribution of HCV genotypes in patients with end-stage renal disease according to type of dialysis treatment. Dig Dis Sci 2006; 51: 1420–1425.

12. Çil T, Özekinci T, Göral V, Altıntaş A. Hepatitis C virus genotypes in the southeast region of Turkey. Türkiye Klinikleri J Med Sci 2007; 27: 496–500 (article in Turkish with an abstract in English).

13. Kalaycı R, Altındiş M, Gülamber C, Demirtürk 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: 237–243 (article in Turkish with an abstract in English).

14. Karslıgil T, Savaş E, Savaş MC. Genotype distribution and 5' UTR nucleotide changes in hepatitis C virus. Balkan Med J 2011; 28: 232–236 (article in Turkish with an abstract in English).

15. Küçüköztaş MF, Özgüneş N, Yazıcı 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: 111–115 (article in Turkish with an abstract in English).

16. Gökahmetoğlu S, Atalay MA, Kılınç A. Determination of the hepatitis C virus genotypes with 'pyrosequencing' method. Erciyes Med J 2011; 33: 99–102 (article in Turkish with an abstract in English).

17. Kayman T, Karakükçü Ç, Karaman A, Gözütok F. Genotypic distribution of hepatitis C virus infection in Kayseri region. J Turk Mikrobiyol Soc 2012; 42: 21–26 (article in Turkish with an abstract in English).

18. Yarkın F, Hafta A. The distribution of hepatitis C virus (HCV) genotypes in patients with chronic hepatitis C infection. Viral Hepatit J 2000; 3: 164–167 (article in Turkish with an abstract in English).

19. Sönmez E, Taşyaran MA, Kızılkaya N, Korkut H, Tombul Z, Akçam ZC, Yılmaz Ş, Köksal F, Leblebicioğlu H, Ekici H. The distribution of hepatitis C virus (HCV) genotypes in 59 HCV infected patients: a multicenter study. Flora 1996; 2: 92–95 (article in Turkish with an abstract in English).

20. Yalçın K, Değertekin H, Akkız H. HCV genotypes in HCV related chronic hepatitis in Southeast Anatolia. Turk J Gastroenterol 1999; 10: 249–252.

21. Özacar T, Altuğlu İ, Zeytinoğlu A, Sayıner AA, Akarca U, Erensoy S, Bilgiç A. Distribution of HCV genotypes in chronic hepatitis C. Mikrobiyol Bul 2001; 35: 451–458 (article in Turkish with an abstract in English).

22. Altindis M, Yilmaz S, Dikengil T, Acemoglu H, Hosoglu S. Seroprevalence and genotyping of hepatitis B, hepatitis C and HIV among healthy population and Turkish soldiers in Northern Cyprus. World J Gastroenterol 2006; 12: 6792–6796.

23. Gökahmetoğlu S, Bozdayı M, Özbakır O, Aygen B, Özbal Y, Soyuer I, Yıldız O. Hepatitis C virus genotypes detected in Erciyes University. J Turk Mikrobiyol Soc 2007; 37: 35–38.

24. Ural O, Arslan U, Fındık D. The distribution of hepatitis C virus genotype in the Konya region, Turkey. Turkish J Infection 2007; 21: 175–181 (article in Turkish with an abstract in English).

25. 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: 239–244.

26. Şanlıdağ T, Akçalı S, Özbakkaloğlu B, Ertekin D, Akduman E. Distribution of hepatitis C virus genotype in Manisa region, Turkey. Mikrobiyol Bul 2009; 43: 613–618 (article in Turkish with an abstract in English).

27. Çiftçi İH, Er H, Aşık G, Aktepe OC, Altındiş M. The distribution of genotype of the hepatitis C virus (HCV) RNA positive patients. Med J Kocatepe 2009; 10: 21–24 (article in Turkish with an abstract in English).

28. Ozbek E, Ozekinci T, Mese S, Atmaca S. Hepatitis C virus genotypes are changing in the southeast of Turkey. Biotechnol Biotechnol Eq 2009; 23: 1521–1523.

29. Aktaş E, Ögedey ED, Külah C, Beğendik Cömert F. Hepatitis C virus genotypes in a province of western Black-Sea region, Turkey. Mikrobiyol Bul 2010; 44: 647–650 (article in Turkish with an abstract in English).

30. Çelik C, Bakıcı MZ, Kaygusuz R, Ertürk R. The searching of HCV genotyping distributions in the region of Sivas. Viral Hepatit J 2010; 16: 106–110 (article in Turkish with an abstract in English).