Research Article

Doi: 10.4274/vhd.galenos.2019.2019.0012
Viral Hepatitis Journal 2019;25(2):62-66

Genotyping Distribution of Hepatitis C Virus in Şanlıurfa Province and Effect of Syrian Patients

Şanlıurfa İlinde Hepatit C Virüsünün Genotip Dağılımı ve Suriyeli Hastaların Etkisi

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ABSTRACT

Objectives: Hepatitis C virus (HCV) is one of the major causes of global mortality and morbidity. The purpose of our study was to characterize the distribution of HCV genotypes in Şanlıurfa, to see the effect of patients from Syria on the distribution of HCV genotypes and to compare our results with other regions in our country.
Materials and Methods: Between January 2011 and December 2015, serum samples of 312 HCV-RNA positive patients (58 Syrian patients) were sent to the laboratory for HCV genotyping. HCV genotype analysis was investigated using a commercial Abbott GT II assay (Abbott, USA).
Results: The most frequent genotype was genotype 1 (69.6%), followed by genotype 2 (14.1%) and genotype 4 (10.3%). Among Syrian patients, the most prevalent genotype was genotype 4 (48.2%), followed by genotype 1 (41.4%) and genotype 5 (8.7%). Our data showed that the prevalence of genotype 1 decreased from 75% to 58.7% and genotype 2 decreased from 21.2% to 12% between 2011 and 2015, while genotype 4 increased from 1.9% to 20% and genotype 5 increased from 0% to 1.6% due to the Syrian patients.
Conclusion: Our updated estimates confirm an increase in genotype 4 and genotype 5, particularly in Şanlıurfa due to the Syrian patients.
Keywords: Hepatitis C virus, genotype, migration, molecular microbiology

ÖZ

Amaç: Hepatit C virüsü (HCV), küresel ölüm ve morbiditenin başlıca nedenlerinden biridir. Çalışmamızın amacı Şanlıurfa'da HCV genotiplerinin dağılımını belirlemek, Suriye'den gelen hastaların HCV genotiplerinin dağılımı üzerindeki etkisini görmek ve sonuçları ülkemizdeki diğer bölgelerle karşılaştırmaktır.
Gereç ve Yöntemler: Ocak 2011 ile Aralık 2015 arasında, toplam 312 (58'i Suriyeli hasta) HCV-RNA pozitif hepatit C hastasının serum örnekleri HCV genotiplendirme için laboratuvara gönderildi. HCV genotip analizi, ticari Abbott GT II (Abbott, ABD) kullanılarak araştırıldı.
Bulgular: En sık rastlanan genotip; genotip 1 (%69,6), iken genotip 2 (%14,1) ve genotip 4 (%10,3) bunu takip etmekteydi. Suriyeli hastalar arasında en yaygın genotip; genotip 4 (%48,2) iken genotip 1 (%41,4), genotip 5 (%8,7) bunu takip etmekteydi. Verilerimiz 2011-2015 döneminde genotip 1 ve 2 prevalansının sırasıyla %75'ten %58,7'ye ve %21,2'den %12'ye düştüğünü, genotip 4 ve 5'in Suriyeli hastalar nedeniyle sırasıyla %1,9'dan %20'ye ve 0'dan %1,6'ya çıktığını göstermektedir.
Sonuç: Güncellenen tahminlerimiz, özellikle Suriyeli hastalar nedeniyle Şanlıurfa'da genotip 4 ve genotip 5'teki artışı doğrulamaktadır.
Anahtar Kelimeler: Hepatit C virüsü, genotip, göç, moleküler mikrobiyoloji

Cirit OS, Uzala Mızraklı A, Vurupalmaz Y, Gümüş HH, Özturhan H, Barış A. Genotyping Distribution of Hepatitis C Virus in Şanlıurfa Province and Effect of Syrian Patients. Viral Hepat J. 2019;25:62-66.

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Introduction

Hepatitis C virus (HCV) is one of the major globally cause of death and morbidity (1). In 2015, 71 million persons worldwide were living with chronic HCV infection according to the global hepatitis report published by WHO in 2017 (2). Chronic HCV infection is generally associated with the development of liver cirrhosis, hepatocellular carcinoma, liver failure, and death, and is a main indication for liver transplantation (3). HCV has extensive genetic heterogeneity, which phylogenetic analysis categorized into 7 major genotypes and 67 subtypes (4). Genotype 1 (46%) and genotype 3 (22%) are the most common genotypes followed by genotype 2 (13%), genotype 4 (13%), genotype 6 (2%), and genotype 5 (1%) as shown by Gower et al. (5). Genotype 7 has been identified in four individuals originating from the Democratic Republic of Congo (6). Distinction between genotypes remains essential because selection of treatment regimens are mostly still genotype specific (7). The purpose of our study is to characterize the distribution of HCV genotypes in Şanlıurfa, to see the influence of patients from Syria on the the distribution of HCV genotypes and compare our results with other regions in our country.

Materials and Methods

Between January 2011 and December 2015, serum samples of 312 (159 male, 153 female) HCV RNA positive patients were analyzed retrospectively which were sent to Şanlıurfa Mehmet Akif İnan Training and Research Hospital Microbiology laboratory for HCV genotyping. Of the 312 patients, 58 were originated from Syria. HCV antibody was determined by microparticle enzyme immunoassay method (Abbott Laboratories, USA), quantitative HCV-RNA assay was performed by a commercial real-time polymerase chain reaction (PCR) method (Abbott Molecular Inc., USA). HCV genotype analysis was investigated by using a commercial Abbott GT II assay (Abbott, USA). Viral RNA extraction from 500 uL patient serum were completed on the Abbott m2000sp system using the Abbott mSpecimen Preparation System kit (Abbott, USA) according to the manufacturer's instruction. RealTime (RT)-PCR master mixes were prepared using the Abbott m2000sp and the Abbott RT-HCV Genotype II Amplification Reagent Kit (Abbott,USA). According to the manufacturer's recommendation, RT-PCR reactions were performed on the Abbott m2000rt (Abbott, USA) instrument.

Results

The most frequent genotype was genotype 1 (217 patients; 69.6%) followed by genotype 2 (44 patients; 14.1%), genotype 4 (32 patients; 10.3%), genotype 3 (12 patients; 3.8%) and genotype 5 (five patients; 1.6%). Two patients revealed presence of more than one HCV genotype (mixed). In two case (0.6%), genotype 1b was seen to be associated with genotype 3 and genotype 2 (0.3% and 0.3%, respectively). Of the 217 genotype one patients, genotype 1b was detected in 78.8% (n=171), genotype 1a was detected in 7.8% (n=17) and 13.4% (n=29) couldn't subtyped. Among Syrian patients (n=58), the most prevalent genotype was genotype 4 (28 patients; 48.2%) followed by genotype 1 (24 patients; 41.4%), genotype 5 (five patients; 8.7%) and genotype 3 (one patient; 1.7%). Genotype 2 was not detected in any of the Syrian patients. The percent distribution of genotypes both in Turkish and Syrian patients are shown in Table 1. Distribution of patients according to gender and mean age are shown in Table 2. As shown in Figure 1, the prevalence of genotype1 and 2 declined and the prevalence of genotype 4 increased over the years.

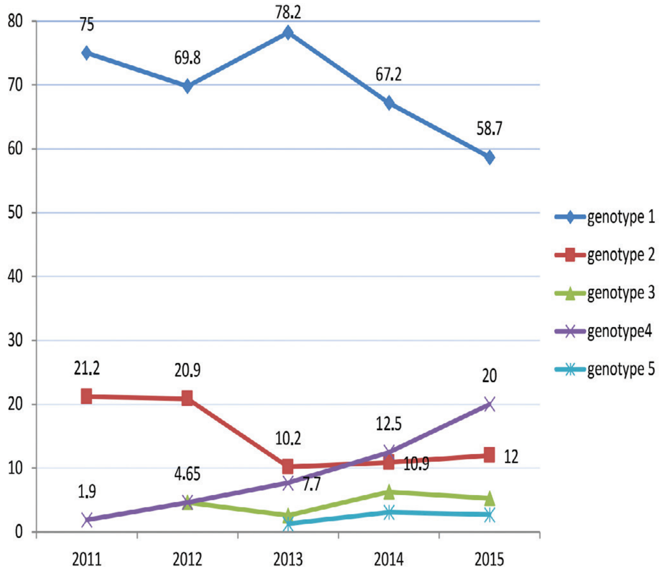

Figure 1. The percent distribution of genotype over years

Table 1. The percent distribution of genotypes both in Turkish patients and Syrian patients

Genotypes	Turkish patients				Syrian patients			
	Male (n)	Female (n)	Total (n)	(%)	Male (n)	Female (n)	Total (n)	(%)
1 (can not subtyped)	15	10	25	(9.8%)	4	-	4	(6.9%)
1a	6	-	6	(2.4%)	6	5	11	(19%)
1b	83	79	162	(63.8%)	4	5	9	(15.5%)
2	18	26	44	(17.3%)	-	-	-	-
3	9	2	11	(4.3%)	1	-	1	(1.7%)
4	1	3	4	(1.6%)	7	21	28	(48.2%)
5	-	-	-	-	3	2	5	(8.7%)
Mixed (1b+2)	1	-	1	(0.4%)	-	-	-	-
Mixed (1b+3)	1	-	1	(0.4%)	-	-	-	-

a: ,b:

Discussion

Knowledge of the geographic distribution of HCV genotypes is still playing an important role for epidemiological studies, treatment and vaccine development (8). The length of the treatment and the opportunity to associate interferon and/or ribavirin with the new direct-acting antiviral therapies still remain dependent on HCV genotype (1).

There are significant variations in global regions alsovary in different regions within countries (9). Gower et al. (5) showed that in North America, Latin America, and Europe genotype 1 (62-71%) was the predominant genotype with genotype 1b accounting for 26%, 39%, and 50% of all cases respectively. North Africa and the Middle East had a large genotype 4 population (71%), which was attributable to the high prevalence of genotype 4 in Egypt. In Asia, genotype 3 dominates followed by genotype 1.

When we check the previous studies which published after 2010 in Turkey, the most representative genotype is genotype 1, ranging between 51.7% and 95.3% (Table 3). The second common genotype distribution shows variability among the regions. Although the second most common genotype in Gaziantep, Antakya and Adıyaman was genotype 2 (7.8%, 9.3% and 11.3%, respectively), the second most common genotype in Kahramanmaraş, Adana and Antalya was reported as genotype 3 (46%, 26% and 11.1% respectively) (11,14,15,16,24). Only few cases of genotype 5, genotype 6 and mixed types are reported (Table 3).

Consistent with the results of other studies in Turkey, in our study genotype 1 was found most common genotype with the prevalance of 69.6%. When Syrian patients were excluded, genotype 1 accounted for 76%.

In the current study, genotype 2 was the second most common genotype (14.1%). This finding was similar to the studies in Adıyaman (11.3%), Antakya (9.3%) and Gaziantep (7.8%) which the cities are geographically close to each other and located at the south and southeast part of Turkey (14,15,24).

In the present study, the third most common genotype was genotype 4 (10.3%), which was attributable to the high prevalence of genotype 4 (48.2%) in Syrian patients. When Syrian patients were excluded, genotype 4 accounted for 1.6%. The frequency of genotype 4 is highest in Central Africa and the Middle East and has increased in prevalence due to migration from the Middle East and Africa (4). In Turkey, genotype 4 distribution does not show high

Table 2. Distribution of patients according to gender and mean age

Genotypes	Gender (%)		Mean age	
	Female	**Male**	**Female**	**Male**
1	45.6	54.4	55	49
2	59	41	59	53
3	16.7	83.3	36	31
4	75	25	49	45
5	40	60	59	48

Table 3. Hepatitis C genotype studies which published after 2010 in Turkey

Researcher (Referance)	Time	Province	Genotypes (%)							Number
			1	**2**	**3**	**4**	**5**	**6**	**mix**	
Tezcan et al. (10)	2010-2012	Mersin	92.3	2.1	4.2	0.8	-	0.4	-	236
Saglik et al. (11)	2009-2013	Antalya	83.4	3.5	11.1	1.6	-	-	0.2	422
Buruk et al. (12)	2009-2012	Trabzon	92.8	1.6	4.9	0.7	-	-	-	304
Kayman et al. (13)	2010-2011	Kayseri	62.4	3.2	1.1	32	-	-	1.3	375
Karslıgil et al. (14)	2011	Gaziantep	88.2	7.8	2	2	-	-	-	51
Öztürk et al. (15)	2010-2012	Adana	58.7	14.6	26	0.6	-	-	-	315
		Antakya	87	9.3	0.9	2.8	-	-	-	324
Caliskan et al. (16)	2010-2014	Kahramanmaraş	51.7	1.3	46	1	-	-	-	313
Altuğlu et al. (17)	2007-2011	İzmir	93.3	1.5	3.7	1.5	-	-	-	535
Us et al. (18)	2009-2014	Eskişehir	94.5	1.5	2	2	-	-	-	203
Tüzüner et al. (19)	2010-2017	Central Anatolia	90	3.8	3.3	2.5	0.2	0.2	-	480
Kirdar et al. (20)	2011-2016	Aydın	90.2	2.1	5.9	1.4	-	-	0.4	286
Duran et al. (21)	2015-2016	Adana	71.4	7.6	16.8	3.4	0.8	-	-	119
Selek et al. (22)	2015-2016	İstanbul	81.2	2.8	16	-	-	-	-	106
Karabulut et al. (23)	2013-2016	İstanbul	82.5	4.6	10.7	2.2	-	-	-	412
Akgun et al. (24)	2013-2016	Adıyaman	84.5	11.3	4.2	-	-	-	-	71
Aktaş et al. (25)	2011-2014	Erzurum	95.3	-	-	3.7	1	-	-	108
Current study	2011-2015	Şanlıurfa	69.6	14.1	3.8	10.3	1.6	-	0.6	312

variability among the regions, ranging between 0.5% and 3.4% (Table 3), while a significant prevalence described only in Kayseri (32%) (13). By using molecular clock analysis, they predicted the introduction of type 4d HCV into the Kayseri region probably 30-75 years ago (26).

The prevalence of genotype 3 is globally higher in the intravenous drug users (9). Studies from Kahramanmaraş and Adana, in which the genotype 3 favored the male gender (95.8%,85% respectively) with a mean age of 25 and 30.5 years (16,21). We also found that genotype 3 was significantly more common in young (mean age: 31) male (83.3%) patients. This findings suggest that intravenous drug use may have become more common among young males (16).

According to the united nations refugee agencies 2017 global report, Turkey hosted the largest number of refugees worldwide, with 3.5 million people (27). Şanlıurfa is located at the southeast part of Turkey near Syrian border, which has a population of 1.985.753 and currently hosts around 420.000 registered Syrian refugees who make up 21% of the overall city population (28).

The finding in this study that the most prevalent genotype was genotype 4 (48.2%) followed by genotype 1 (41.4%), genotype 5 (8.7%) and genotype 3 (1.7%) in 58 Syrian patients. Our results are in concordance with the published data from Syria, in which the most prevalent genotype in 636 HCV-RNA-positive patients from eight medical centres in Syria over a 3-year period was genotype 4 (59%) followed by genotype 1 (28.5%), genotype 5 (10%) and genotype 3 (1.8%) (29).

Of the 64 genotype 5 positive patients, 56 (87%) live in the north of Syria (originated from the northern province around the city of Aleppo), including 21 cases (33%) from Azaz, a small city close to Turkey (29). Similarly, in our study, genotype 5 was found only in patients arriving from Syria. Of the five genotype 5 positive patients, four of them were originated from the city of Aleppo and one was from Kobani. Genotype 5 (35.7%) is the most common genotype in South Africa (1). Antaki et al. (29) finding was the first report describing the presence of genotype 5 in Syria and the Middle East. At that time no cases were reported from neighbouring countries and they were unable to discover an explanation (so far from South Africa, and they consider that there is absolutely no immigration from Africa to Syria) for these unexpected findings. In Turkey, Yildirim et al. (30) presented probably the first finding of genotype 5 identified in three patients in Gaziantep originating from Syria. To the best of our knowledge, this was the first genotype 5 report from Turkey.

Study Limitations

One of the most important limitation of our study was the absence of information about the transmission routes due to the retrospective design of the study.

Conclusion

Migration into Turkey is an emerging phenomenon. Migration has influenced the prevalence of hepatitis C genotype distribution. It's interesting to note that, our data shows that the prevalence of genotype 1 and 2 between the period 2011-2015, has decreased from 75% to 58.7% and from 21.2% to 12% respectively while genotype 4 and 5 has increased from 1.9% to 20% and 0 from 1.6% respectively due to the Syrian patients. Our updated estimations confirm a raise in genotype 4 and genotype 5, in particular in Şanlıurfa due to the Syrian patients. Moreover, further regional and national epidemiological studies are required to see the effect of Syrian patients on genotype distribution.

Ethics

Ethics Committee Approval: Retrospective study.

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: O.S.C., Design: O.S.C., Data Collection or Processing: A.U.M., Y.V., Analysis or Interpretation: H.H.G., Literature Search: H.Ö., A.B., Writing O.S.C.

Conflict of Interest: The authors declare no conflict of interest.

Financial Disclosure: The authors declare that this study has not received any financial support.

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