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RESEARCH ARTICLE



Genotype distribution of hepatitis C virus in the province of Gaziantep, a 10-year evaluation

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ABSTRACT

Hepatitis C virus (HCV) can cause both acute and chronic hepatitis infections. Gaziantep is located southeast part of Turkey and has a border with Syria. More than 400,000 Syrian refugees live in Gaziantep. The aim of this study was to evaluate distribution of HCV genotypes among Syrian patients and in people who inject drugs.

Serum samples from 1,628 individuals (786 female, 842 male) which were sent to our laboratory for genotyping between January 2013 and December 2022, were analyzed retrospectively. Three different HCV genotyping assays (Qiagen, RTA and Abbott) were used during the 10-year study period.

Out of the 1,628 patients, genotype 1 was detected in 51.5%, genotype 3 in 21.4%, genotype 4 in 20%, genotype 5 in 4.6%, genotype 2 in 1.3%. Mixed genotype was found in 20 patients. Of the patients, 1,143 were Turkish patients and among those patients genotype 1 (66.8%) was the most common genotype followed by genotype 3 (29%). Among Syrian patients ($n = 477$), genotype 4 (64.2%) was predominant genotype followed by genotype 1 and genotype 5. Genotype 3 was detected in 277 (79.6%) prisoners. All of them were male and probably the main source of HCV infection was intravenous drug abuse. While genotypes 1 and 4 were common in females, genotypes 1 and 3 were common in males.

In the future genotype 3 may become an increasing problem due to the persons who inject drugs. Less frequent genotypes such as 4 and 5 may become more frequent due to Syrian patients.

KEYWORDS

hepatitis C virus, genotype, migration, molecular microbiology, persons who inject drugs

INTRODUCTION

According to the WHO reports, an estimated 58 million persons were chronically infected and living with hepatitis C, 1.5 million new hepatitis C virus (HCV) infections occurred worldwide in 2019 and both Eastern Mediterranean Region and the European Region have the highest reported prevalence of HCV [1, 2].

HCV genotypes show high variability among the regions and still important for treatment selection [3, 4]. Uncontrolled migratory flows and injection drug use are the main factors contribute to changing epidemiology of HCV [5]. The prevalence of genotypes 4 and 5 has increased due to migration from the Middle East [4]. Persons who inject drugs (PWID) have become the main transmission mechanism of HCV in Western Europe, with the rapid increase in the number of iv drug users in Eastern Europe placing PWID at the center of the HCV epidemic [5]. The aim of this study was to evaluate the effect of Syrian patients and

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PWID to the distribution of HCV genotypes and compare our results with the regional and national data.

MATERIALS AND METHODS

Serum samples from 1,628 individuals (786 female, 842 male) which were sent to our laboratory for HCV genotyping between January 2013 and December 2022, were analyzed retrospectively. HCV antibody was tested by electrochemiluminescence immunoassay method (Cobas, Roche diagnostics, Mannheim). Three different HCV genotyping assays (Qiagen, RTA and Abbott) were used during the 10-year study period. At the first period (2013–2017) HCV-RNA was investigated by quantitative PCR (Qiagen®, Germany). HCV genotypes detected by using pyrosequencing method (Pyromark Q24 kit, Qiagen®, Germany). Between 2017 and 2020 serum samples were subjected to HCV RNA quantification by RTA HCV Real time PCR and genotyped by RTA HCV genotyping qPCR Kit. RTA HCV Genotyping real time Kit detects HCV genotypes 1a, 1b, 2, 3, 4 and, 5. Viral RNA isolation from clinical samples done by RTA Viral RNA isolation kit (RTA Laboratories, Turkey) or RTA Viral Nucleic Acid Isolation kit (RTA Laboratories, Turkey). Data analysis were performed on BIO-RAD CFX96-IVD software system. Between 2020 and 2022 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). According to the manufacturer's instruction, viral RNA extraction from 500 uL patient serum were completed on the Abbott m2000sp system using the Abbott mSpecimen Preparation System kit (Abbott, USA). By using the Abbott m2000sp and the Abbott RT-HCV Genotype II Amplification Reagent Kit (Abbott, USA) Real-Time (RT)-PCR master mixes were prepared. According to the manufacturer's recommendation, RT-PCR reactions were performed on the Abbott m2000rt (Abbott, USA) instrument.

RESULTS

Out of the 1,628 patients, genotype 1 was detected in 51.5% ($n = 838$), genotype 3 in 21.4% ($n = 348$), genotype 4 in 20% ($n = 326$), genotype 5 in 4.6% ($n = 75$) and genotype 2 in 1.3% ($n = 21$). 13 patients were coinfecting with genotype 2 and 5, three patients were coinfecting with genotype 1 and 2, two patients were coinfecting with genotype 1 and 3, two patients were coinfecting with genotype 2 and 4. Of the patients, 1,143 were Turkish patients and among those patients genotype 1 (66.8%) was the most common genotype followed by genotype 3 (29%). In particular, among Syrian patients ($n = 477$), genotype 4 (64.2%) was predominant genotype followed by genotype 1 and genotype 5, respectively (14.9%, 14.5%). Among prisoners ($n = 350$) genotype 3 was detected in 277 (79.6%) prisoners. All were

male and, based on the diagnoses entered in the hospital information system, intravenous drug use was likely the main source of HCV infection. Genotype 1 (60.3%) and 4 (28.4%) were common in females. In addition, genotype 1 (43.3%) and 3 (39.5%) were common in males.

The percent distribution of genotypes both in Turkish, Syrian patients, patients from other nationalities and prisoners are shown in Table 1. Distribution of patients according to gender and median age are shown in Table 2. The percent distribution of HCV genotypes over 10 year shown in Fig. 1. The total number of patients by year, the number of Turkish, Syrian, other country citizens and those coming from prisons are shown in Fig. 2, while the proportions of these patient groups in the total number of patients for that year are shown in Table 3.

DISCUSSION

HCV genotype distribution is an important factor for treatment selection and epidemiological research's [3, 6]. Injection drug use has been one of the most efficient routes for HCV transmission due to sharing needles in the developed countries [3, 5].

Globally, there are significant variations on HCV genotypes [7]. Genotype 1 is predominant genotype in Europe, North America, Latin America, and Australasia (53–71%) while genotype 3 accounting for 40% of all infections in Asia [8]. HCV genotype 4 is seen most commonly in Middle Eastern countries like Egypt and Syria [6]. Genotype 5 is the dominant genotype in South Africa [4].

We compared our results with the articles which published between 2015 and 2023 in Türkiye, Genotype 1 is the most prevalent genotype all over the regions (47.3% and 90.2%) (Table 4). The finding in our study that the most frequent genotype was genotype 1 (51.5%) is inline with the results of other studies in Türkiye.

In this study, the second most common genotype was genotype 3 (21.4%), which detected in 277 (79.6%) prisoners and PWID. It is significantly associated to males, and is mostly detected in younger PWID. High prevalence rates have been reported for genotype 3 in Adana, Istanbul, Antalya Mersin, Kahramanmaraş and Antalya (16.8%, 16%,

Table 1. The percent distribution of HCV genotypes in Turkish, Syrian patients and patients from other nationalities

Genotypes	Turkish patients		Syrian patients		Other nationalities	
	Total (n)	(%)	Total (n)	(%)	Total (n)	(%)
1	763	(66.8%)	71	(14.9%)	4	(50%)
2	16	(1.4%)	4	(0.8%)	1	(12.5%)
3	332	(29%)	13	(2.7%)	3	(37.5%)
4	20	(1.8%)	306	(64.2%)	-	-
5	6	(0.5%)	69	(14.5%)	-	-
Mixed	6	(0.5%)	14	(2.9%)	-	-



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

Genotypes	Gender (%)		Mean age	
	Female	Male	Female	Male
1	60.3	43.3	62	53
2	1.4	1.2	54	46
3	1.9	39.5	48	32
4	28.4	12.2	55	47
5	6.7	2.6	58	58
Mix	1.3	1.2	54	60

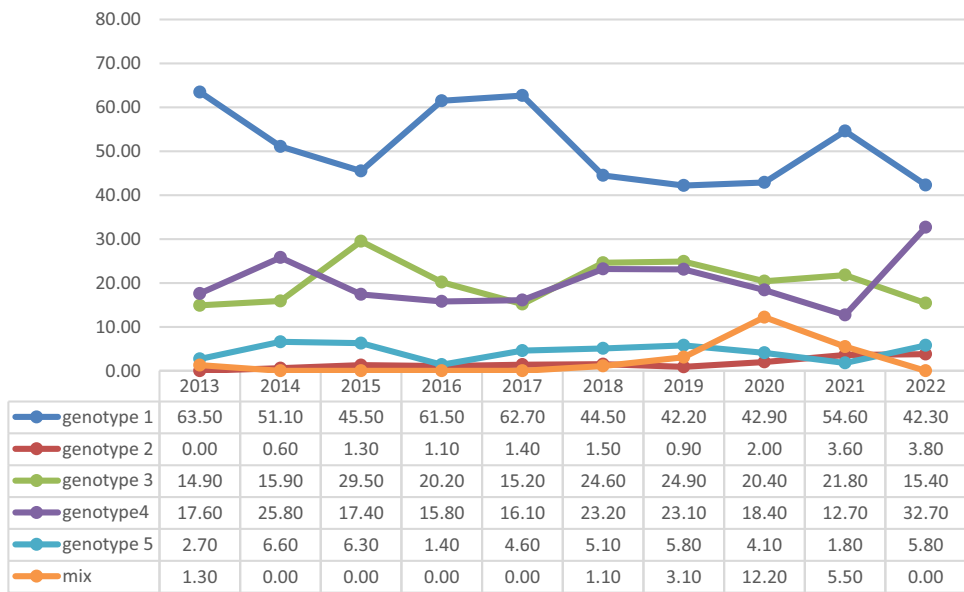


Fig. 1. The percent distribution of HCV genotypes over years

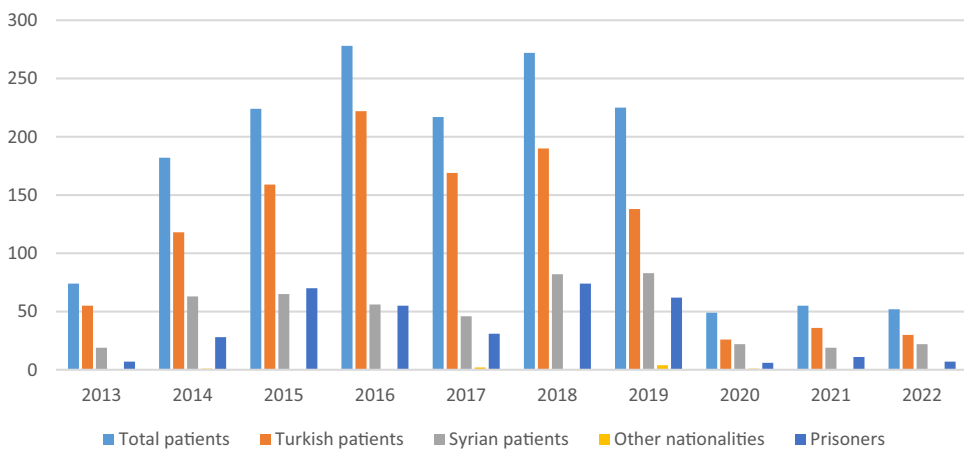


Fig. 2. The total number of patients by year, the number of Turkish, Syrian, other country citizens and those coming from prisons

17.9%, 20.3% and 45.3% respectively) [10, 11, 18–20]. Genotype 3 is especially higher in the intravenous drug users [7]. According to the Ministry of Interior Turkish National Police Counter Narcotics Departments 2018 Turkish National Drug Report, when the provinces where the inpatients who applied addiction treatment centers are residing are examined; the third highest number of cases

belongs to Gaziantep. It is observed that 5.4% of the applicants are residing in Gaziantep. 2,366 injecting drug users among 11,633 inpatients at the addiction treatment centers were screened for viral hepatitis. When the results of the screening test for 2,366 injecting drug users are examined; 1,083 out of 2,366 injecting drug users tested for Hepatitis C (45.77%) are positive [22].



Table 3. The proportions of patient groups in the total number of patients for that year

Years	Turkish patients		Syrian patients		Other nationalities		Prisoners		All patients	
	Total (n)	(%)	Total (n)	(%)	Total (n)	(%)	Total (n)	(%)	Total (n)	(%)
2013	55	74.3	19	25.7	0	0	7	9.4	74	4.5
2014	118	64.8	63	34.6	1	0.6	28	15.3	182	11.2
2015	159	71	65	29	0	0	70	31.2	224	13.8
2016	222	79.9	56	20.1	0	0	55	19.7	278	17.1
2017	169	77.9	46	21.2	2	0.9	31	14.3	217	13.3
2018	190	69.8	82	30.2	0	0	74	27.2	272	16.7
2019	138	69.8	83	36.9	4	1.8	62	27.5	225	13.8
2020	26	53	22	45	1	2	6	12.2	49	3
2021	36	65.5	19	34.5	0	0	11	20	55	3.4
2022	30	57.7	22	42.3	0	0	7	13.4	52	3.2

Table 4. HCV genotype studies published after 2015 in Türkiye

Researcher (Reference)	Time	Province	Genotypes (%)							Number
			1	2	3	4	5	6	mix	
Kirdar et al. [9].	2011–2016	Aydın	90.2	2.1	5.9	1.4	-	-	0.4	286
Duran et al. [10].	2015–2016	Adana	71.4	7.6	16.8	3.4	0.8	-	-	119
Selek et al. [11].	2015–2016	Istanbul	81.2	2.8	16	-	-	-	-	106
Karabulut et al. [12].	2013–2016	Istanbul	82.5	4.6	10.7	2.2	-	-	-	412
Akgun et al. [13].	2013–2016	Adıyaman	84.5	11.3	4.2	-	-	-	-	71
Cirit et al. [14].	2011–2015	Şanlıurfa	69.6	14.1	3.8	10.3	1.6	-	0.6	312
Bulut et al. [15].	2016–2019	Istanbul	81.3	3.4	8.8	2.9	0.8	-	2.9	385
Alacam et al. [16].	2017–2020	Istanbul	72	6.7	14.1	8.8	1.3	0.2	8.6	546
Arıcı et al. [17].	2016–2021	Istanbul	80.4	2.6	15.3	2.1	-	-	2.6	386
Erman Daloglu et al. [18].	2014–2019	Antalya	77.2	1.5	17.9	2.8	0.1	0.2	0.3	720
Şimşek Bozok et al. [19].	2017–2022	Mersin	58.3	5.4	20.3	11.9	1	-	3	202
Gişi et al. [20].	2015–2019	Kahramanmaraş	47.3	1.6	45.3	5.8	-	-	-	497
Çetin Duran et al. [21].	2005–2016	Izmir	88.4	2.1	5.2	2.9	0.3	-	1.1	558
Current study	2013–2022	Gaziantep	51.5	1.3	21.4	20	4.6	-	1.2	1,628

In the present study, the third most common genotype was genotype 4 (20%), which was attributable to the high prevalence of genotype 4 (64.2%) in Syrian patients. When Syrian patients were excluded, genotype 4 accounted for 1.8%. 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 [23]. According to the United Nations refugee agencies 2022 global trends report, Türkiye hosted nearly 3.6 million refugees, the largest population worldwide [24]. Gaziantep is located at the southeast part of Turkey near Syrian border, which has a population of 2.154.051. Republic of Türkiye Ministry Of Interior, Presidency of Migration Management latest report currently hosts around 434,563 registered Syrian patients. Rate of the number registered Syrians to the total number of people living in the city is 16.83% [25].

The results of the present study demonstrate that the most prevalent genotype was genotype 4 (64.2.2%) followed by genotype 1 (14.9%), genotype 5 (14.5%) in 477 Syrian patients. Our results are in agreement with the published data from Syria, in which the most prevalent genotype in was genotype 4 (59%) followed by genotype 1 (28.5%) and genotype 5 (10%) [26]. In Mersin and Şanlıurfa which the

cities are geographically close to each other and located at the south and southeast part of Türkiye, genotype 4 is over the ten percent of all the genotypes found. [14, 19].

Genotype 5 (35.7%) is the most common genotype in South Africa have increased in prevalence due to emigration from the Middle East and Africa [27]. Antaki et al. reported the first presence of genotype 5 in Syria and the Middle East. [26]. The first finding of genotype 5 identified in three patients in our laboratory originating from Syria in 2013. To the best of our knowledge, this was the first genotype 5 report from Turkey [28]. The prevalence of genotype 5 in Türkiye is, ranging between 0.1% in Antalya and 1.6% in Şanlıurfa [14, 18].

Genotype 2 seems to have a major prevalence in cities like Adıyaman, Adana, Şanlıurfa and Mersin which are our neighbour cities (5.4–14.1%), if compared to our study (1.3%) or western part of Türkiye [10, 13, 14, 19]. Only a small percentage of genotype 6 and mixed genotypes were reported [16, 18].

Migration into Turkey is an emerging phenomenon. Migration and the increase in sex/drug consume-tourism may also influence epidemiologic trend of HCV infection within the next years and lead to changes in its epidemiology



[29]. It's interesting to note that, our data shows that the prevalence of genotype 1 between the period 2013–2022, has decreased from 63.5% to 42.3% while genotype 3, 4 and 5 has increased from 14.9%, 17.6% and 2.7% to 15.4, 32.7% and 5.8% respectively due to the Syrian patients and PWID. The total number of patients, which was in the range of 200–300 before the pandemic, decreased to 50 patients per year during the pandemic period. The ratio of prisoners to total patients over the years was parallel to the HCV genotype 3 ratio. Our updated estimations confirm a raise in genotype 3, 4 and 5 in particular in Gaziantep due to the Syrian patients and PWID. Moreover, further regional and national studies are necessary to confirm the effect of both Syrian patients and PWID on genotype distribution.

Conflict of interest: The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest in the subject matter or materials discussed in this manuscript.

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