ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Hepatitis C Genotype Assay and Sequence Analysis at a University Hospital in Eastern Türkiye

Türkiyenin Doğusunda Bir Üniversite Hastanesinde Hepatit C Genotip Testi ve Sekans Analizi

Cigdem Eda Balkan Bozlak[1], Abdullah Gumus[1], Ahmet Yilmaz[2]

[1]Kafkas University, Faculty of Medicine, Department of Medical Microbiology, Kars, Türkiye; [2]Ataturk University, Vocational School of Health Services, Department of Medical Laboratory Techniques, Erzurum, Türkiye

ABSTRACT

Aim: *Hepatitis C virus (HCV) is a virus with many genotypes and subtypes that can cause cirrhosis, liver failure, and cancer worldwide. Countries must also identify their genotypes and subtypes to determine the treatment process for HCV infections. This study aimed to determine the HCV genotype among 71 patients who visited Kafkas University Hospital and tested positive for anti-HCV via macro-ELISA over three years.*

Material and Method: *71 samples collected from patients admitted to our hospital and identified as anti-HCV positive using the ELISA method were included in our study. The 5'untranslated region (5'UTR) region of HCV was amplified and sequenced by PCR. 5'-ctgtgaggaactactgtctt-3' and 5'-atactcgaggtgcacggtctacgagacct-3' primers were used for 5'UTR region. Sequence reactions were conducted on an ABI Prism 3130xl DNA sequencer, and sequence analysis was performed. The resulting sequence was screened in HCV Databank to detect genotype.*

Results: *The HCV genotype distribution of 56 samples was as follows: 27 (48%) patients were male and 29 (51%) were female. Genotype1a, 6 (8.5%); Genotype1b, 40 (71%); Genotype3a, 7 (12%); Genotype4, 3 (6%). According to our results, Type1b, the most common species in Türkiye, was also found to be the highest in our city.*

Conclusion: *We hope this study provides the regional distribution of the procedures to be followed in the formation of treatments for HCV-positive patients in Türkiye since it is the first study to determine HCV genotype in our region.*

Keywords: *HCV; genotype; subtypes; sequence analysis; polymerase chain reaction*

ÖZET

Amaç: *Hepatit C virüsü (HCV), dünya çapında siroz, karaciğer yetmezliği ve kansere neden olabilen birçok genotip ve alt tipi olan bir virüstür. HCV enfeksiyonlarının tedavi sürecini belirlemek için ülkelerin HCV genotiplerini ve alt tiplerini belirlemeleri de önemlidir. Bu çalışmada üç yıldır Kafkas Üniversitesi Hastanesi'ne gelen ve makro-ELISA'da anti-HCV pozitif bulunan 71 hastada HCV genotipini belirlemeyi amaçladık.*

Materyal ve Metot: *Hastanemize başvuran ve ELISA yöntemi ile anti-HCV pozitif saptanan hastalardan alınan 71 örnek çalışmamıza dâhil edildi. HCV genomunun 5'çevrilmemiş bölgesi (5'UTR) bölgesi, PCR ile amplifiye edildi ve sekanslandı. 5'UTR bölgesi için 5'-ctgtgaggaactactgtctt-3' ve 5'-atactcgaggtgcacggtctacgagacct-3' primerleri kullanıldı. Sekans reaksiyonları, bir ABI Prism 3130xl DNA sekanslayıcı üzerinde gerçekleştirildi ve sekans analizi yapıldı. Ortaya çıkan sekans, genotipi tespit etmek için HCV Veri Bankasında tarandı.*

Bulgular: *Elli altı örneğin HCV genotip dağılımı şu şekildeydi; Hastaların 27'si (%48) erkek, 29'u (%51) kadındı. Genotip la, altı (%8,5); Genotip 1b, kırk (%71); Genotip 3a, yedi (%12); Genotip 4, üç (%6). Türkiye'de en yaygın tür olan Type1b, sonuçlarımıza göre ilimizde de en yüksek tür olarak bulundu.*

Sonuç: *HCV genotipini belirlemeye yönelik ilk çalışma olması nedeniyle bölgemizin Türkiye'de HCV pozitif hastalara uygulanacak tedavilerin oluşturulmasında izlenecek prosedürlerin bölgesel dağılımına katkı sağlayacağını umuyoruz.*

Anahtar kelimeler: *HCV; genotip; alt tipler; sekans analizi; polimeraz zincir reaksiyonu*

Introduction

HCV virus is an RNA virus that can cause disease in acute and chronic form, encoding ten proteins (5'-C-E1-E2-p7-NS2-NS3-NS4A-NS4B-NS5A-NS5B-3') in the Flaviviridae family, hepacivirus genus, with a single strand, positive sense RNA enveloped genome of 30–60 nm (1). 70% of HCV-positive patients are at risk of developing the virus into a chronic form, and 15–30% of patients in the chronic patient group are at risk of developing cirrhosis and liver cancer on average[1]. HCV infections are generally known to be transmitted in developing countries with poor

İletişim/Contact: *Associate Prof. Ahmet Yılmaz, Vocational School of Health Services, Department of Medical Laboratory Techniques, Ataturk University, Erzurum, Türkiye* • ***Tel:*** *+90 505 216 71 07* • ***E-mail:*** *aymet25@hotmail.com* • ***Geliş/Received:*** *03.05.2023* • ***Kabul/Accepted:*** *25.09.2023*

ORCID: *Ciğdem Eda Balkan Bozlak, 0000-0003-3922-7758 • Abdullah Gumus, 0000-0002-7762-4634 • Ahmet Yilmaz, 0000-0002-2350-1516*

sanitation conditions by intravenous drug use, non-sterile blood transfusion, unprotected sexual intercourse, and even rarely mother-to-baby transplacental or mother-to-child transmission (MTCT) invasive interventions during childbirth[2]. Almost 1.75 million new cases are encountered every year, according to WHO's report published in 2015. It is known that approximately 1.25 million of these patients are at risk of the chronic form of HCV. There are also around 360.000 cases of cirrhosis and cancer considering the rates[3]. It is known that the number of HCV-related liver diseases varies according to country. At the same time, HCV is expected to decrease or disappear in the next 15–20 years, considering the current situation. Although these data indicate a general decrease in HCV infection, especially in Western Europe, a recent modelization has estimated how the numbers of HCV– mortality will increase in the following decades.

According to this model, in the period 2013–2030, the number of decompensated cirrhosis, the prevalence of HCC in the general population, and the liver-related morbidity rate will increase in Europe by 80 %, 75 %, and 65 %, respectively[4].

The creation of the treatment protocol for the virus depends on the detection of genotypes, especially in terms of countries since DAA (interferon and/or ribavirin with the new direct-acting antiviral therapies) used in its treatment depends on the genotype of HCV, for this reason[5]. Today, 30%-35% of HCV nucleotides have eight different genotypes (G1, G2, G3, G4, G5, G6, G7, G8) and again <15% difference in terms of nucleotides; 67 of them have known, 20 investigated subtypes[4,6,7]. The gold standard for genotyping is whole-genome analysis. Still, since 5'UTR, NS3, NS5A, NS5B, and core antigen tests are new-generation tests, they are very expensive[8]. Genotypes 1a, 1b, 2a, and 3a, referred to as "epidemic subtypes" in the worldwide distribution of HCV, are also common in developing South Africa, South Asia, Africa, and Southeast Asia. Genotype 7 was first detected in a person who migrated from Africa to Canada. Genotype 8 was first reported in India[9]. Epidemiological studies have shown that the genotype of the virus in blood donors also changes according to geographical areas[10]. For example, Genotype 1 is more common worldwide than Genotype 2, whereas Genotype 3 is more common in South Asia, Australia, and Iran; Genotype 4 is in Central Asia and northern Africa. Genotype 5a is the most common species in South Africa, and Genotype 6a is the most common species in Hong Kong and Vietnam; Genotype 1b is the most common species in South and Northern Europe in South and Eastern Europe; Genotype 1a is the most common species in North America and Europe[10,11]. There is a gradual increase in the number of Genotype 3 and Genotype 4. In contrast, Genotype 1b is the most common genotype when Türkiye's data is examined in some studies[2]. This study, conducted for the first time in Türkiye, aims to compare the results of genotype determination and sequence analysis in serum samples of patients admitted to our hospital for any reason and diagnosed with HCV-positivity with Türkiye and the world data.

Materials and Methods

Patient Samples, Genotyping, and Sequencing

Plasma samples of 71 patients who were found to be anti-HCV positive with Biomerieux (France) Architect 1000 device by macro-ELISA method from the patient blood sent to Kafkas University Medical Microbiology Laboratory for routine examinations were stored at -80°C in our study. It was performed in an HCV RNA EZ1 advanced (Qiagen, Germany) automated extraction device using the EZ1 Virus Mini Kit v2.0 (Qiagen, Germany). The extracts obtained were amplified using 5'NCR, and the core gene region was amplified using 5'-ctgtgaggaactactgtctt-3'and 5'-atactcgaggtgcacggtctacgagacct-3'primers with HCV RNA Rt-quantitative 2.0 (NLM, Italy) kit in Rotorgene 6000 real-time polymerase chain reaction (Rt-PCR) (Qiagen, Germany) device. Sequence reactions were conducted on an ABI Prism 3130xl DNA sequencer in the remaining 56 patients, and sequence analysis software was performed with version 5.4 (Applied Biosystems, Foster City, CA, USA). Fifteen patients were observed to be negative. The resulting sequence was screened in the Hepatitis C Virus Databank to detect HCV genotype[13].

This study was carried out with the approval of the Kafkas University Faculty of Medicine Ethics Committee (Date: 30.01.2019 and Decision no: 80576354–050–99/47).

Statistical Data were analyzed using Windows IBM Statistical Package for Social Sciences (SPSS) program, version 22.0 (IBM Inc., Chicago, Illinois, USA), and the results were presented as median values ± standard deviations (SD), number, and percentage. The chi-square test was used to compare the groups; $p<0.05$ was considered statistically significant.

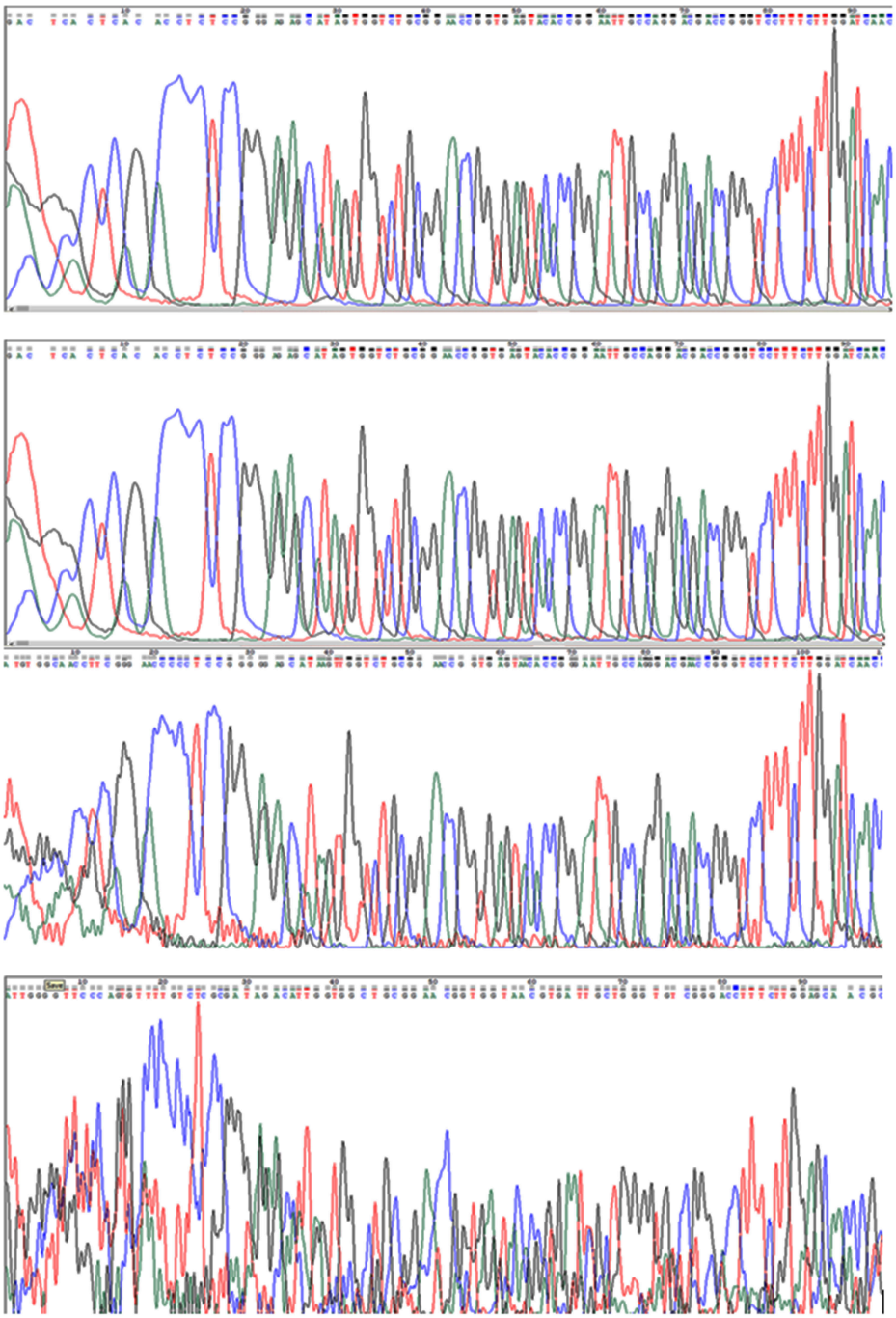

Figure 1. The results of four different sequence analyses were 1a, 1b, 3a, and 4, respectively.

Results

The HCV results of 56 samples were as follows: 27 (48%) patients were male and 29 (51%) were female. Genotype 1a, 6 (8.5%); Genotype 1b, 40 (71%); Genotype 3a, 7 (12%); Genotype 4, 3 (6%). No other genotypes were found (Figure 1) (Table 1).

Discussion

HCV is a viral disease that has almost 71 million chronic patients worldwide and is rarely transmitted maternally by blood transfusion or non-sterile injection, causing liver diseases, cirrhosis, and hepatocellular carcinoma in some patients[2,3].

Table 1. *Data from our study*

Number(n) / Gender	G1	G2	G3	G4	G5	G6
n: 56 M: 27(48%) F: 29(51%)	1a; 6(8.5%) 1b; 40(71%)	0	3a; 7(12%)	3(6%)	0	0

Table 2. *HCV genotype distribution by countries*

Country	Year	Method	Genotype 1	Genotype 2	Genotype 3	Genotype 4	Genotype 5	Genotype 6
China[21]	2011-2019	PCR	2.94% 1a, 45.1% 1b	14.7% 2a	13.7% 3a	-	-	12.7% 6a
Czechia[22,23]	2001-2009	PCR	79%	1%	19.7%	-	-	-
France[24,25]	1995-2005	PCR	57%	9.3%	20.8%	8.9%	2.7%	0.9%
Germany[26,27]	1996-2002	PCR	61.7%	6.9%	28%	3.2%	-	-
Greece[28]	2011	PCR	47%	8.3%	20.8%	8.9%	2.7%	0.2%
Hungary[29]	2010	PCR	94.1%	0.8%	3.4%	1.7%	-	-
Italy[30]	2002	PCR	62%	27%	7%	0.8%	-	0.4%
Poland[31]	2008	PCR	57.5%		31.3%	4.8%	-	-
Norway[32]	2003	PCR	61.5%	1.5%	28%		-	-
Israel[33]	2010	PCR	70%	8%	20%	3%	-	-
S. Africa[34]	2020	PCR	21.8%	-	15.4%	10.3%	60.3%	-
Iranian[35]	2020	PCR	12.7%	-	26%	-	8%	8%
Georgia[36]	2014	PCR	45%	1.6%	50.3%	-	-	-
Bulgaria[37]	2019	PCR	86.9%	0.9%	11.3%	0.9%	-	-

Almost 2–3% of the world's population is known to be infected with HCV, totaling approximately 130–170 million people. Studies have shown that the frequency of HCV is generally higher in the Middle East and African countries and less frequent in Europe and America, indicating that these rates may vary between <1% and >10% according to countries[14]. The prediction suggests that the situation might change based on country-specific conditions, social factors, accessibility to healthcare facilities, and various other epidemiological conditions, despite the World Health Organization's set target to decrease the disease by 30% and fatalities by 10% for HCV by 2020 and to eradicate the disease in nine countries by 2030[15].

These treatments cannot be applied sufficiently in developing countries due to the inability to diagnose the disease in the acute phase, the failure to perform genotype determination, limited treatment infrastructure, and high costs, even though HCV is one of the promising therapeutic interventions for clinicians[16,] Serological tests such as ELISA are the first recommended tests for detecting the disease. Nucleic acid tests (NAT) and HCV ribonucleic acid detection (RNA) are advised to confirm the disease. Aminotransferase/platelet ratio index (APRI) and FIB-4 tests are used to show hepatic fibrosis to detect liver damage[17]. The non-structural (NS) 5b-encoded RNA-dependent RNA polymerase enzyme found in the HCV genome is an enzyme with no proofreading mechanism. It is known that different genotypes and subtypes of HCV emerge with mutations for this reason. There are thoughts that even diverse microbiota of the countries in this variety may be effective in the chronicity and mutation of HCV[18]. It aims to create new steps in treating this virus by sequence analysis of HCV with this different genetic distribution among countries[17]. This indicates that Genotype 1b in our province is positioned at the midpoint of the scale for 1b, ranging from 37% to 80% across the cities presented in Table 3, with a value of 71%. Even though the HCV genotype distribution within the countries is so different, it is relatively normal to see this diversity among the nations. According to our results, our genotype distribution is similar to some developing or developed countries, as shown in Table 2. This emphasizes the need for global genotyping studies and the development of treatment options[17,18].

The dose and duration of administration of the drugs used in the treatment of HCV vary according to genotype, acute or chronic occurrence of the disease, and the presence of cirrhosis. Clinical resistance to sofosbuvir (SOF) and mericitabine (MCB),

Table 3. *HCV genotype distribution in Türkiye*

Researcher	Location	Year	Method	Genotype 1	Genotype 2	Genotype 3	Genotype 4	Genotype 5	Genotype 6
Özbek E, et al.[38]	Diyarbakır	2009	Inno-Lipa	4.1% 87.8% 1b	2.7%	2.7% 2.7% 3a	-	-	-
Şanlıdağ T, et al.[39]	Manisa	2009	Real-Time	2% 1a 90% 1b	2% 2a		5%	-	-
Çelik C, et al.[40]	Sivas	2010	HCV-PM BİO (LİPA)	8.9% 1a 88.2% 1b	1.2% 2a	1.7%	-	-	-
Kalaycı R, et al.[41]	Afyon	2010	Sequence Analysis	20% 1a 63.3% 1b	-	-	13.3% 4a	-	-
Aktaş E, et al.[42]	Zonguldak	2010	Versant HCV Gen. Assay	2.6% 1a 97.4% 1b	-	-	-	-	-
Karslıgil T, et al.[43]	Gaziantep	2011	Sequence Analysis	9.8% 1a 78.4% 1b	7.8% 2a	2% 3a	-	-	-
Tezcan S, et al.[44]	Mersin	2012	Inno-Lipa	3.8% 1.7% 1a 84.7% 1b 2.1% 1a/1b	0.4% 1.3% 2b	4.2% 3a	0.8% 4a	-	0.4%
Buruk CK, et al.[45]	Trabzon	2013	Real-Time	5.3% 1a 87.5% 1b	1.6%	4.9%	0.7%	-	-
Sağlık İ, et al.[46]	Antalya	2014	Real-Time	14.7% 1a 63.3% 1b	3.5%	11.1%	1.6%	-	-
Çalışkan A, et al.[47]	Kahramanmaraş	2015	Real-Time	51.7%	1.3%	46%	1%	-	-
Çetin Duran A, et al.[48]	Adana	2016	Real-Time	12.6% 1a 58.8% 1b	7.6%	16.8%	3.4%	0.8%	-
Tüzüner U, et al.[49]	Central Anatolia	2018	Reverse hybridization	1.9% 3.1% 1a 3.5% 1b 0.8% 1a/1b	1.3% 1% 2b 0.6% 2a/2c	0.3% 2.9% 3a	1.7% 0.2% 4a	0.2% 5a	-
Karabulut N. et al.[50]	İstanbul	2018	Real-Time	6.3% 38.8% 1a 37.4% 1b	4.6%	10.7%	2.2%	-	-
Çetin Duran A. et al.[51]	Coastal Aegean	2019	Real-Time	36.9% 7.4% 1a 44.1% 1b	2.1%	5.3%	2.9%	0.3%	-
Süntur et al.[52]	Adana	2020	Real-Time	1.3% 8% 1a 43.1% 1b	11.3%	28.6%	4.1%	0.8%	-

two of the medications used in the treatment, are associated with mutations in NS5B-S283 in some studies. The non-response rate was around 50% in Genotype 1. In contrast, the success rate of the combination of pegylated interferon alfa and ribavirin used to treat HCV infection was approximately 80% in Genotypes 2 and 3. The response to combined treatment is around 35%, and treatment difficulties are mentioned in Genotype 4 patients. These studies also show the importance of genetic studies in the treatment and eradication stage. Treatment failure in the disease is mainly associated with decreased susceptibility to DAAs and resistance-associated substitutions (RAS), also known to be caused by the high cost of treatments. The fact that genotype and sequencing are of great importance in eradicating the disease is known about the subject[17,19].

HCV virus has eight main genotypes currently known due to its variable genome structure[18]. The difficulty in identifying these genotypes in each patient stands as a solid barrier to the eradication of the disease. Studies show that Genotype 1 and Genotype 3 are more common in these eight main genotypes, with a rate of 46% and 30%, respectively. In addition, Genotypes 4, 5, and 6 are common in Egypt, the Middle East, and the central region of Africa.

Genotypes 1a, 3a, and 1b are common in South Africa, Asia, and Iran[20]. In this case, our study data is more similar to the Middle East and the Far East from developing countries. Genotype distribution of HCV infection in countries is shown in Table 2.

Genotype 1b was high in many studies conducted in Türkiye, as in our study. A limited number of studies

also undertaken sequence analysis even though PCR was used in most studies[42,49]. The first four genotypes and their subtypes were detected throughout Türkiye, including our research. Still, Genotype 5 was encountered in Adana, Central Anatolia, and Coastal Aegean, and Genotype 6 was encountered in Mersin when the genotype distributions were examined in the studies[48–52]. 13.4% suggest researching the genotype source by pointing to a regional prevalence in Afyon. Genotype 7 and Genotype 8 have not yet been reported in Türkiye[41]. According to our study's data, age and gender differences had no effect on HCV genotype distribution ($p>0.05$).

In conclusion, our study sheds light on the possibility of various problems in the treatment of HCV in the region due to the resistance of 1b (71%) and four genotypes (2%) to treatment response, as well as being the first study on the determination of HCV genotype in Türkiye. It is a known fact that the importance of regional genotype determination in treatment options that may be a beacon of hope in HCV treatment is indisputable in line with the studies conducted on this subject.

Acknowledgments

Our study was supported by the number 2020-TS-13 within the scope of Kafkas University Scientific Research Projects.

Authors' Contributions

CEBB and AG Conceived and designed research. CEBB and AG Performed experiments; CEBB, AY, and AG Wrote the manuscript; AY and CEBB Analyzed data; AG and AY Interpreted results of experiments; CEBB and AY Prepared figures; CEBB and AG Edited and revised the manuscript; CEBB, AG, and AY Approved final version of the manuscript.

Conflict of Interest

We declare that there is no conflict of interest. The funding bodies had no role in the study's design, collection/ analysis/ interpretation of data, writing of the manuscript, or the decision to publish the results.

References

1. Lindenbach BD, Rice CM. Unravelling hepatitis C virus replication from genome to function. Nature. 2005;436:933–8.
2. Dore GJ, Bajis S. Hepatitis C virus elimination: laying the foundation for achieving 2030 targets. Nat Rev Gastroenterol Hepatol. 2020;18:91–2.
3. https://www.who.int/news-room/fact-sheets/detail/hepatitis-c
4. Razavi H, Elkhoury AC, Elbasha E, Estes C, Pasini K, Poynard T, et al. Chronic hepatitis C virus (HCV) disease burden and cost in the United States. Hepatology. 2013;57:2164–2170. [PMC free article] [PubMed] [Google Scholar]
5. Petruzziello A, Marigliano S, Loquercio G, Cozzolino A, Cacciapuoti C. Global epidemiology of hepatitis C virus infection: An up-date of the distribution and circulation of hepatitis C virus genotypes. World J Gastroenterol. 2016;22:7824–40.
6. Cooke GS, Lemoine M, Thursz M, Gore C, Swan T, Kamarulzaman A, et al. Viral hepatitis and the Global Burden of Disease: a need to regroup. J Viral Hepat. 2013;20:600–1.
7. Mohd Hanafiah K, Groeger J, Flaxman AD, Wiersma ST. Global epidemiology of hepatitis C virus infection: new estimates of age-specific antibody to HCV seroprevalence. Hepatology. 2013;57:1333–42.
8. Kato N. Genome of human hepatitis C virus (HCV): gene organization, sequence diversity, and variation. Microbial & comparative genomics, 2000, 5. 3:129–151.
9. Messina JP, Humphreys I, Flaxman A, Brown A, Cooke GS, Pybus OG, et al. Global distribution and prevalence of hepatitis C virus genotypes. Hepatology, 2015, 61. 1:77–87.
10. McOmish F, Yap PL, Dow BC, Follett EA, Seed C, Keller AJ, et al. Geographical distribution of hepatitis C virus genotypes in blood donors: an international collaborative survey. J Clin Microbiol. 1994;32:884–92.
11. Simmonds P, Holmes EC, Cha TA, Chan SW, McOmish F, Irvine B, et al. Classification of hepatitis C virus into six major genotypes and a series of subtypes by phylogenetic analysis of the NS-5 region. J Gen Virol. 1993;74:2391–9.
12. Altindis M, Dal T, Akyar I, Karatuna O, Gokahmetoglu S, Tezcan US, et al. Six-year distribution pattern of hepatitis C virus in Turkey: a multicentre study. Biotechnol Bioeng. 2016;30:335–40.
13. https://euhcvdb.lyon.inserm.fr/euHCVdb/jsp/index.jsp
14. The Global Burden of Hepatitis C Working Group. Global burden of disease (GBD) for hepatitis C. J Clin Pharmacol. 2004;44:20–9.
15. Polaris Observatory. The authoritative resource for epidemiological data, modeling tools, training, and decision analytics to support global elimination of hepatitis B and C by 2030. Available at: http://www.hepbunited.org/assets/Webinar-Slides/8b54a78215/POLARIS-Brief-181217.pdf
16. Dore GJ. The changing therapeutic landscape for hepatitis C. Med J Aust. 2012;196:629–2.

17. World Health Organization. Guidelines for the Screening Care and Treatment of Persons with Chronic Hepatitis C Infection Updated Version April 2016: Guidelines. World Health Organization. p:15–16.
18. Sandres-Sauné K, Deny P, Pasquier C, Thibaut V, Duverlie G, Izopet J. Determining hepatitis C genotype by analyzing the sequence of the NS5b region, J Virol Methods. 2003;109:187–93.
19. Patiño-Galindo JÁ, Salvatierra K, González-Candelas F, López-Labrador FX. Comprehensive screening for naturally occurring hepatitis C virus resistance to direct-acting antivirals in the NS3, NS5A, and NS5B genes in worldwide isolates of viral genotypes 1 to 6. Antimicrob Agents Chemother. 2016;60:2402–16.
20. Prakash S, Shukla S, Ramakrishna V, Jain A. Distribution of hepatitis C genotypes in Uttar Pradesh, India;rare genotype 4 detected. J Med Virol. 2018;90:1875–81.
21. Li R, Xie Y, Liu W, Ma Y. Hepatitis C virus genotype and subtype distribution among ethnic minorities in Liaoning Province of China. Medicine. 2021;100:e24137.
22. Krekulova L, Rehak V, Strunecky O, Nemecek V. Current situation and trends in the hepatitis C virus genotype distribution among injecting drug users in the Czech Republic. Epidemiol Microbiol Immunol. 2009;58:84–9.
23. Nemecek V, Strunecky O. Genotypic heterogeneity of hepatitis C virus (HCV) from blood donors in the Czech Republic. Epidemiol Microbiol Immunol. 2009;58:63–72.
24. Karmochkine M, Carrat F, Dos Santos O, Cacoub P, Raguin G. A case- control study of risk factors for hepatitis C infection in patients with unexplained routes of infection. J Viral Hepat. 2006;13:775–82.
25. Martinot-Peignoux M, Roudot-Thoraval F, Mendel I, Costel J, Izopet J, Duverlie J, et al. Hepatitis C virus genotypes in France: relationship with epidemiology, pathogenicity and response to interferon therapy. J Viral Hepat. 1999;6:435–43.
26. Feucht HH, Zöllner B, Schröter M, Hoyer A, Sterneck M, Polywka S. Distribution of genotypes and response to alpha-interferon in patients with hepatitis C virus infection in Germany. Eur J Clin Microbiol Infect Dis. 1996;15:128–32.
27. Ross RS, Viazov SO, Holtzer CD, Beyou A, Monnet A, Mazure C, et al. Genotyping of hepatitis C virus isolates using CLIP sequencing. J Clin Microbiol. 2000;38:3581–4.
28. Raptopoulou M, Touloumi G, Tzourmakliotis D, Nikolopoulou G, Dimopoulou M, Giannoulis G, et al. Significant epidemiological changes in chronic hepatitis C infection: results of the nationwide HEPNET-GREECE cohort study. Hippokratia. 2011;15:26–31.
29. Sarvari C, Ujhelyi E, Szlavik J. Determination of hepatitis C virus genotypes in Hungary using the Trugene HCV 5'NC genotyping test. ISMD2010 Eighth International Symposium on Molecular Diagnostics 2010 Graz, Austria Clin Chem Lab Med. 2010;48:A1.
30. Matera G, Lamberti A, Quirino A, Foca D, Giancotti A, Barreca GS, et al. Changes in the prevalence of hepatitis C virus (HCV) genotype 4 in Calabria, Southern Italy. Diagn Microbiol Infect Dis. 2002;42:169–73.
31. Chlabicz S, Flisiak R, Kowalczuk O, Grzeszczuk A, Pytel-Krolczuk B, Prokopowicz D, et al. Changing HCV genotypes distribution in Poland relation to source and time of infection. J Clin Virol. 2008;42:156–9.
32. Dalgard O, Jeansson S, Skaug K, Raknerud N, Bell H. Hepatitis C in the general adult population of Oslo: prevalence and clinical spectrum. Scand J Gastroenterol. 2003;38:864–70.
33. Zuckerman E. Liver Unit, Carmel Medical Center, Haifa, Israel. Hepatitis C in Israel. Conversation with Razavi HA. Center for Disease Analysis, Kromite, Louisville, CO, USA 2 October 2010.
34. Abuelhassan WB, Gasim GI, Ally R, Menezes C. Hepatitis C infection at a tertiary hospital in South Africa: Clinical presentation, non-invasive assessment of liver fibrosis, and response to therapy. S Afr Med J. 2020;110:920–5.
35. Khadempour-Arani H, Shojaeian A, Mehri-Ghahfarrokhi A, Shahraki HR, Karimi A, Dehghan A, et al. Identifying genotype profile of chronic hepatitis C infection in Southwest Iran. J Res Med Sci. 2020;25:1–5.
36. Hadinedoushan H, Salmanroghani H, Amirbaigy MK, Akhondi-Meybodi M. "Hepatitis C virus genotypes and association with viral load in yazd, central province of iran. " Hepa Month. 2019;14:e11705.
37. Brady Z, Stoykova Z. Hepatitis C virus genotype analysis in patients with chronic hepatitis in North Eastern Bulgaria. J Drug Assess. 2019;8:146–9.
38. Sarvar E, Ozekinci T, Mese S, Atmaca S. Hepatitis C virus genotypes are changing in the Southeast of Turkey. Biotechnol Biotechnol Eq. 2009;23:1521–23.
39. Şanlıdağ T, Akcali S, Ozbakkaloğlu B, Ertekin D, Akduman E. Distribution of hepatitis C virus genotypes in Manisa region, Turkey. Mikrobiyol Bul. 2009;43:613–8.
40. Çelik C, Bakıcı MZ, Kaygusuz R, Ertürk R. Investigation of HCV genotype distributions in Sivas region. Viral Hepatit Derg. 2010;16:106–10.
41. Kalayci R, Altindiş M, Gülamber G, 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;44:237–43.
42. Aktaş E, Ogedey ED, Külah C, Beğendik Cömert F. Hepatitis C virus genotypes in a province of Western Black-Sea region, Turkey. Mikrobiyol Bul. 2010;44:647–50.
43 Özbek T, Savaş E, Savaş MC. Genotype distribution and 5' UTR nucleotide changes in hepatitis C virus. Balkan Med J. 2011;28:232–6.
44. Tezcan S, Ülger M, Aslan G, Yaraş S, Altındaş E, Sezgin O, et al. Genotype Distribution in Mersin Province, Turkey. Mikrobiyol Bul. 2013;47:332–8.

45. Buruk CK, Bayramoglu G, Reis A, Kaklıkkaya N, Tosun I, Aydın F. Determination of hepatitis C virus genotypes among hepatitis C patients in eastern black sea region, Turkey. Mikrobiyol Bul. 2013;47:650–7.
46. Sağlik İ, Mutlu D, Öngut G, İnan D, Öğünç D, Can Sarinoğlu R, et al. Distribution of Hepatitis C Virus Genotypes among Patients with Chronic Hepatitis C Infection in Akdeniz University Hospital, Antalya, Turkey: A Five-Year Evaluation. Mikrobiyol Bul. 2014;48:429–37.
47. Calıskan A, Kirisci O, Ozkaya E, Ozden S, Tümer S, Caglar S, et al. Distribution and Predomi-nance of Genotype 3 in Hepatitis C Virus Carriers in the Province of Kahramanmaras, Turkey. Hepat Mon. 2015;15:e25142.
48. 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. Turk Hij Den Biyol Derg. 2017;74:201–210.
49. Tüzüner U, Saran Gülcen B, Özdemir M, Feyzioğlu B, Baykan M. Seven-year Genotype Distribution Among Hepatitis C Patients in a City in the Central Anatolia Region of Turkey. Viral Hepat J. 2018;24:12–7.
50. Karabulut N, Alaçam S, Yolcu A, Onel M, Agaçfidan A. Distribution of hepatitis C virus genotypes in Istanbul, Turkey. Indian journal of medical microbiology. 2018;36:192–6.
51. Duran AÇ, Çetinkaya ÖK, Sayıner AA, Şeydaoğlu G, Özkarataş E, Abacıoğlu H. Changes on Hepatitis C Virus Genotype Distribution in Western Turkey: Evaluation of Twelve-Year Data. Turk J Gastroenterol. 2020;31:128–35.
52. Suntur BM, Kaya H, Şahin Eker HB, Kara B, Bozok T, Unal N. A cross-sectional study of real-life data of HCV from Turkey south region, J Infect Dev Ctries. 2020;14:380–386.