VIRAL HEPATITIS SOCIETY

E-ISSN: 2147-2939

Viral Hepatitis Journal

VİRAL HEPATİT DERGİSİ

RESEARCH ARTICLES

www.viralhepatitisjournal.org

December 2024
3
Volume 30

galenos

VIRAL HEPATITIS SOCIETY

Viral Hepatitis Journal

VİRAL HEPATİT DERGİSİ

Address for Correspondence:
Viral Hepatitis Prevention Society
Sağlık Mahallesi, Süleyman Sırrı Caddesi No: 2/15
Sıhhıye, Ankara, Turkey
Phone: +90 312 433 74 26
Fax: +90 312 433 06 54
E-mail: info@viralhepatitisjournal.org

Publisher Contact
Address: Molla Gürani Mah. Kaçamak Sk. No: 21/1
34093 İstanbul, Turkey
Phone: +90 (539) 307 32 03
E-mail: info@galenos.com.tr
yayin@galenos.com.tr
Web: www.galenos.com.tr

Yayıncı Sertifika No: 14521
Online Publication Date: December 2024
E-ISSN: 2147-2939
International scientific journal published quarterly.

VIRAL HEPATITIS SOCIETY

Viral Hepatitis Journal

VİRAL HEPATİT DERGİSİ

Please refer to the journal's webpage **(https://viralhepatitisjournal.org/)** for **"About Us"**, **"Instructions to Authors"** and **"Peer Review & Ethics"**.
The journal's editorial policies are based on "ICMJE Recommendations" rules.
Viral Hepatitis Journal is indexed in **Emerging Sources Citation Index (ESCI), EBSCO, Gale, ProQuest, Embase, CABI, Embase, CINAHL Database, Tübitak/Ulakbim Turkish Medical Database, British Library, J-Gate, IdealOnline, Türk Medline Index** and **Turkiye Citation Index databases**.
The journal is published online.
Owner: Viral Hepatitis Society
Responsible Manager: Fehmi TABAK

VIRAL HEPATITIS SOCIETY

Viral Hepatitis Journal

VİRAL HEPATİT DERGİSİ

CONTENTS

Research Article

49

DOI: 10.4274/vhd.galenos.2024.2023-1-2
Viral Hepat J 2024;30(3):49-52

Epidemiological Shift in Hepatitis C Genotypes in Northern Anatolia

Kuzey Anadolu'da Hepatit C Genotiplerinin Epidemiyolojik Değişimi

Tuğçe Ünalan-Altıntop[1], Mustafa Arslan[2], Sabri Engin Altıntop[3], Pelin Onarer[1], Fikriye Milletli-Sezgin[4]

[1]*Amasya University, Sabuncuoğlu Şerefeddin Training and Research Hospital, Clinic of Medical Microbiology, Amasya, Turkey*
[2]*Amasya University Faculty of Medicine, Department of Infectious Diseases and Clinical Microbiology, Amasya, Turkey*
[3]*Suluova State Hospital, Clinic of Internal Medicine, Amasya, Turkey*
[4]*Amasya University Faculty of Medicine, Department of Medical Microbiology, Amasya, Turkey*

ABSTRACT

Objectives: Hepatitis C virus (HCV) infection is an important public health issue with large genetic diversity. Although treatment differences for genotypes have vanished with the discovery of novel drugs, genotyping enables the prediction of clinical outcomes and prevents the spread of HCV in the community by identifying risk factors. The aim of this study was to assess the genotypic changes of HCV in Northern Anatolia, Amasya, Turkey.

Materials and Methods: A total of 180 HCV-positive patients were included in the study between 2015 and 2022. HCV genotypes were investigated using real time polymerase chain reaction. Demographic data were obtained from medical records.

Results: There was a decrease in the overall incidence of HCV after 2016. A decrease in genotypes 1 and 1b and an increase in genotype 3 were recorded over time. The only patient with genotype 4 detected in 2016 was an immigrant from Syria. The mean age of patients with genotype 1 was 55.95 and genotype 3 was 28.75. There were slightly more male patients in the genotype 3 group and more female patients in the genotype 1. After a year of treatment with the new regimens, all of the patients achieved viral clearance except two.

Conclusion: Despite genotype 1b's continued dominance, our research showed that there was a shift in the distribution and frequency of HCV in our region, primarily due to emigration. The new treatment regimens decreased the number of patients and improved treatment success.

Keywords: Hepatitis C, genotype, epidemiology

ÖZ

Amaç: Hepatit C virüsü (HCV) enfeksiyonu, geniş genetik çeşitliliğe sahip önemli bir halk sağlığı sorunudur. Yeni pan-genotipik ilaçların keşfiyle, genotipler için tedavi farklılıkları ortadan kalkmış olsa da, genotiplendirme, hastaların klinik seyirlerinin tahmini ve risk faktörlerine yönelik etkin önlemlerin alınması açısından önemini korumaktadır. Bu çalışmanın amacı, Kuzey Anadolu'da yer alan Amasya ilinde HCV'nin genotipik değişimlerini değerlendirmektir.

Gereç ve Yöntemler: 2015-2022 yılları arasında toplam 180 HCV ile pozitif hasta çalışmaya dahil edildi. HCV genotipleri gerçek zamanlı polimeraz zincir reaksiyonu ile araştırıldı. Demografik veriler tıbbi kayıtlardan elde edildi.

Bulgular: 2016 yılından sonra genel HCV insidansında azalma gözlendi. Zaman içinde genotip 1 ve 1b'de azalma ve genotip 3'te artış kaydedildi. 2016 yılında görülen tek genotip 4 hastası Suriye kökenliydi. Genotip 1 hastalarının yaş ortalaması 55,95, genotip 3 ise 28,75 idi. Genotip 3 grubunda erkek hasta sayısı, genotip 1 grubunda ise kadın hasta sayısı fazlaydı. Yeni tedavi protokolleri ile iki hasta haricinde tüm hastalarda viral klirens gözlendi.

Sonuç: Genotip 1b ilimizdeki hakimiyetini sürdürmesine rağmen, HCV sıklığında ve genotiplerin dağılımında, öncelikle göç nedenli değişimler olduğunu göstermiştir. Yeni tedavi rejimleri hasta sayısını azaltırken, tedavi başarısını artırmıştır.

Anahtar Kelimeler: Hepatit C, genotip, epidemiyoloji

Cite this article as: Ünalan-Altıntop T, Arslan M, Altıntop SE, Onarer P, Milletli-Sezgin F. Epidemiological shift in hepatitis C genotypes in Northern Anatolia. Viral Hepat J. 2024;30(3):49-52

Address for Correspondence: Tuğçe Ünalan-Altıntop, MD, Amasya University, Sabuncuoğlu Şerefeddin Training and Research Hospital, Clinic of Medical Microbiology, Amasya, Turkey
E-mail: t.unalan91@gmail.com **ORCID ID:** orcid.org/0000-0001-5318-2942
Received: 09.01.2023 **Accepted:** 15.08.2024 **Epub:** 07.10.2024 **Publication Date:** 30.12.2024

Introduction

Hepatitis C virus (HCV) infection is a serious public health problem that leads to liver cirrhosis, which carries a 1-5% annual risk of hepatocellular cancer, with an estimated 80 (64-103) million viremic people worldwide (1). In our country, the prevalence of HCV infection is estimated to be 1-1.9 percent (2). HCV has a significant genetic diversity including seven virus genotypes (1-7) in addition to subtypes (a, b, c, etc.) and quasi-species (3). Genotypes 1a, 1b, 2, 3, and 4 cause the majority of infections, whereas genotypes 5, 6, and 7 are less common or limited to specific geographic locations (4). In studies conducted in our country, the predominant HCV genotype was 1b (5,6).

Chronic HCV infection was treated with pegylated interferon plus ribavirin and first-generation protease inhibitors depending on the HCV genotype of the patient (7). However, the need for genotype determination for treatment strategies has disappeared with the advent of novel pangenotypic direct-acting agents (DAAs) (8). Nevertheless, genotype determination remains important for epidemiological purposes because it can be influenced by migratory patterns and changes in infection routes (9,10,11). Although genotype 1b was widely spread throughout the world after World War II, genotypes 3 and 4 were spreading with an increasing trend due to intravenous drug use and migratory movements (12,13,14). In addition, patients with genotypes 1 and 3 are at risk of hepatocellular cancer development, and they need to be followed up more closely (15,16).

The aim of this study was to determine the molecular epidemiological profile of the HCV in the Northern Anatolian region of Amasya, Turkey, and to establish the changes that occurred over time. In addition, the clinical follow-ups and treatment responses of all HCV-positive patients were analyzed.

Materials and Methods

A total of 180 chronic Hepatitis C patients who were anti-HCV and HCV-RNA positive and who were tested in Amasya University, Sabuncuoğlu Şerefeddin Research and Training Hospital Medical Microbiology Laboratories between January 2015-May 2022 were enrolled in this study. The anti-HCV assay was performed using a chemiluminescent microparticle immunoassay (Abbott, USA). HCV-RNA and genotypes were determined using real-time PCR according to the manufacturer's recommendations (Anatolia Geneworks, Turkey). The demographic data of the patients were reviewed retrospectively using medical records. This study was approved by the Ethics Committee of Amasya University Non-interventional Clinical Studies (approval number: 81, date: 07.07.2022).

Results

Of the 180 chronic HCV patients, 106 (58.89%) were female and 74 (41.11%) were male. The mean age of the patients was 63.95±16.43. While most patients were in the 61 and over age range (n=106; 58.89%), 15 (8.33%) patients were in the 15-30 age group, 12 (6.67%) patients were in the 31-45 age group, and 31 (17.22%) patients were in the 46-60 age range. Of the 180 chronic HCV patients, 106 (58.89%) were female and 74 (41.11%) were male. During 2015-2022, 93.33% of the patients were in genotype 1 (88.69% were genotype 1b), 1.67% were in genotype 2, 4.44% were in genotype 3, and 0.06% were in genotype 4. The genotype distribution according to years is presented in Figure 1. The female-to-male proportion of genotypes was as follows: 7 to 12 for genotype 1a, 97 to 52 for genotype 1b, 0 to 3 for genotype 2, 1 to 7 for genotype 3, and only 1 female for genotype 4 (Figure 2). There were 2 immigrants from Syria; one has genotype 1b and the other 4. The mean age of genotypes was as follows: 55.95 for 1a, 67.62 for 1b, 34.33 for 2, 28.75 for 3, and 40 for 4.

Out of the 180 patients, 71 were initiated on ombitasvir, paritaprevir, ritonavir, and dasabuvir; 37 received sofosbuvir and ledipasvir; 18 received glecaprevir and pibrentasvir, sofosbuvir and ribavirin. 50 were not followed up in our center, and two were not treated due to advanced age. Before treatment, the mean viral load was 1487780 international unit (IU)/mL, then decreased to 61408 IU/mL in one month. After 12 months of treatment, all patients were HCV-negative by PCR, except for two. One patient had recurrence after ombitasvir, paritaprevir, ritonavir, and dasabuvir treatment but was successfully treated with glecaprevir and pibrentasvir, and the other could not be treated with sofosbuvir and ribavirin. Thirty-five of the patients had previously used ribavirin and pegylated interferon but were nonresponsive to treatment. Fifty-two of the patients had cirrhosis, 4 had type 1a, 46 had type 1b, 1 had type 2, and 1 had type 3. Two patients had hepatocellular carcinoma, who had type 1b HCV.

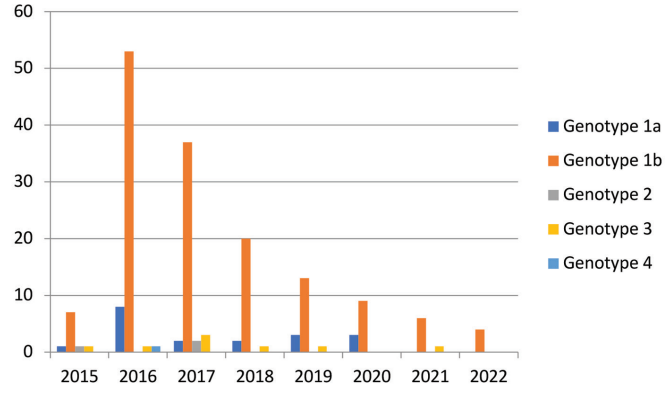

Figure 1. HCV genotype distribution according to age
HCV: Hepatitis C virus

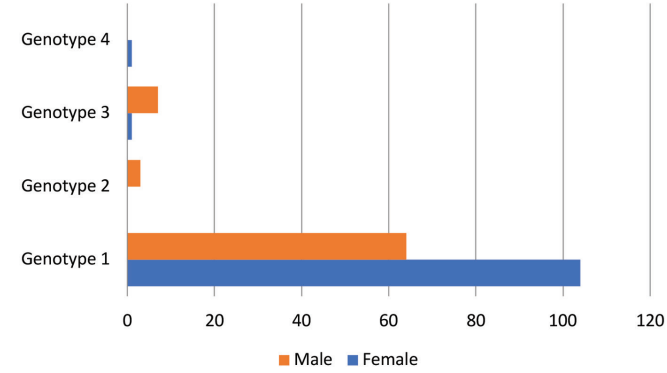

Figure 2. HCV genotype distribution according to gender
HCV: Hepatitis C virus

Discussion

Our research demonstrated an increase in genotypes 3 and 4 in our region. In a previous report from Amasya, during the 2010-2014 years, 96.34% of the patients were genotype 1 (69.51% were genotype 1b), 2.44% were genotype 2, and 1.22% were genotype 3 (17). However, during the 2015-2022 years, we found that 93.33% of the patients were genotype 1 (88.69% were genotype 1b), 1.67% were genotype 2, 4.44% were genotype 3, and 0.06% were genotype 4. The only patient with genotype 4 was an immigrant from Syria. According to a Syrian study, genotype 4 infections are more common in eastern Syria, whereas genotype 5 infections are more common in northern regions close to Turkey (18). Due to migration movements from the Middle East and Africa, genotype 4 has become more prevalent and is most prevalent in Central Africa and the Middle East (4). Turkey's genotype 4 distribution does not exhibit substantial regional variation, with a range of 0.5-3.4%, with Kayseri (32%) having the highest prevalence (19,20). A study from İzmir demonstrated that approximately one-third of patients with genotype 4 were of Syrian origin (21). In our study, no genotype 4 was detected until one patient of Syrian origin was found to have genotype 4 in 2016.

In addition, a decreasing trend was observed in HCV positivity, especially after 2016. This result is also comparable with national and global reports of HCV (22,23,24). The discovery of novel agents, advent of disinfection and sterilization practices, and improved awareness of risk factors among the community can all cause the decline in HCV prevalence (22). The DAAs have had a high rate of treatment success in Turkey during the past ten years because of their potent antiviral activity, which can block a variety of targets involved in the life cycle of HCV (25). Since medications have become more widely available, more patients are receiving DAA treatment worldwide, particularly in low- and middle-income countries (26). In our study population, all patients except two had achieved complete viral clearance after 12 months of treatment.

Age-based genotype distributions may differ depending on the social structure of the community and the incidence of risk factors in that community among different age groups. According to Niu et al. (27), genotypes 1 and 2 were more prevalent in patients aged 40-60 years, but genotype 3 cases were more common in younger patients. Different studies have demonstrated that genotype 1 patients are older than 45 years old (28,29,30). In Turkey, patients with genotype 1 are typically aged 50-60 years (31,32,33). The mean ages of patients with genotypes 1, 2, and 4 in a study from Maraş were comparable to those in the abovementioned studies. However, genotype 3 patients had a mean age of 26.4 (34). In our study, patients with genotype 1 had a mean age of 67.92 years, and those with genotype 3 had a mean age of 28.75 years, which is concordant with global and national data.

The genotype distribution may differ according to sex. Janahi et al. (35) found that male cases of all genotypes were more common than female case. However, genotype 3 had the lowest prevalence in female patients. Similarly, Bouacida et al. (36) reported that the prevalence of male cases was greater across all genotypes. genotypes 1b and 2 were more prevalent in women, according to Kartashev et al. (28), whereas genotypes 1a, 3, and 4 were more prevalent in men. Our study also demonstrated that female patients had a higher proportion of genotype 1b patients; however, male patients were of genotype 3.

Conclusion

Our research revealed that despite genotype 1b's continuous dominance, our region's HCV genotype distribution and prevalence are shifting, mostly as a result of migration. Total viral clearance was achieved in all patients, except two patients who received the new treatment protocols after 12 months. Treatment and prevention choices may be improved by tracking the epidemiology of HCV genotypes.

Ethics

Ethics Committee Approval: This study was approved by the Ethics Committee of Amasya University Non-interventional Clinical Studies (approval number: 81, date: 07.07.2022).

Informed Consent: Informed consents were not obtained due to the retrospective design of the study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: T.Ü-A., M.A., S.E.A., Concept: T.Ü-A., Design: T.Ü-A., Data Collection or Processing: T.Ü-A., P.O., F.M-S., Analysis or Interpretation: T.Ü-A., P.O., F.M-S., Literature Search: T.Ü-A., Writing: T.Ü-A.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declare no financial support.

References

1. 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(Suppl 1):45-57.
2. Barut HŞ, Günal Ö. Global and National Epidemiology. Klimik Journal. 2009;22:38-43.
3. Smith DB, Bukh J, Kuiken C, Muerhoff AS, Rice CM, Stapleton JT, Simmonds P. Expanded classification of hepatitis C virus into 7 genotypes and 67 subtypes: updated criteria and genotype assignment web resource. Hepatology. 2014;59:318-327.
4. Messina JP, Humphreys I, Flaxman A, Brown A, Cooke GS, Pybus OG, Barnes E. Global distribution and prevalence of hepatitis C virus genotypes. Hepatology. 2015;61:77-87.
5. Abacioglu YH, Davidson F, Tuncer S, Yap PL, Ustacelebi S, Yulug N, Simmonds P. The distribution of hepatitis C virus genotypes in Turkish patients. J Viral Hepat. 1995;2:297-301.
6. Keskın F, Çıftçı S, Türkoğlu S, Badur S. Transmission routes of chronic hepatitis C and their relation to HCV genotypes. Turk J Gastroenterol. 2010;21:396-400.
7. Keikha M, Eslami M, Yousefi B, Ali-Hassanzadeh M, Kamali A, Yousefi M, Karbalaei M. HCV genotypes and their determinative role in hepatitis C treatment. Virusdisease. 2020;31:235-240.
8. Zoratti MJ, Siddiqua A, Morassut RE, Zeraatkar D, Chou R, van Holten J, Xie F, Druyts E. Pangenotypic direct acting antivirals for the treatment of chronic hepatitis C virus infection: A systematic literature review and meta-analysis. EClinicalMedicine. 2020;18:100237.
9. Pawlotsky JM, Tsakiris L, Roudot-Thoraval F, Pellet C, Stuyver L, Duval J, Dhumeaux D. Relationship between hepatitis C virus genotypes and

sources of infection in patients with chronic hepatitis C. J Infect Dis. 1995;171:1607-1610.

10. Esteban JI, Sauleda S, Quer J. The changing epidemiology of hepatitis C virus infection in Europe. J Hepatol. 2008;48:148-162.
11. Markov PV, van de Laar TJ, Thomas XV, Aronson SJ, Weegink CJ, van den Berk GE, Prins M, Pybus OG, Schinkel J. Colonial history and contemporary transmission shape the genetic diversity of hepatitis C virus genotype 2 in Amsterdam. J Virol. 2012;86:7677-7687.
12. Morice Y, Cantaloube JF, Beaucourt S, Barbotte L, De Gendt S, Goncales FL, Butterworth L, Cooksley G, Gish RG, Beaugrand M, Fay F, Fay O, Gonzalez JE, Martins RM, Dhumeaux D, Vanderborght B, Stuyver L, Sablon E, de Lamballerie X, Pawlotsky JM. Molecular epidemiology of hepatitis C virus subtype 3a in injecting drug users. J Med Virol. 2006;78:1296-1303.
13. Uddin G, Shoeb D, Solaiman S, Marley R, Gore C, Ramsay M, Harris R, Ushiro-Lumb I, Moreea S, Alam S, Thomas HC, Khan S, Watt B, Pugh RN, Ramaiah S, Jervis R, Hughes A, Singhal S, Cameron S, Carman WF, Foster GR. Prevalence of chronic viral hepatitis in people of south Asian ethnicity living in England: the prevalence cannot necessarily be predicted from the prevalence in the country of origin. J Viral Hepat. 2010;17:327-335.
14. Kamal SM, Nasser IA. Hepatitis C genotype 4: What we know and what we don't yet know. Hepatology. 2008;47:1371-1383.
15. Bruno S, Crosignani A, Maisonneuve P, Rossi S, Silini E, Mondelli MU. Hepatitis C virus genotype 1b as a major risk factor associated with hepatocellular carcinoma in patients with cirrhosis: a seventeen-year prospective cohort study. Hepatology. 2007;46:1350-1356.
16. Kanwal F, Kramer JR, Ilyas J, Duan Z, El-Serag HB. HCV genotype 3 is associated with an increased risk of cirrhosis and hepatocellular cancer in a national sample of U.S. veterans with HCV. Hepatology. 2014;60:98-105.
17. Kilinc C, Guckan R, Aydin O, Idil O, Guleryuzlu Y, Çapraz M. Determining hepatitis C virus genotype distribution of Amasya area. Ulutas Med J. 2015;1:41-43.
18. Antaki N, Haddad M, Kebbewar K, Abdelwahab J, Hamed O, Aaraj R, Alhaj N, Haffar S, Assil M, Ftayeh M, Assaad F, Doghman D, Ali T, Nassereldine M, Ali A, Antaki F; Syrian Working Group for the Study of Viral Hepatitis. The unexpected discovery of a focus of hepatitis C virus genotype 5 in a Syrian province. Epidemiol Infect. 2009;137:79-84.
19. Cirit OS, Mızraklı AU, 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.
20. Kayman T, Karakükçü, Karaman A, Gözütok F. Genotypic distribution of hepatitis C virus infection in Kayseri region. J Turk Mikrobiyol Soc. 2012;42:21-26.
21. Çetin Duran A, Kaya Çetinkaya Ö, 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;31:128-135.
22. Güngör S. Status of Viral Hepatitis in Uşak Province, Turkey: A 9-Year Retrospective Analysis. Acta Medica. 2021;37:2089.
23. World Health Organization (WHO). Global hepatitis report. 2017.
24. Polaris Observatory HCV Collaborators. Global prevalence and genotype distribution of hepatitis C virus infection in 2015: a modelling study. Lancet Gastroenterol Hepatol. 2017;2:161-176.
25. Turkey Viral Hepatitis Prevention and Control Program. 2018.
26. Razavi H. Global Epidemiology of Viral Hepatitis. Gastroenterol Clin North Am. 2020;49:179-189.
27. Niu Z, Zhang P, Tong Y. Age and gender distribution of hepatitis C virus prevalence and genotypes of individuals of physical examination in WuHan, Central China. Springerplus. 2016;5:1557.
28. Kartashev V, Döring M, Nieto L, Coletta E, Kaiser R, Sierra S; HCV EuResist Study group. New findings in HCV genotype distribution in selected West European, Russian and Israeli regions. J Clin Virol. 2016;81:82-89.
29. Khan N, Akmal M, Hayat M, Umar M, Ullah A, Ahmed I, Rahim K, Ali S, Bahadar S, Saleha S. Geographic distribution of hepatitis C virus genotypes in pakistan. Hepat Mon. 2014;14:e20299.
30. Rouabhia S, Sadelaoud M, Chaabna-Mokrane K, Toumi W, Abenavoli L. Hepatitis C virus genotypes in north eastern Algeria: A retrospective study. World J Hepatol. 2013;5:393-397.
31. Tüzüner U, Gülcen BS, Özdemir M, Feyzioğlu, Baykan M. Seven-year genotype distribution among hepatitis C patients in a city in the Central Anatolia Region of Turkey. Viral Hepat J. 2018;24:12-17.
32. Tezcan S, Ulger M, Aslan G, Yaraş S, Altıntaş E, Sezgin O, Emekdaş G, Gürer Giray B, Sungur MA. Mersin ilinde hepatit C virusu genotip dağılımının belirlenmesi [Determination of hepatitis C virus genotype distribution in Mersin province, Turkey]. Mikrobiyol Bul. 2013;47:332-338.
33. Çiftçi İH, Er H, Aşık G, Aktepe OC, Altındiş M. The distribution of genotype of the hepatitis C virus (HCV) RNA positive patients. Kocatepe Medical Journal. 2009;10:21-24.
34. Gişi K, İspiroğlu M, Şahin AR, Aral M, Kantarçeken B. Hepatitis C genotype distribution changing through years in the Kahramanmaraş region. Viral Hepat J. 2022;28:67-71.
35. Janahi EM, Al-Mannai M, Singh H, Jahromi MM. Distribution of hepatitis C virus genotypes in Bahrain. Hepat Mon. 2015;15:e30300.
36. Bouacida L, Suin V, Hutse V, Boudewijns M, Cartuyvels R, Debaisieux L, De Laere E, Hallin M, Hougardy N, Lagrou K, Oris E, Padalko E, Reynders M, Roussel G, Senterre JM, Stalpaert M, Ursi D, Vael C, Vaira D, Van Acker J, Verstrepen W, Van Gucht S, Kabamba B, Quoilin S, Muyldermans G. Distribution of HCV genotypes in Belgium from 2008 to 2015. PLoS One. 2018;13:e0207584.

Research Article

53

DOI: 10.4274/vhd.galenos.2024.2024-5-1
Viral Hepat J 2024;30(3):53-58

Prevalence of Hepatitis C Virus and Human Pegivirus Type 1 Co-Infection in Patients Infected with Human Immunodeficiency Virus Type-1

İnsan İmmün Yetmezlik Virüsü Tip 1 ile Enfekte Hastalarda Hepatit C Virüsü ve İnsan Pegivirüs Tip-1 Ko-Enfeksiyonlarının Prevalansı

Zahra Elahimanesh[1], Farah Bokharaei-Salim[1], Tahereh Donyavi[2], Seyed Hamidreza Monavari[1], Ahmad Tavakoli[3], Majid Khoshmirsafa[3], Sara Chavoshpour[4], Mohammad Abbasi-Kolli[5], Zohreh Yousefi Ghalejoogh[1], Seyed Jalal Kiani[1]

[1]*Iran University of Medical Sciences, School of Medicine, Department of Virology, Tehran, Iran*
[2]*Iran University of Medical Sciences, School of Allied Medical Sciences, Department of Medical Biotechnology, Tehran, Iran*
[3]*Iran University of Medical Sciences, Institute of Immunology and Infectious Diseases, Research Center of Pediatric Infectious Diseases, Tehran, Iran*
[4]*Tarbiat Modares University Faculty of Medical Sciences, Department of Virology, Tehran, Iran*
[5]*Tarbiat Modares University Faculty of Medical Sciences, Department of Medical Genetics, Tehran, Iran*

ABSTRACT

Objectives: Human immunodeficiency virus (HIV) infection remains a global health concern. As individuals infected with HIV struggle with the complexities of their condition, the coexistence of additional pathogens can significantly alter the course of the disease. This study aimed to determine the prevalence of hepatitis C virus (HCV) and human pegivirus type 1 (HPgV-1) co-infection in patients with HIV-1 infection using an in-house developed multiplex real-time reverse transcriptase-polymerase chain reaction (RT-qPCR) assay.

Materials and Methods: This cross-sectional study included 113 HIV-1-positive patients. The HIV-1 load was evaluated using the Artus HI Virus-1 RG RT-PCR Kit in serum samples. Subsequent to the assessment of optimal annealing temperature, primer-probe concentration, analytical sensitivity, and endpoint sensitivity, selected primer-probe sets for HCV, HPgV-1, and ribonuclease P were recruited to identify co-infections.

Results: Of the 113 HIV-1-positive individuals, 24% were female and 76% were male. Interestingly, 74% of the patients had no history of addiction. Optimization of the in-house developed RT-

ÖZ

Amaç: İnsan immün yetmezlik virüsü (HIV) enfeksiyonu küresel bir sağlık sorunu olmaya devam etmektedir. HIV ile enfekte kişiler, durumlarının karmaşıklığıyla mücadele ederken, ek patojenlerin bir arada bulunması, hastalığın seyrini önemli ölçüde değiştirebilir. Bu çalışma, in-house geliştirilen multipleks kantitatif gerçek zamanlı polimeraz zincir reaksiyonu testi (RT-qPCR) kullanılarak HIV-1 ile enfekte hastalarda hepatit C virüsü (HCV) ve insan pegivirüs tip 1 (HPgV-1) ko-enfeksiyonlarının prevalansını incelemeyi amaçladı.

Gereç ve Yöntemler: Bu kesitsel çalışmaya 113 HIV-1-pozitif hasta dahil edilmiştir. HIV-1 yükü serum örneklerinde Artus HI Virus-1 RG RT-PCR Kiti kullanılarak değerlendirildi. Optimum tavlama sıcaklığı, primer-prob konsantrasyonu, analitik hassasiyet ve uç nokta hassasiyetinin değerlendirilmesinin ardından, ortak enfeksiyonları tanımlamak için HCV, HPgV-1 ve ribonükleaz P için seçilen primer-prob setleri kullanıldı.

Bulgular: HIV-1 pozitif olan 113 kişiden %24'ü kadın, %76'sı erkekti. İlginçtir ki hastaların %74'ünde bağımlılık öyküsü yoktu. In-house geliştirilen RT-qPCR testinin optimizasyonu, kabul edilebilir bir verimlilik ve 287 kopya/µL tespit sınır ile doğrusal bir dinamik

Cite this article as: Elahimanesh Z, Bokharaei-Salim F, Donyavi T, Monavari SH, Tavakoli A, Khoshmirsafa M, Chavoshpour S, Abbasi-Kolli M, Yousefi Ghalejoogh Z, Kiani SJ. The prevalence of hepatitis C virus and human pegivirus type 1 co-infections in patients infected with human immunodeficiency virus type-1. Viral Hepat J. 2024;30(3):53-58

Address for Correspondence: Seyed Jalal Kiani MD, Iran University of Medical Sciences, School of Medicine, Department of Virology, Tehran, Iran
E-mail: kiani.j@iums.ac.ir **ORCID ID:** orcid.org/0000-0002-5499-892
Received: 08.05.2024 **Accepted:** 11.10.2024 **Epub:** 18.11.2024 **Publication Date:** 30.12.2024

qPCR test revealed an acceptable efficiency and a linear dynamic range with a limit of detection of 287 copy/µL. HCV was detected in five patients (4.43%), whereas no HPgV-1 was detected.

Conclusion: More than 74% of the participants had no history of addiction, which may explain the differences in the reported prevalence of HCV/HIV co-infection worldwide and in Iran. Findings of the present study are consistent with the prevalence reported for the general population (4%). In the present study, HPgV-1 was not detected in the collected samples, which is consistent with reports from Iran (a range of 0-26%).

Keywords: HIV, HCV, HPgV-1, viral load, RT-qPCR

aralık ortaya çıkardı. Beş hastada (%4,43) HCV saptanırken, HPgV-1 saptanmadı.

Sonuç: Katılımcıların %74'ünden fazlasının bağımlılık geçmişi olmadığından, bu durum dünyadan ve İran'dan bildirilen HCV/HIV ko-enfeksiyonu prevalansındaki farklılıkları açıklayabilir. Bu çalışmanın bulguları genel nüfus için bildirilen yaygınlık (%4) ile tutarlıdır. Bu çalışmada, İran'dan gelen raporlarla tutarlı olarak (%0-26 aralığı) toplanan örneklerde HPgV-1 tespit edilmemiştir.

Anahtar Kelimeler: HIV, HCV, HPgV-1, viral yük, RT-qPCR

Introduction

Human immunodeficiency virus (HIV) infection remains a global health concern, challenging scientists from all aspects of virology, immunology, molecular biology, medicine, pharmacology, and socioeconomic fields are challenging. First identified in the early 1980s, despite remarkable efforts in research and medical interventions, the HIV pandemic has continued, and no promising short-term or long-term solutions are expected in the near future (1). As a lentivirus belonging to the Retroviridae family, HIV primarily targets macrophages and CD4+ T cells of the immune system, progressively compromising the immune system's ability to develop an effective response against infections and malignancies. From the initial acute infection to the chronic stages and, potentially, the development of acquired immunodeficiency syndrome, the disease spectrum poses diverse challenges to affected individuals and healthcare providers. The most outstanding progress in disease management has been the advent of antiretroviral therapy, which has transformed HIV infection from a notorious incurable infectious disease into a manageable chronic condition with an approximate normal life expectancy (2).

The dynamic interplay between HIV and co-infections has been a critical aspect in understanding the multifaceted nature of the disease and its impact on affected individuals. Co-infections introduce a mixture of challenges that involve the already compromised immune system of HIV-infected patients. As individuals infected with HIV struggle with the complexities of their condition, the coexistence of additional pathogens, such as bacteria, viruses, and parasites, can significantly alter the course of disease progression, treatment outcomes, and overall health. Understanding the interplay between HIV and these co-pathogens is critical not only for comprehensive patient care but also for developing effective prevention and treatment strategies (3).

The coexistence of HIV and hepatitis C virus (HCV) in a patient represents a more complicated medical intersection. As a member of the hepacivirus genus from the Flaviviridae family, HCV is a small spherical enveloped virus that contains positive-sense single-stranded genomic RNA. The virus has a specific tropism for hepatocytes, and its replication and pathogenesis may lead to liver dysfunction, fibrosis, cirrhosis, and even hepatocellular carcinoma (HCC). Although the advent of direct-acting antiviral agents has revolutionized HCV treatment and has offered new hope for improved outcomes in this complex population, understanding the specific challenges posed by HCV in the context of HIV-infected individuals requires a comprehensive exploration of the clinical manifestations, treatment paradigms, and intertwined epidemiology (4). HIV and HCV, both blood-borne pathogens, share common routes of transmission, creating a substantial overlap in the populations affected by these viruses. The synergy between these two viruses not only puts more pressure on the immune system but also increases the risk of severe liver disease progression to cirrhosis and/or HCC (5,6).

High coinfection rates have also been reported for human pegivirus type 1 (HPgV-1) in patients with HIV and HCV infection. This member of the pegivirus genus within the Flaviviridae family has similar virion and genomic characteristics as HCV. The virus can induce persistent infection that is not associated with hepatitis or other obvious clinical symptoms or diseases in healthy individuals (7). Several studies have indicated that persistent infection with HPgV-1 is associated with slower disease progression in not only HIV-infected patients but also other viral infections (8,9). The beneficial effect of persistent HPgV-1 infection may be associated with the inhibition or reduction of abnormal/excessive immune activation, especially in T lymphocytes (8). Understanding the interplay between HPgV-1 and HIV via the same transmission route also requires comprehensive investigations of the disease course, treatment outcomes, and molecular epidemiology.

Therefore, the aim of the present study was to determine the prevalence of HCV and HPgV-1 co-infection in patients with HIV-1 infection using an in-house developed multiplex real-time reverse transcriptase-polymerase chain reaction (RT-qPCR) assay. HCV co-infection was found in less than 5% of the samples, whereas no HPgV1 positivity was identified in this group.

Materials and Methods

Study Population

This cross-sectional study included 113 HIV-1-positive patients who were referred to the Reference Laboratory of the Iran University of Medical Sciences, Tehran. The study was approved by the Research Ethics Committee of the Faculty of Medicine at the Iran University of Medical Sciences (approval number: IR.IUMS.FMD.REC.1401.517, date: 21.01.2023). A consent form was signed by all participants or their legal representative.

Sample Collection

Five microliters of fresh blood were collected from each HIV-1-positive individual in anti-coagulant tri-potassium ethylenediaminetetraacetic acid-containing tubes. Samples were centrifuged at 7000 g for 10 min, and the serum was stored at 20 °C until use.

Nucleic Acid Extraction

HIV-RNA was extracted from serum samples using a Zybio Semi-Automatic Nucleic Acid Extraction Kit (Zybio, Shenzhen, China) according to the manufacturer's instructions. Two hundred microliters of the serum samples were extracted, and RNA was eluted into a volume of ~50 µL. The extracted RNA was stored at 80 °C until further analysis.

HIV Load Assessment

The HIV-1 RNA load was detected using the Artus HI virus-1 RG RT-PCR Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Briefly, RT-PCR was performed on 10 µL extracted nucleic acid in a 25 µL reaction volume. The following thermal profile was programed for Rotor gene Q (Qiagen; Germany) software: first hold for reverse transcription at 50 °C for 30 min, second hold for DNA polymerase activation at 95 °C for 15 min and 50 amplification cycles of 30 s at 95 °C, 60 s at 50 °C and 30 s at 72 °C.

Assessment of the Multiplex Assay

Primer-Probe Sets

Primer-probe sequences for the detection of HCV were adapted from Chen et al. (10), for the detection of HPgV-1 was adapted from Schlueter et al. (11) and for the internal control ribonuclease P (RNase P) was adapted from our previous study (12). Selected primer-probe sets (Table 1) were further validated by a Basic Local alignment search tool analysis for analytical specificity (in Silico testing) and the OligoAnalyser Tool (Integrated DNA Technologies; USA) for secondary structures and primer dimer formation.

Annealing Temperature Optimization

To determine the most appropriate annealing temperature, a gradient test was performed for each primer set over a temperature range of 55 °C-62 °C. The reaction mixture contained 10 µL of SYBR green master mix (Ampliqon; Denmark), 1 µL of each forward and reverse primer (10 nM), and 6 µL of distilled water. Two microliters of the control plasmid were added to each reaction, resulting in a final volume of 20 µL. The following thermal profile was applied on a QIAquant real-time PCR thermal cycler: 10 min at 95 °C and 40 amplification cycles of both 15 s at 95 °C and 45 s at 55-62 °C. Data acquisition was programed for the green channel [fluorescein amidite (FAM)] at the end of each annealing/extension step. The results were analyzed for lower Cq, higher signal-to-noise ratio, normal amplification plot, and the absence of unintended PCR amplicons (through a melt curve analysis at the end of the amplification cycles).

Optimization of Primer-Probe Concentration

For each primer-probe set, a concentration matrix test with 500, 250, and 125 nM concentrations of each primer and 500, 400, 300, 200, and 100 nM concentrations of each probe were investigated for lower Cq, higher signal-to-noise ratio, and normal amplification plots. The test was performed by 5 µL of 4X capital 1-step RT quantitative reverse transcription PCR (qRT-PCR) probe master mix (biotechrabbit, Germany), 1 µL RTase with RNase inhibitor (RT-RI), 1 µL of each primer dilution, 1 µL of probe dilution, and 9 µL of distilled water. Two microliters of the control plasmid were added to each reaction, resulting in a final volume of 20 µL. Duplicate reactions were performed in a QIAquant real-time PCR thermal cycler with the following cycling conditions: 10 min at 95 °C and 40 cycles of both 15 s at 95 °C and 45 s at 55 °C. At the end of the annealing/extension step, data acquisition was programed on channel green (FAM), orange [carboxy-X-rhodamine (ROX)], and red [cyanine 5 (Cy-5)].

Analytical Sensitivity (Efficiency, Linearity) and Endpoint Sensitivity Limit of Detection (LOD)

Eight dilutions of the synthesized control plasmid were tested in quadruplicate over two runs to determine the efficiency, linearity, and LOD (13). The slope of the log-linear portion of the calibration curve was used to assess the amplification efficiency. The highest to lowest quantifiable copy numbers were analyzed for the linear dynamic range. The test LOD was defined as success in at least three out of four amplification reactions with the lowest dilution of the control plasmid.

Multiplex RT-qPCR

Multiplex RT-qPCR reaction mixture was prepared by including 6.25 µL of 4x capital 1-step qRT-PCR probe master mix

Table 1. Sequences of the primer-probe sets used for target detection

HCV	5′-UTR	bp 62	F	GCCTTGTGGTACTGCCTGAT
			R	TGCACGGTCTACGAGAC
			P	FAM-CCGGGGCACTCGCAAGCACCC-BHQ1
HPgV-1	5′-NCR	bp 186	F	CGGCCAAAAGGTGGTGGATG
			R	ACGACGAGCCTGACGTCGG
			P	ROX-TGGTAGCCACTATAGGTGGGTC-BHQ2
Internal control	RNase P	bp 65	F	AGATTTGGACCTGCGAGCG
			R	GAGCGGCTGTCTCCACAAGT
			P	Cy5-TTCTGACCTGAAGGCTCTGCGCG-BHQ2

HCV: Hepatitis C virus, HPgV-1: Human pegivirus type 1, RNase P: Ribonuclease P, 5′UTR : 5′ untranslated region, 5′-NCR: 5' Non-coding region

(biotechrabbit, Germany), 1.25 µL RT-RI, 6 µL of 1:4:1 primer-probe mixture (HCV: HPgV-1: RNase-P), and 1.5 µL of distilled water. Ten microliters of the extracted RNA were added to each reaction, resulting in a final volume of 25 µL. A temperature profile of 50 °C for 30 min, 95 °C for 10 min, and 40 amplification cycles of 15 s at 95 °C and 45 s at 55 °C was programed for the Rotorgene Q real-time PCR machine. Data acquisition was performed at the end of the annealing/extension step on channel green (FAM) for HCV, orange (ROX) for HPgV-1, and red (Cy-5) for RNAse-P.

Statistical Analysis

SPSS version 22.0 (IBM SPSS Statistics, Chicago, IL, USA) was used for all statistical analyses. To compare proportions, Fisher's exact test was applied. For variables with normal distribution and variables without normal distribution, the independent t-test and Mann-Whitney U test was selected, respectively, to compare means/medians between groups. A p-value of less than 0.05 was considered statistically significant.

Results

Patients Characteristics

Of the 113 HIV-1-positive individuals, 24% were female and 76% were male. The mean (± standard deviation) age of the participants was 41.38 (±10.14) in women and 44.51 (±12.25) in men. The majority of patients (~40%) were in the age group of 31-40 years, followed by the age group of 41-50 years (~27%). Approximately 74% of patients had no history of addiction. Most patients (82.3%) had a viral load of 500,000 international unit/mL. The characteristics of the participants are summarized in Table 2.

Multiplex Test Performance Evaluation

The optimum annealing temperature of the primers was selected at 55 °C by analyzing the performance of the primers using a gradient PCR assay. The combination of forward primer, reverse primer, and probe ratio was chosen as 500:500:500 nM for all targets, and the ratio of primer-probe mixture in the multiplex test was chosen as 1:4:1 for HCV, HPgV-1, and RNase P, respectively.

Table 2. Characteristics of patients with HIV-1 infection in this study

Variables		Female	Male	Total	p-value
Number of patients		27 (24.1%)	86 (75.9%)	113	
Age ± SD		41.38 (±10.14)	44.51 (±12.25)	43.73 (±11.74)	N.S
Age groups (years)					
	0-20	1 (50%)	1 (50%)	2 (1.8%)	
	21-30	2 (50%)	2 (50%)	4 (3.5%)	
	31-40	15 (32.6%)	31 (77.4%)	46 (40.7%)	N.S
	41-50	6 (20%)	24 (80%)	30 (26.6%)	
	51-60	2 (10%)	18 (90%)	20 (17.7%)	
	>60	2 (18%)	9 (82%)	11 (9.7%)	
Education level					
	Illiterate	2 (33.3%)	4 (66.7%)	6 (5.3%)	
	Elementary school	2 (15.4%)	11 (84.6%)	13 (11.5%)	
	Middle school	9 (31%)	20 (69%)	29 (25.7%)	N.S
	High school	10 (32.3%)	21 (67.7%)	31 (27.4%)	
	University	7 (20.6%)	27 (79.4%)	34 (30.1%)	
Marital status					
	Permanent	15 (25.4%)	34 (74.6%)	59 (52.2%)	
	Single	2 (5.3%)	36 (94.7%)	38 (33.6%)	N.S
	Divorced	6 (50%)	6 (50%)	12 (10.6%)	
	Temporary	4 (100%)	0 (0%)	4 (3.6%)	
Addiction					
	No	24 (28.6%)	60 (71.4%)	84 (74.3%)	
	Yes	5 (20%)	20 (80%)	25 (22.1%)	N.S
	Unknown	0 (0%)	4 (100%)	4 (3.6%)	
Viral load (before treatment) IU/mL; mean (range)		154933 (0-1315565)	1329137 (0-26464483)	1035586 (0-26464483)	N.S
Viral load (after treatment) IU/mL; mean (range)		322 (0-3751)	381 (0-3862)	366 (0-3862)	N.S
CD4 count (cells/mm^3)		470.31 (316.98)	367.26 (±289.31)	396.17 (±297.22)	N.S

HIV-1: Human immunodeficiency virus type-1, SD: Standard deviation, IU: International unit, N.S: Not significant

The results revealed an acceptable efficiency and a linear dynamic range for all targets (HCV: R^2=0.98 and E=0.99, HPgV-1: R^2=0.97 and E=1.04, RNase-P: R^2=0.98 and E=1). Moreover, a scrutiny of the test LOD showed that this multiplex RT-qPCR assay is capable to detect 287 copy/µL of the target in each reaction.

Prevalence of HCV and HPgV-1 Co-Infection

Of the 113 HIV-1-positive samples, HCV was detected in five patients (4.43%). In the present study, HPgV-1 was not detected in HIV-1 infected individuals.

Discussion

HIV infection continues to pose many challenges to physicians, pharmacists, psychologists, politicians, and researchers. The virus has perfectly adapted to its host to ensure its persistence in the human population. In addition to all complexities of the virus-host interactions per se, co-infections add another level of difficult-to-manage conditions for patients and their physicians (14). In the present study, the prevalence of HCV and HPgV-1 co-infection was investigated in HIV-1-infected individuals using an in-house developed RT-qPCR. The results revealed co-infection with HCV in less than 5% of the patients, whereas HPgV-1 was not detected in the sample population.

In a study performed by Zahra et al. (15), they reported 60-80% frequency of HCV infection in HIV-1 infected patients in four different cities of Pakistan. Their sample population included only male patients with a history of intravenous drug use (IDU). Schmidbauer et al. (16) also reported a prevalence of 11.1% for HCV co-infection among patients with HIV-1 infection in Austria. More than 63% of the study population were either IDUs or men who have sex with men (16). Teimoori et al. (17) also reported 58.7% positivity for HCV/HIV coinfection in Ahvaz, Iran. They reported that the most common route of transmission (99.1%) among their patients was IDU, and 97.8% of the subjects had a history of imprisonment. In the present study, samples were collected from patients referred to counseling centers for behavioral diseases. Since more than 74% of the patients in this study had no history of addiction (including IDU) or same-sex experience, this may explain the differences in the reported prevalence of 4.43% for HCV/HIV co-infection. Accordingly, the prevalence of HCV/HIV co-infection was consistent with that reported for general population samples by Platt et al. (18) (4%). In a systematic review and meta-analysis, Bagheri Amiri et al. (19) reported a zero prevalence of HCV/HIV co-infection in both the general population and healthcare workers, whereas 10.95% was reported for IDUs in Iran.

In an attempt to assess HPgV-1 prevalence in HIV-1-infected patients, de Miranda et al. (20) reported 17% positivity by performing conventional nested PCR. They also showed that HPgV-1 is associated with lower HIV-1 loads and higher CD4 counts. By recruiting a similar methodology, Alcalde et al. (21) reported a prevalence of 30% for HPgV-1/HIV co-infection and similar effects on viral load and CD4 count. Likewise, Li et al. (22) reported a prevalence of 9% for infection by these two viruses. Moreover, a prevalence of 2.3% was reported in healthy blood donors. In studies conducted in Iran, a range of 0-26% were reported for co-infection of HPgV-1 and HIV (23,24,25). In the present study, HPgV-1 was not detected in the samples. Considering the prevalence of HCV in the present study, which was approximately similar to that of the general population, it is plausible to expect an HPgV-1 prevalence close to that of healthy blood donors or the general population. On the other hand, the analytical sensitivity of 287 copy/µL for the in-house developed RT-qPCR assay may explain the inability of the test to detect HPgV-1. Another matter of concern is the type of samples used. Because growing evidence supports the presence/replication of hepatitis viruses as well as HPgV-1 in peripheral blood mononuclear cells (PBMCs) (8,9,26,27,28,29,30), the prevalence reported in the current study could be different if PBMCs had been subjected to nucleic acid extraction and RT-qPCR assay. However, further studies are required to validate this hypothesis.

Study Limitations

The present study had some limitations. Due to the scarcity of available financial support, the results were not re-checked using commercial kits. Therefore, no data is available for confirmed HPgV1-positive samples tested using this assay, and the power of this test for virus detection in clinical specimens is a matter of concern. Despite several reports on the high prevalence of HPgV-1 in HIV-infected patients, no positive samples were detected in the present study. An extensive literature review revealed that the prevalence of HPgV-1 is profoundly dependent on the characteristics of the studied population (21,22,23,24,25). The prevalence was highest among IDUs and lowest among the normal population. Evaluation of the demographic and epidemiological characteristics of the individuals who participated in this project revealed that the sample population resembled the general population, despite being infected with HIV-1. Therefore, the finding was explained based on this characteristic. Moreover, the LOD of the in-house developed test is another matter of concern that could be a reason for not finding HPgV-1-positive cases.

This article aims to highlight the current state of knowledge regarding the effects of coinfection on HIV-infected patients and to elucidate the intricate relationships between multiple infectious agents and the immune system. The intricate interplay of these viruses raises pivotal questions about optimal management strategies, potential synergies or antagonisms in their pathogenesis, and broader public health implications.

Conclusion

Evaluation of co-infections in the context of HIV infection is necessary for better patient management. Although most infectious agents exacerbate the condition, agents such as HPgV-1, may be beneficial for the host to combat other pathogens. However, more studies are required to support this hypothesis.

Ethics

Ethics Committee Approval: The study was approved by the Research Ethics Committee of the School of Medicine at the Iranian University of Medical Sciences (approval number: IR.IUMS.FMD.REC.1401.517 date: 21.01.2023).

Informed Consent: A consent form was signed by all participants or their legal representative.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Z.E., S.C., M.A-K., S.J.K., Concept: Z.E., F.B-S., M.K., S.J.K., Design: Z.E., F.B-S., T.D., M.K., S.J.M., Data Collection or Processing: Z.E., F.B-S., S.H.M., A.T., M.K., S.J.K., Analysis or Interpretation: Z.E., F.B-S., T.D., S.H.M., A.T., M.K., S.J.K., Literature Search: Z.E., Z.Y.G., S.J.K., Writing: Z.E., Z.Y.G., S.J.K.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: This study was financially supported by Iran University of Medical Sciences (Grant no: 25427).

References

1. Deeks SG, Overbaugh J, Phillips A, Buchbinder S. HIV infection. Nature Rev Dis Primers. 2015;1:15035.
2. Saag MS. HIV infection-screening, diagnosis, and treatment. N Engl J Med. 2021;384:2131-2143.
3. Boulougoura A, Sereti I. HIV infection and immune activation: the role of co-infections. Current Opin HIV AIDS. 2016;11:191-200.
4. Lara J, Teka MA, Sims S, Xia G-L, Ramachandran S, Khudyakov Y. HCV adaptation to HIV coinfection. Infect Genet Evol. 2018;65:216-225.
5. Sohrab SS, Suhail M, Ali A, Qadri I, Harakeh S, Azhar EI. Consequence of HIV and HCV co-infection on host immune response, persistence and current treatment options. Virusdisease. 2018;29:19-26.
6. Rallón N, García M, García-Samaniego J, Rodríguez N, Cabello A, Restrepo C, Álvarez B, García R, Górgolas M, Benito JM. HCV coinfection contributes to HIV pathogenesis by increasing immune exhaustion in CD8 T-cells. PLoS One. 2017;12:0173943.
7. Samadi M, Salimi V, Haghshenas MR, Miri SM, Mohebbi SR, Ghaemi A. Clinical and molecular aspects of human pegiviruses in the interaction host and infectious agent. Virol J. 2022;19:41.
8. Yu Y, Wan Z, Wang J-H, Yang X, Zhang C. Review of human pegivirus: prevalence, transmission, pathogenesis, and clinical implication. Virulence. 2022;13:323-341.
9. Stapleton JT. Human pegivirus type 1: a common human virus that is beneficial in immune-mediated disease? Front Immunol. 2022;13:887760.
10. Chen L, Li W, Zhang K, Zhang R, Lu T, Hao M, Jia T, Sun Y, Lin G, Wang L, Li J. Hepatitis C virus RNA real-time quantitative RT-PCR method based on a new primer design strategy. J Mol Diagn. 2016;18:84-91.
11. Schlueter V, Schmolke S, Stark K, Hess G, Ofenloch-Haehnle B, Engel AM. Reverse transcription-PCR detection of hepatitis G virus. J Clin Microbiol. 1996;34:2660-2664.
12. Ramshini M, Bokharaei-Salim F, Donyavi T, Khoshmirsafa M, Ghorbani S, Khatami A, Abbasi-Kolli M, Safi Deh Naeini A, Jafari E, Tavakoli A, Monavari SH, Ataei-Pirkooh A, Yousefi Ghalejoogh Zohreha, Kiani SJ. Single nucleotide polymorphism in toll-like receptor 3 gene as a potential risk factor for severe outcome of coronavirus disease 2019. Reviews and Research in Medical Microbiology. 2024;35:119-126.
13. Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, Mueller R, Nolan T, Pfaffl MW, Shipley GL, Vandesompele J, Wittwer CT. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin Chem. 2009;55:611-622.
14. Bekker LG, Beyrer C, Mgodi N, Lewin SR, Delany-Moretlwe S, Taiwo B, Masters MC, Lazarus JV. HIV infection. Nat Rev Dis Primers. 2023;9:42.
15. Zahra A, Saleem MA, Javed H, Khan MAU. Prevalence of HCV-HIV Co-Infection with Intravenous Drug Users in Central Punjab, Pakistan. Pak J Zool. 2021;54:2003-2500.
16. Schmidbauer C, Chromy D, Schmidbauer V, Bauer D, Apata M, Nguyen D, Mandorfer M, Simbrunner B, Rieger A, Mayer F, Schmidt R, Holzmann H, Trauner M, Gschwantler M, Reiberger T. Epidemiological trends in HCV transmission and prevalence in the Viennese HIV+ population. Liv Int. 2020;40:787-796.
17. Teimoori A, Ebrahimi S, Keshtkar N, Khaghani S, Salmanzadeh S, Ghafari S. Prevalence and genetic diversity of HCV among HIV-1 infected individuals living in Ahvaz, Iran. BMC Infect Dis. 2019;19:389.
18. Platt L, Easterbrook P, Gower E, McDonald B, Sabin K, McGowan C, Yanny I, Razavi H, Vickerman P. Prevalence and burden of HCV co-infection in people living with HIV: a global systematic review and meta-analysis. Lancet Infect Dis. 2016;16:797-808.
19. Bagheri Amiri F, Mostafavi E, Mirzazadeh A. HIV, HBV and HCV coinfection prevalence in Iran-a systematic review and meta-analysis. PloS One. 2016;11:0151946.
20. de Miranda BKB, de Sá KSG, da Silva ANR, Feitosa RNM, Cayres-Vallinoto IMV, Ishak R, Vallinoto ACR. GBV-C/HIV-1 coinfection is associated with low HIV-1 viral load and high CD4+ T lymphocyte count. Arch Virol. 2017;162:3431-3438.
21. Alcalde R, Nishiya A, Casseb J, Inocêncio L, Fonseca LA, Duarte AJ. Prevalence and distribution of the GBV-C/HGV among HIV-1-infected patients under anti-retroviral therapy. Virus Res. 2010;151:148-152.
22. Li Z, Li Y, Liang Y, Hu L, Chen S. Prevalence and risk factors of human pegivirus type 1 infection in hematopoietic stem cell transplantation patients. Int J Infect Dis. 2019;85:111-113.
23. Ziaee M, Zarban A, Malekinejad P, Akhbary H. Evaluation of HGV viremia prevalence and its co-infection with HBV, HCV, HIV and HTLV-1 in hemophilic patients of Southern Khorassan, Iran. Hepatitis Monthly. 2007;7:11-14.
24. Yaghobi R, Afkari R, Mohsenzadeh M, Jafari M, Khorrami H, Pirouzi A. A study on the serologic and molecular prevalence of hepatitis G virus (HGV) and hepatitis C virus (HCV) infections in patients with thalassemia in Larestan of Iran. Afr J Microbiol Res. 2012;6:5866-5870.
25. Ramezani A, Mohraz M, Vahabpour R, Jam S, Banifazl M, Eslamifar A, Mahboudi F, Aghakhani A, Edalat R, Hekmat S. Frequency of hepatitis G virus infection among HIV positive subjects with parenteral and sexual exposure. J Gastrointestin Liver Dis. 2008;17:269-272.
26. Khattab MA, Zakaria Y, Sadek E, Abd El Fatah AS, Fouad M, Khattab M, Moness HM, Adel NM, Ahmed E. Detection of hepatitis C virus (HCV) RNA in the peripheral blood mononuclear cells of HCV-infected patients following sustained virologic response. Clin Exp Med. 2023;23:131-140.
27. Austria A, Wu GY. Occult hepatitis C virus infection: a review. J Clin Transl Hepatol. 2018;6:155-160.
28. Sayed I, Seddik M, Gaber M, Saber S, Mandour S, El-Mokhtar M. Replication of hepatitis E virus (HEV) in primary human-derived monocytes and macrophages in vitro. Vaccines (Basel). 2020;8:239.
29. Joshi SS, Coffin CS. Hepatitis B virus lymphotropism: emerging details and challenges. Biotechnol Genet Eng Rev. 2018;34:139-151.
30. Yan Q, Lan YH, Huang YX, Fan RS, Liu L, Song SP, Li YG. Hepatitis B virus replication is upregulated in proliferated peripheral blood lymphocytes. Mol Med Rep. 2016;13:3581-3587.

Research Article

59

DOI: 10.4274/vhd.galenos.2024.2024-7-4
Viral Hepat J 2024;30(3):59-64

Potential Drug-Drug Interactions Between Oral Antiviral Agents Used for Hepatitis B Treatment and Concomitant Systemic Medications

Hepatit B Tedavisinde Kullanılan Oral Antiviral Ajanlar ve Eş Zamanlı Kullanılan İlaçlar Arasındaki Potansiyel İlaç-İlaç Etkileşimleri

Nurten Nur Aydın, Murat Aydın

University of Health Sciences Turkey, Erzurum Regional Training and Research Hospital, Clinic of Infectious Diseases and Clinical Microbiology, Erzurum, Turkey

ABSTRACT

Objectives: Antiviral therapy planning for hepatitis B (HB) requires consideration of drug interactions. The aim of this study was to evaluate the potential drug-drug interactions (pDDIs) between oral antiviral drugs and concomitant medications for hepatitis.

Materials and Methods: HB patients who received oral antiviral therapy in our clinic were included. Identified pDDIs were categorized as level 1 (weak potential interaction), level 2 (potential interaction), or level 3 (contraindicated) according to the University of Liverpool Hepatitis Drug Interaction Database.

Results: Of the 205 patients included in the study, 112 (54.6%) received tenofovir disoproxil fumarate (TDF), 65 (31.7%) received entecavir (ETV), and 28 (13.7%) received tenofovir alafenamide fumarate (TAF). Patients receiving TDF, ETV, and TAF received 135, 119, and 52 concomitant systemic medications, respectively. Twenty-level 2 and two level 1 interactions were observed, but no level 3 interactions. Potential DDIs were observed in 12.6% of patients receiving TDF, 3.4% receiving ETV, and 1.9% receiving TAF. The most common pDDIs were observed with non-steroidal anti-inflammatory drugs (noted in 12 occurrens and all with TDF).

ÖZ

Amaç: Hepatit B'ye (HB) yönelik antiviral tedavi planlandığında, ilaç etkileşimlerinin dikkate alınması gerekmektedir. Bu çalışmanın amacı, HB tedavisinde kullanılan oral antiviral ilaçların, eş zamanlı kullanılan diğer ilaçlarla potansiyel ilaç-ilaç etkileşimlerini (pİİE) değerlendirmektir.

Gereç ve Yöntemler: Kliniğimizde HB tedavisi için oral antiviral ilaç kullanan hastalar çalışmaya dahil edildi. Belirlenen pİİE'ler, Liverpool Üniversitesi Hepatit İlaç Etkileşimi Veri Tabanı'na göre seviye 1 (zayıf potansiyel etkileşim), seviye 2 (potansiyel etkileşim) veya seviye 3 (kontrendike) olarak kategorize edildi.

Bulgular: Çalışmaya dahil edilen 205 hastanın 112'si (%54,6) tenofovir disoproksil fumarat (TDF), 65'i (%31,7) ETV ve 28'i (%13,7) tenofovir alafenamid fumarat (TAF) almaktaydı. TDF, ETV ve TAF alan hastalar sırasıyla 135, 119 ve 52 eşzamanlı sistemik ilaç almaktaydı. Yirmi adet seviye 2 etkileşim ve iki adet seviye 1 etkileşim gözlenmiş, ancak seviye 3 etkileşim gözlenmemiştir. TDF alan hastaların %12,6'sında, ETV alan hastaların %3,4'ünde ve TAF alan hastaların %1,9'unda pİİE gözlenmiştir. En yaygın pİİE'leri non-steroidal anti-enflamatuvar ilaçlarla gözlenmiştir (12 kez ve hepsi TDF ile).

Cite this article as: Aydın NN, Aydın M. Potential drug-drug interactions between oral antiviral agents used for hepatitis B treatment and concomitant systemic medications. Viral Hepat J. 2024;30(3):59-64

Address for Correspondence: Nurten Nur Aydın, MD, University of Health Sciences Turkey, Erzurum Regional Training and Research Hospital, Clinic of Infectious Diseases and Clinical Microbiology, Erzurum, Turkey

E-mail: nurtennurkenc@hotmail.com **ORCID ID:** orcid.org/0000-0003-4138-2490

Received: 17.07.2024 **Accepted:** 05.12.2024 **Epub:** 17.12.2024 **Publication Date:** 30.12.2024

Conclusion: The combination of antivirals used for chronic HB treatment with systemic drugs can lead to pDDIs, especially with TDF. All patients with HB should be screened for pDDI.
Keywords: Drug interactions, hepatitis B, tenofovir, entecavir, oral antivirals

Sonuç: HB ilaçlarının sistemik ilaçlarla kombinasyonu, özellikle TDF ile olmak üzere, pİE'lerine yol açabilir. Tüm HB hastaları pİE açısından taranmalıdır.
Anahtar Kelimeler: İlaç etkileşimleri, hepatit B, tenofovir, entekavir, oral antiviraller

Introduction

Hepatitis B (HB) continues to be a major public health problem worldwide (1). This condition can result in severe outcomes, including liver damage, cirrhosis, and liver cancer (1). Effective treatment and control of HB virus (HBV) infection are essential for preventing the spread of the disease and reducing complications (2). Oral antiviral drugs that inhibit HBV replication play a crucial role in the treatment of patients with this virus. Tenofovir disoproxil fumarate (TDF), entecavir (ETV), and tenofovir alafenamide fumarate (TAF) are used for this purpose. These drugs help patients obtain the treatment they need to stop disease progression and limit liver damage (3).

Patients with HBV often have other health problems and may need to take more than one medication. This leads to the risk of potential drug-drug interactions (pDDIs) resulting from the combination of different drugs. Drug interactions can occur through pharmacodynamic and pharmacokinetic mechanisms, each with different clinical implications. Pharmacokinetic interactions can alter the absorption, distribution, metabolism, or excretion of drugs, resulting in changes in plasma drug levels and therapeutic efficacy (4). On the other hand, pharmacodynamic interactions can influence the action of drugs at their target sites, potentially worsening side effects or worsening therapeutic outcomes. For example, concomitant use of certain medications with TDF is associated with increased renal toxicity, which is a significant problem in patients requiring multiple medications (5). Understanding these interactions is crucial for optimizing treatment strategies and minimizing side effects in patients with HB infection.

Studies examining the interaction of TDF, ETV, and TAF with other systemic drugs in patients with HB are limited. This study aimed to investigate the pDDIs between oral antiviral drugs used for the treatment of HB and other concomitant systemic drugs.

Materials and Methods

The study was conducted as a retrospective, observational study. Between 01.07.2022-01.10.2022, patients over the age of 18 who applied to the infectious diseases outpatient clinic of our hospital and were receiving antivirals (TDF, ETV, TAF) were included in the study.

The potential interactions between antivirals and other systemic drugs used concomitantly were investigated. The University of Liverpool Hepatitis Drug Interaction Database (available on www.hep-druginteractions.org) was used to identify pDDIs, which were categorized as level 1 (potential weak interaction), level 2 (potential interaction), or level 3 (contraindicated) (6).

Other concurrent medications and comorbid conditions were recorded. These data were obtained from follow-up forms of patients attending the infectious diseases outpatient clinic who were taking antivirals.

The study was approved by the Erzurum Regional Training and Research Hospital (decision no.: E-37732058-514.99, date: 06.06.2022) and was conducted in accordance with the principles of the Declaration of Helsinki.

Statistical Analysis

The IBM SPSS 23.0 (IBM SPSS Statistics for Windows Version 23.0, Armonk, NY: IBM Corp., USA) statistical package program was used for data analysis. For categorical variables, descriptive statistics include numbers (n) and percentages (%); for numerical variables, descriptive statistics include means and standard deviations (SD). The chi-square test was used to analyze categorical variables in the independent groups. The Shapiro-Wilk W test and the Kolmogorov-Smirnov test were used to assess the normal distribution of continuous variables. When comparing two independent groups, the Student t-test was used for variables that followed a normal distribution, and the Mann-Whitney U test was used for variables that did not follow a normal distribution. The significance level was set at $p<0.05$.

Results

In total, 205 patients were evaluated. Among the included patients, 115 (56.1%) were male and 90 (43.9%) were female. The mean age of the patients was 50.2±13.3 years. Comorbidities were present in 109 patients (53.2%). Among them, 56 had hypertension, 29 had diabetes mellitus, 28 had peptic ulcer or gastritis, 26 had cardiovascular disease, 11 had chronic obstructive pulmonary disease, and 41 had other conditions. In addition to antivirals for HB, 124 patients (60.5%) were taking concomitant drugs. The mean number of concomitant drugs used was 1.49±1.64 per patient (Table 1).

Eighty-one (39.5%) patients were not taking any medication other than their antivirals. Thirty-seven and 37 patients were using one and two additional drugs. Twenty-five patients were using 3 additional drugs, and 14 were using four additional drugs. Data on the number of additional medication use are presented in Figure 1.

No pDDIs with antivirals were detected in 185 (90.2%) patients. Twenty patients had pDDIs with antivirals. A comparison of patients with and without pDDIs is presented in Table 1. No significant differences were found between the two groups in terms of age, gender, and number of comorbid diseases (Table 1). The mean number of additional medications was 2.40±1.35 for the PDDIs group and 1.39±1.64 for the non-PDDI group, and the difference was statistically significant (p=0.001).

Among the patients, 112 (54.6%) were on TDF, 65 (31.7%) were on ETV, and 28 (13.7%) were on TAF. Fifty-eight (51.8%) of the patients receiving TDF, 47 (72.3%) of the patients receiving ETV, and 19 (67.9%) of the patients receiving TAF were concurrently using other systemic medications. There were 135 additional drug

use cases in patients on TDF, 119 in patients on ETV, and 52 in patients on TAF. In patients receiving TDF, 15 (11.1%) of 135 drugs had a level 2 interaction, and two (1.5%) had a level 1 interaction. A level 2 interaction was found with four of the 119 drugs (3.4%) in patients receiving ETV and one of the 52 drugs (3.4%) in patients receiving TAF. No pDDIs were found in 87.4% of patients receiving TDF, 96.6% of patients receiving ETV, and 98.1% of patients receiving TAF (Table 2).

Drug interactions were most commonly observed with nonsteroidal anti-inflammatory drugs (NSAIDs) in patients receiving TDF (noted in 12 occurrences). Among the NSAIDs, the most common drug interaction was with dexketoprofen (noted in 6 occurrences). The drugs that interacted with antivirals are presented in Table 3.

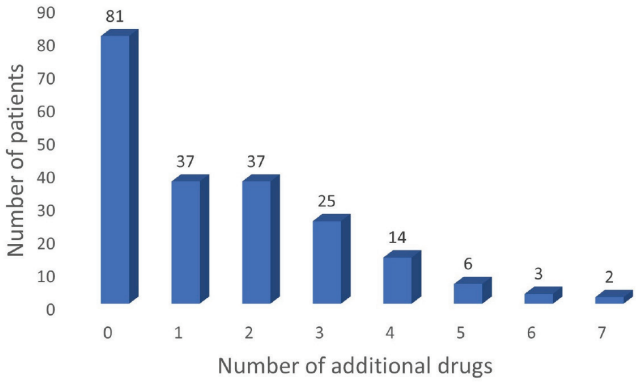

Figure 1. Patients taking drugs other than antivirals

Discussion

Our findings showed that a significant proportion of patients included in the study received additional systemic medications. This result demonstrates that patients with HB often have multiple health problems and therefore may need to take more than one medication. This polypharmacy carries the risk of pDDIs. Although this study had a limited amount of patient data on pDDIs, the significance of these interactions is worth noting. This study revealed a higher risk of pDDIs, particularly in patients receiving TDF. This result suggests that patients taking TDF should be monitored more carefully and should receive special attention for drug combinations.

Patients with HB may have various comorbidities, including liver cirrhosis, liver cancer, renal dysfunction, cardiovascular disease, and diabetes mellitus (7,8). Previous studies have shown that patients with HB can have several comorbidities, often resulting in the use of multiple drugs (9,10,11). In our study, more than half of the patients (53.2%) had comorbidities. Furthermore, most patients (60.5%) were taking additional medications other than antivirals for HB.

Table 1. Demographic data of patients and pDDIs

	Total	No pDDI	pDDIs	p-value
Male, n (%) Female, n (%)	115 (56.1%) 90 (43.9%)	103 (55.7%) 82 (44.3%)	12 (60.0%) 8 (40.0%)	0.894
Mean age ± SD	50.2±13.3	49.9±13.3	53.1±13.3	0.312
Comorbidity, n (%)				
Hypertension Diabetes mellitus Peptic ulcer/gastritis CVD COPD Others	56 (27.3%) 29 (14.1%) 28 (13.7%) 26 (12.7%) 11 (5.4%) 41 (19.7%)	49 (26.5%) 25 (13.5%) 27 (14.6%) 22 (11.9%) 10 (5.4%) 36 (19.5%)	7 (35.0%) 4 (20.0%) 1 (5.0%) 4 (20.0%) 1 (5.0%) 5 (25.0%)	0.584 0.496 0.322 0.293 1.000 0.560
Number of Comorbidities, mean ± SD	0.94±1.08	0.92±1.08	1.10±1.07	0.371
Number of additional drugs, mean ± SD*	1.49±1.64	1.39±1.64	2.40±1.35	0.001

*Number of drugs other than antivirals
COPD: Chronic obstructive pulmonary disease, CVD: Cardiovascular disease, pDDI: Potential drug-drug interaction, SD: Standard deviation

Table 2. pDDIs of antivirals with other drugs

HB drugs	Patients using additional drugs, n (%)	Number of additional drugs	No interaction, n (%)	Level 1 pDDIS, n (%)	Level 2 pDDIs, n (%)	Level 3 pDDIs, n	Total number of pDDIs, n (%)
TDF, n=112	58 (51.8%)	135	118 (87.4%)	2 (1.5%)	15 (11.1%)	0	17 (12.6%)
ETV, n=65	47 (72.3%)	119	115 (96.6%)	0	4 (3.4%)	0	4 (3.4%)
TAF, n=28	19 (67.9%)	52	51 (98.1%)	0	1 (1.9%)	0	1 (1.9%)

HB: Hepatitis B, ETV: Entecavir, pDDIs: Potential drug-drug interactions, TAF: Tenofovir alafenamide fumarate, TDF: Tenofovir disoproxil fumarate

Table 3. Drugs with pDDIs with antivirals

Drugs with pDDIs	Number of patients	Level of pDDIs	Possible outcome
TDF			
NSAIDS*	12	Level 2	Increased renal toxicity
Valsartan	2	Level 2	Increase in the concentration of both drugs
Furosemide	1	Level 1	Decreased renal absorption of TDF
Amiodarone	1	Level 2	Increased absorption of TDF
Tacrolimus	1	Level 1	Increased renal toxicity
ETV			
Furosemide	2	Level 2	Increase in ETV concentration
Methotrexate	1	Level 2	Change in the concentration of both drugs
Captopril	1	Level 2	Increase in ETV concentration
TAF			
Amiodarone	1	Level 2	Increase in TAF concentration

*Acetylsalicyclic acid was used by one patient, dexketoprofen by six patients, diclofenac by two patients, ibuprofen by one patient, indometacin by one patient
pDDIs: Potential drug-drug interactions, TDF: Tenofovir disoproxil fumarate, NSAIDS: Nonsteroidal anti-inflammatory drugs, ETV: Entecavir, TAF: Tenofovir alafenamide fumarate

ETV, TDF, and TAF are important antiviral agents used to treat chronic HB. These drugs effectively suppress viral replication (12). In our study, we analyzed the pDDIs of these medications and other systemic drugs used concomitantly by patients. Drug interactions may occur with antiviral agents used for HB treatment due to various mechanisms. For example, tenofovir is a substrate of the P-glycoprotein (P-gp) transporter and increases its interaction potential with other drugs that are excreted via renal P-gp pathways, whereas ETV interacts with renal transporters such as hOAT1 and hCNT2, which can inhibit the uptake of other drugs (13,14). Neither tenofovir nor ETV interact significantly with the cytochrome P450 system, which is advantageous because it minimizes the risk of metabolic interactions with other systemic drugs (15). Such mechanistic insights help us understand the potential pharmacokinetic and pharmacodynamic interactions that may occur with these agents. Potential DDIs with antivirals were identified in 9.8% of patients in our study. This was significantly associated with the number of other drugs used (p=0.001). This suggests that the use of additional medications for HB treatment should be carefully considered. A study of drug interactions in patients with viral hepatitis found that 44% of 69 patients with HB had DDIs (16). The higher incidence of pDDIs in comparison with our study can be explained by the fact that the study was conducted on patients who were hospitalized, and all drugs used by these patients were assessed for pDDIs. However, our study only included outpatients, and we only assessed antivirals and other systemic drugs for interactions.

In our study, pDDIs were observed to be more frequent, particularly in patients receiving TDF. The interaction between tenofovir and other systemic drugs has not been well investigated. In a case report, virological reactivation occurred in a patient with chronic HB during TDF treatment, and it was thought that this may be related to drug interactions. After discontinuation of antidepressant drugs (venlafaxine, paroxetine and zolpidem), a good response to TDF treatment was observed during follow-up (17). In a study evaluating the coadministration of TDF with etravirine and lamivudine, no significant drug-drug interaction was observed (18). In another study, the drug interaction between TDF and didanosine was investigated, and it was emphasized that the dose of didanosine should be reduced due to drug interaction in concomitant use (19).

The most common pDDI was caused by concomitant use of TDF and NSAIDs. In a retrospective analysis of HIV-positive patients receiving antiretroviral therapy with and without TDF, it was found that 14.6% of patients receiving TDF developed acute kidney injury after the initiation of NSAIDs (diclofenac), but no acute kidney injury occurred in patients receiving a drug regimen without TDF (5). A case report describes the development of biopsy-proven acute tubular necrosis occurring 5 days after the initiation of NSAIDs (diclofenac) in an HIV-positive patient receiving TDF treatment (20). In another case report, proximal tubular dysfunction was documented in an HIV-positive patient receiving TDF treatment, occurring 2 weeks after the initiation of ibuprofen therapy (21). Complete recovery of renal function occurred within a week of stopping ibuprofen and continuing TDF. In our study, pDDIs with TDF were commonly associated with impaired renal function. The concomitant use of TDF-NSAIDs should be avoided because of the risk of acute renal failure. If both drugs are used concomitantly, it is important to monitor patients closely for renal dysfunction.

The rate of PDDIs in patients receiving ETV was 3.4% in this study. A previous study investigating the potential of ETV to interact with renal solute carriers (SLC) *in vitro* showed that ETV interacts with these transporters, but these interactions occur with low affinity (14). This study showed that the potential of ETV to cause nephrotoxicity and DDIs were significantly lower than that of adefovir, tenofovir, and cidofovir. It was also stated in the package insert that ETV does not affect the CYP enzyme system and is not likely to interact with drugs affected by the CYP system (22). In a study examining the pharmacology/pharmacokinetics and therapeutic efficacy of ETV in patients with chronic HBV infection, pDDIs associated with the use of ETV were also reviewed, and it was stated that the potential for drug interaction with ETV was minimal (23). The study stated that drugs that inhibit tubular secretion of drugs (e.g., probenecid) may increase the serum concentration of ETV. In our study, serum concentrations of ETV-

associated pDDIs may increase or serum concentrations of both drugs may be altered.

The only pDDI observed in patients receiving TAF was associated with concomitant amiodarone use in our study. The concomitant use of both drugs was not investigated. As a P-gp substrate, TAF is expected to exhibit increased absorption when used in combination with P-gp inhibitors, such as amiodarone, leading to a higher systemic concentration (6).

Study Limitations

This study has some limitations. The drug interactions observed in this study are potential interactions; therefore, there are no data on actual interactions. For the assessment of pDDIs, only the University of Liverpool Hepatitis Drug Interaction Database was used. Further research can be conducted by combining different databases. Larger sample sizes and longer follow-up periods are needed to comprehensively study drug interactions.

Conclusion

In conclusion, this study highlighted the significance of pDDIs during the treatment of HB. This study provides important information for clinicians to guide treatment regimens for patients with HB and select appropriate drug combinations. It is important that treatment plans for patients with HB take into account interactions with other medicines and that patients are monitored regularly. This approach can potentially optimize treatment responses and contribute to the management of HB infection. Further research is needed to improve the treatment of HB infection and reduce the risk of developing pDDIs.

Ethics

Ethics Committee Approval: The study was approved by the Erzurum Regional Training and Research Hospital (decision no.: E-37732058-514.99, date: 06.06.2022) and was conducted in accordance with the principles of the Declaration of Helsinki.

Informed Consent: The study was conducted as a retrospective, observational study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: N.N.A., M.A., Concept: N.N.A., Design: M.A., Data Collection or Processing: N.N.A., M.A., Analysis or Interpretation: M.A., Literature Search: N.N.A., M.A., Writing: N.N.A.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declare no financial support.

References

1. Yuen MF, Chen DS, Dusheiko GM, Janssen HLA, Lau DTY, Locarnini SA, Peters MG, Lai CL. Hepatitis B virus infection. Nat Rev Dis Primers. 2018;4:18035.
2. Phillips S, Jagatia R, Chokshi S. Novel therapeutic strategies for chronic hepatitis B. Virulence. 2022;13:1111-1132.
3. Chien RN, Liaw YF. Current trend in antiviral therapy for chronic hepatitis B. Viruses. 2022;14:434.
4. Palleria C, Di Paolo A, Giofrè C, Caglioti C, Leuzzi G, Siniscalchi A, De Sarro G, Gallelli L. Pharmacokinetic drug-drug interaction and their implication in clinical management. J Res Med Sci. 2013;18:601-610.
5. Bickel M, Khaykin P, Stephan C, Schmidt K, Buettner M, Amann K, Lutz T, Gute P, Haberl A, Geiger H, Brodt HR, Jung O. Acute kidney injury caused by tenofovir disoproxil fumarate and diclofenac co-administration. HIV Med. 2013;14:633-638.
6. HEP Drug Interactions. University of Liverpool. [Cited 2023 Nov 20]. Available from: https://www.hep-druginteractions.org/checker
7. Rizzo GEM, Cabibbo G, Craxì A. Hepatitis B virus-associated hepatocellular carcinoma. Viruses. 2022;14:986.
8. Tseng CH, Hsu YC, Ho HJ, Nguyen MH, Wu CY. Increasing age and nonliver comorbidities in patients with chronic hepatitis B in Taiwan: A Nationwide Population-Based Analysis. Dig Dis. 2021;39:266-274.
9. Wong GL, Wong VW, Yuen BW, Tse YK, Luk HW, Yip TC, Hui VW, Liang LY, Lui GC, Chan HL. An aging population of chronic hepatitis B with increasing comorbidities: a territory-wide study from 2000 to 2017. Hepatology. 2020;71:444-455.
10. Oh H, Jun DW, Lee IH, Ahn HJ, Kim BO, Jung S, Nguyen MH. Increasing comorbidities in a South Korea insured population-based cohort of patients with chronic hepatitis B. Aliment Pharmacol Ther. 2020;52:371-381.
11. Nguyen MH, Lim JK, Burak Ozbay A, Fraysse J, Liou I, Meyer N, Dusheiko G, Gordon SC. Advancing age and comorbidity in a US insured population-based cohort of patients with chronic hepatitis B. Hepatology. 2019;69:959-973.
12. Sarin SK, Kumar M, Lau GK, Abbas Z, Chan HL, Chen CJ, Chen DS, Chen HL, Chen PJ, Chien RN, Dokmeci AK, Gane E, Hou JL, Jafri W, Jia J, Kim JH, Lai CL, Lee HC, Lim SG, Liu CJ, Locarnini S, Al Mahtab M, Mohamed R, Omata M, Park J, Piratvisuth T, Sharma BC, Sollano J, Wang FS, Wei L, Yuen MF, Zheng SS, Kao JH. Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update. Hepatol Int. 2016;10:1-98.
13. Yang M, Xu X. Important roles of transporters in the pharmacokinetics of anti-viral nucleoside/nucleotide analogs. Expert Opin Drug Metab Toxicol. 2022;18:483-505.
14. Mandíková J, Volková M, Pávek P, Navrátilová L, Hyršová L, Janeba Z, Pavlík J, Bárta P, Trejtnar F. Entecavir interacts with influx transporters hOAT1, hCNT2, hCNT3, but not with hOCT2: The potential for renal transporter-mediated cytotoxicity and drug-drug interactions. Front Pharmacol. 2016;6:304.
15. Hakkola J, Hukkanen J, Turpeinen M, Pelkonen O. Inhibition and induction of CYP enzymes in humans: an update. Arch Toxicol. 2020;94:3671-3722.
16. Noor S, Ismail M, Haider I, Khadim F. Drug-drug interactions in hepatitis patients: do these interactions matter in clinical perspectives? Ann Hepatol. 2018;17:1001-1011.
17. Caroleo B, Staltari O, Gallelli L, Perticone F. Reactivation of chronic hepatitis B during treatment with tenofovir disoproxil fumarate: drug interactions or low adherence? BMJ Case Rep. 2015;2015:bcr2015209586.
18. Anderson MS, Gilmartin J, Fan L, Yee KL, Kraft WK, Triantafyllou I, Reitmann C, Guo Y, Liu R, Iwamoto M. No meaningful drug interactions with doravirine, lamivudine and tenofovir disoproxil fumarate coadministration. Antivir Ther. 2019;24:443-450.
19. Kearney BP, Sayre JR, Flaherty JF, Chen SS, Kaul S, Cheng AK. Drug-drug and drug-food interactions between tenofovir disoproxil fumarate and didanosine. J Clin Pharmacol. 2005;45:1360-1367.
20. Morelle J, Labriola L, Lambert M, Cosyns JP, Jouret F, Jadoul M. Tenofovir-related acute kidney injury and proximal tubule dysfunction

precipitated by diclofenac: a case of drug-drug interaction. Clin Nephrol. 2009;71:567-570.

21. Duim AR, Rokx C, van Gorp EC, Rijnders BJ. Proximal tubular dysfunction in a HIV-1 patient with coadministered tenofovir disoproxil-fumarate and ibuprofen. AIDS. 2015;29:746-748.
22. Bristol-Myers Squibb. Baraclude Prescribing Information in U.S.: Entecavir Tablets, Oral Solutions. 2015. Bristol-Myers Squibb. Available from: https://www.bms.com
23. Matthews SJ. Entecavir for the treatment of chronic hepatitis B virus infection. Clin Ther. 2006;28:184-203.

Research Article

65

DOI: 10.4274/vhd.galenos.2024.2024-8-2
Viral Hepat J 2024;30(3):65-69

Genotype Distribution of HCV in Patients with Chronic Hepatitis C in a University Hospital

Bir Üniversite Hastanesinde Kronik Hepatit C Hastalarındaki HCV Genotip Dağılımı

Burak Ezer[1], Enes Kasapoğlu[2], Alperen Ceylan[3], Mehmet Özdemir[3]

[1]*Beyhekim Training and Research Hospital, Clinic of Medical Microbiology, Konya, Turkey*
[2]*Çumra District Health Directorate, Konya Provincial Health Directorate, Konya, Turkey*
[3]*Necmettin Erbakan University Medicine Faculty, Depertmant of Medical Microbiology, Konya, Turkey*

ABSTRACT

Objectives: Hepatitis C virus (HCV) infection is a public health issue of great importance. HCV genotyping helps in monitoring prognosis, selecting appropriate antiviral drugs, monitoring side effects, and deciding on treatment duration. The aim of this study was to determine the HCV genotypes in our hospital to reveal their distribution over time and to contribute to epidemiological data by evaluating the relationship between HCV genotypes and viral load.

Materials and Methods: Serum samples from 144 patients diagnosed with chronic hepatitis C between January 01, 2019 and December 31, 2023 were included in this study. HCV-RNA loads were determined using a Bosphore quantification kit (Anatolia, Turkey) via a Montania 4896 thermal cycler (Anatolia, Turkey). HCV genotypes were detected using the Bio-Rad CFX96 system with the Diagnotech HCV genotyping quantitative polymerase chain reaction kit targeting the 5'NS5B region.

Results: The most frequently detected genotypes in our hospital were genotypes 1b (34.7%, genotype 3 with 32.6% and genotype 1 with 15.3%). The lower mean age of genotype 3 patients was statistically significant compared with the ages of patients with genotypes 4 and genotype 1b ($p<0.001$). It was found to be statistically significant that the median viral load of patients with genotype 1 was lower than that of patients with genotype 1b and genotype 3 ($p=0.046$). The higher frequency of genotype 4 among foreign nationals was statistically significant ($p=0.034$).

Conclusion: HCV genotypes vary between regions based on geographical location, migration, socioeconomic level, and drug

ÖZ

Amaç: Hepatit C virüsü (HCV) enfeksiyonu önemli bir halk sağlığı sorunudur. HCV genotiplendirilmesi hastalık prognozunun izlenmesinde, uygun antiviral ilaçların seçilmesinde, yan etkilerin takip edilmesinde ve tedavi süresine karar verilmesinde yardımcı olmaktadır. Bu çalışmanın amacı, hastanemizdeki HCV genotip dağılımını ortaya koymak ve HCV genotipleri ile viral yük arasındaki ilişkiyi değerlendirerek epidemiyolojik verilere katkı sağlamaktır.

Gereç ve Yöntemler: Bu çalışmaya 1 Ocak 2019-31 Aralık 2023 tarihleri arasında kronik hepatit C tanılı 144 hastanın serumları dahil edilmiştir. Hastaların HCV-RNA viral yükleri, Montania 4896 ısı döngü cihazı (Anatolia, Türkiye) aracılığıyla Bosphore (Anatolia, Türkiye) kantitifikasyon kiti kullanılarak tespit edilmiştir. HCV genotipleri, 5'NS5B bölgesini hedefleyen Diagnotech HCV genotipleme kantitatif polimeraz zincir reaksiyonu kiti ile Bio-Rad CFX96 cihazı kullanılarak tespit edilmiştir.

Bulgular: Hastanemizde en sık tespit edilen genotipler; genotip 1b (%34,7, genotip 3 %32,6 ve genotip 1 %15,3) olmuştur. Genotip 3 hastalarının yaş ortalamasının genotip 4 ve genotip 1b hastalarının yaş ortalamasından daha düşük olması istatistiksel olarak anlamlı bulunmuştur ($p<0,001$). Genotip 1'e sahip hastaların ortalama viral yükünün genotip 1b ve genotip 3'e sahip hastalardan daha düşük olması istatistiksel olarak anlamlı bulunmuştur ($p=0,046$). Yabancı uyruklular arasında genotip 4 sıklığının daha yüksek olması istatistiksel olarak anlamlı bulunmuştur ($p=0,034$).

Sonuç: HCV genotipleri coğrafi konum, göç, sosyoekonomik düzey ve ilaç kullanımına bağlı olarak bölgeler arasında farklılık

Cite this article as: Ezer B, Kasapoğlu E, Ceylan A, Özdemir M. Genotype distribution of HCV in patients with chronic hepatitis C in a university hospital. Viral Hepat J. 2024;30(3):65-69

Address for Correspondence: Burak Ezer, MD, Beyhekim Training and Research Hospital, Clinic of Medical Microbiology, Konya, Turkey
E-mail: dr.burakezer@gmail.com **ORCID ID:** orcid.org/0000-0003-1217-2080
Received: 14.08.2024 **Accepted:** 13.12.2024 **Publication Date:** 30.12.2024

use. Therefore, molecular studies on this issue are important for appropriate antiviral drug treatment and duration.
Keywords: Chronic viral hepatitis C, genotype, epidemiology

göstermektedir. Bu nedenle, bu konudaki moleküler çalışmalar uygun antiviral ilaç tedavisi ve süresi için önemlidir.
Anahtar Kelimeler: Kronik viral hepatit C, genotip, epidemiyoloji

Introduction

Hepatitis C virus (HCV) infection may cause acute and chronic HCV infections and lead to complications, such as liver failure, cirrhosis, and hepatocellular carcinoma, with high mortality and morbidity (1). HCV infection is a major health problem worldwide, and approximately 85% of acute HCV infections progress to chronic HCV infection. The World Health Organization reports that approximately 58 million people worldwide are affected by chronic HCV infection, and approximately 1,5 million people become infected with HCV each year (2,3). In Turkey, approximately 1 million people are infected with HCV.

HCV is usually transmitted through sexual intercourse, intravenous drug use, blood transfusion, and surgical and dental procedures (4). The most important characteristic of HCV infection is that it can cause chronic HCV infection and become resistant to antiviral drugs as a result of mutations in host cells (5). Different HCV genotypes exist due to differences in nucleotide sequences among regions of the HCV genome (6). The nucleotide sequences of HCV genotypes differ by 30-35%, and there is at least a 15% difference between the nucleotide sequences of the subgroups of HCV genotypes (5,7). The main factors affecting HCV genotype distribution are socioeconomic level, geographical location, migration, and intravenous drug use.

The most common HCV genotype worldwide is genotype 1 with a rate of 46-49%, followed by genotype 3 at 22% (8,9). When the distribution in the world is analyzed; genotype 1 is more common in North and South America, genotype 2 in East Asia, genotype 3 in Asia and Europe, genotype 4 in the Middle East and North Africa, genotype 5 in South Africa, and genotype 6 in Southeast Asia (10). HCV genotyping helps in monitoring prognosis, selecting appropriate antiviral drugs, monitoring side effects, and deciding on the treatment duration (11).

The aim of this study was to determine the HCV genotypes in our hospital, monitor their distribution over the years, and contribute to the epidemiological data by revealing the relationship between HCV genotypes and viral load.

Materials and Methods

Serum samples taken from 144 patients diagnosed with chronic HCV infection between January 01, 2019 and December 31, 2023 were included in this study. HCV-RNA levels were determined using a Bosphore quantification kit (Anatolia, Turkey) via a Montania 4896 thermal cycler (Anatolia, Turkey). Results are expressed as International units per milliliter (IU/mL). HCV genotypes were detected on a Bio-Rad CFX96 PCR thermal cycler (California, USA) using a Diagnotech HCV genotyping qPCR kit (Diagnotech, Turkey) targeting the 5'NS5B region.

This retrospective study was approved by the Ethics Committee on Non-Medicine and Non-Medical Device Research of Necmettin Erbakan University (decision number: 2024/4963, date: 17.05.2024).

Statistical Analysis

The data obtained were analyzed using Statistical Package for Social Sciences 21.0 package program. In descriptive analyses, frequency data were presented as number (n) and percentage (%), whereas numerical data were presented as median (minimum-maximum). The conformity of numerical data to the normal distribution was analyzed by visual (histogram) and analytical methods (Kolmogorov-Smirnov test). For numerical variables that were found not to conform to normal distribution, the Kruskal-Wallis test was used to compare more than two independent groups. Post-hoc Mann-Whitney U test and Bonferroni correction were performed for pairwise comparisons between groups with statistically significant differences. The chi-square (χ^2) test and Fisher's exact test were used to compare categorical variables (statistical significance $p<0.05$).

Results

Of the patients whose results were included in the study, 66.7% were male and 95.8% were Turkish nationals. Of the samples, 34.7% were obtained in 2019, 20.1% in 2021, 18.1% in 2022, 16% in 2020, and 11.1% in 2023. The most frequently detected genotypes were type 1b (34.7%), type 3 (32.6%) and type 1 (15.3%) (Table 1).

The median age of the patients included in the study was determined as 40.00 (29.00-71.00). The median viral load of the patients was determined as 190000 (12500-1519366.50) (Table 2).

When the median ages of the patients were compared according to the detected HCV genotypes, a statistically significant difference was observed ($p<0.001$). In the post-hoc analyses, the difference was attributed to the median age of patients infected with type 3 being lower than that of patients infected with type 4 and type 1b (Table 3).

A statistically significant difference was observed when comparing the median viral loads of patients according to the HCV genotypes detected in patients ($p=0.046$).

In the post-hoc analyses, the difference was attributed to the median viral loads of patients with type 1 being lower than those of patients with type 1b and type 3 (Table 4).

There was a statistically significant difference between the years in which the disease was detected according to HCV genotypes ($p<0.001$). In post-hoc analyses, a lesser number of cases were found in individuals with type 1 genotype in 2019, whereas a greater number of cases were found in 2022 and 2023.

Table 1. Distribution of gender, year, nationality, and genotype

	n	%
Gender		
Male	96	66.7
Female	48	33.3
Year		
2019	50	34.7
2020	23	16.0
2021	29	20.1
2022	26	18.1
2023	16	11.1
Nationality		
Turkish	138	95.8
Foreign	6	4.2
Genotype		
Type 1	22	15.3
Type 1a	7	4.9
Type 1b	50	34.7
Type 2	7	4.9
Type 3	47	32.6
Type 4	5	3.5
Type 1 + Type 1b	2	1.4
Type 1b + Type 3	1	0.7
Type 2 + Type 3	2	1.4
Type 4 + Type 3	1	0.7

Table 2. Age and viral load

	Median (Q1-Q3)	Minimum-maximum
Age	40.00 (29.00-71.00)	18.00-94.00
Viral load	190.000 (12.500-1.519.366,50)	10,00-48.100.000,00

Table 3. Comparison of patient age according to genotype

	n	Median (Q1-Q3)	p-value
Genotype			
Type 1	22	30.50 (24.75-64.25)	<0.001
Type 1a	7	41.00 (32.00-71.00)	
Type 1b	50	67.50 (58.00-79.00)	
Type 2	7	40.00 (31.00-77.00)	
Type 3	47	30.00 (27.00-36.00)	
Type 4	5	57.00 (47.00-77.50)	
Mixed infection	6	59.00 (31.25-73.00)	

In post-hoc analyses, the number of individuals infected with the type 1 genotype was lower in 2019 but was higher in 2022 and 2023. A statistically significant difference was found between the nationalities of the patients based on the HCV genotypes, a statistically significant difference was found (p=0.034). In post-hoc analyses, the rate of infection with genotype 4 was lower in Turkish patients, whereas the rate was higher in foreign patients (Table 5).

Discussion

Hepatitis C infection is a significant public health issue due to its high rate of chronicity, potential for severe liver diseases, various modes of transmission, and the absence of effective vaccines (12). Diagnosis of HCV infection is based on the detection of HCV-specific antibodies using enzyme-linked immunosorbent assay (ELISA). If the ELISA result is positive; HCV viral core antigen or viral genomic RNA (HCV-RNA) must be tested to confirm the diagnosis. In 80-90% of patients, anti-HCV antibodies become positive six to twelve weeks after exposure (13). The identification of HCV genotypes is important for adjusting the dosage of antiviral agents, determining the duration of treatment, monitoring treatment response, and predicting patient prognosis (7,14). Various studies were conducted to investigate the frequency of HCV genotypes in our region and country (Table 6). It has been reported that the majority of HCV infection in Turkey is caused by genotype 1, and its prevalence varies between 57.1% and 97.1% 15). The most common subtype in Turkey is genotype 1b, with a prevalence of 52.7%-97.4% (16,17).

Table 4. Comparison of viral loads according to genotypes

	n	Median (Q1-Q3)	p-value
Genotype			
Type 1	22	22.000,00 (535,00-255.000,00)	0.046
Type 1a	7	390.000,00 (11.085,00-1.800.000,00)	
Type 1b	50	426.204,00 (17.440,00-2.281.547,00)	
Type 2	7	480.000,00 (100.000,00-2.700.000,00)	
Type 3	47	200.000 (21.000,00-1.882.634,00)	
Type 4	5	120.000,00 (7.725,00-7.733.381,50)	
Mixed infection	6	55.000,00 (5.325,00-1.565.894,50)	

In our study, as in previous data, the most frequent genotype was genotype 1 at a rate of 54.9%, and the most common subtype was genotype 1b at a rate of 34.7%. When the relationship between age and genotype was analyzed, the lower age of genotype 3 patients compared with genotype 1b patients was statistically significant. According to the data in the literature, the ages of patients infected with genotype 1 were statistically significantly higher compared with those infected with other genotypes. (18,19). In various studies conducted in Turkey, no statistically significant relationship was found between HCV-RNA load and genotype (15,20).

In our study, the low viral loads of patients infected with genotype 1 were statistically significant compared with those infected with genotypes 1b and 3. We believe that the coronavirus disease 2019 pandemic may lead to variations in the viral loads of infections caused by different HCV genotypes, and the increase in strains that cannot be subtypes may also be related to the pandemic. Different results have been reported in studies investigating genotype distribution and gender association. While some studies have found that genotype 1b is more commonly observed in women and genotype 3 in men, other studies have established that there is no statistical significance between genotype and gender (4,21,22). In a study examining the HCV genotype distribution in Syria between 2004 and 2006, the most frequently detected genotype was 4, with a rate of 59% (23). In various studies conducted in Turkey, the frequency of genotype 4 has been reported as 0-11%. In these studies, it was stated

Table 5. Comparison of gender, year, and nationality according to genotype

	Type 1	Type 1a	Type 1b	Type 2	Type 3	Type 4	Mixed	p-value
Gender								
Male	17 (77.3%)	5 (71.4%)	25 (50.0%)	5 (71.4%)	37 (78.7%)	4 (80.0%)	3 (50.0%)	0.059
Female	5 (22.7%)	2 (28.6%)	25 (50.0%)	2 (21.3%)	10 (21.3%)	1 (20.0%)	3 (50.0%)	
Year								
2019	0 (0.0%)	1 (14.3%)	25 (50.0%)	2 (28.6%)	17 (36.2%)	3 (60.0%)	2 (33.3%)	<0.001
2020	0 (0.0%)	1 (14.3%)	9 (18.0%)	1 (14.3%)	10 (21.3%)	1 (20.0%)	1 (16.7%)	
2021	2 (9.1%)	0 (0.0%)	9 (18.0%)	1 (14.3%)	15 (31.9%)	1 (20.0%)	1 (16.7%)	
2022	10 (45.5%)	3 (6.0%)	3 (6.0%)	3 (42.9%)	5 (10.6%)	0 (0.0%)	2 (33.3%)	
2023	10 (45.5%)	2 (28.6%)	4 (8.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Nationality								
Turkish	21 (95.5%)	7 (100.0%)	50 (100.0%)	7 (100.0%)	44 (93.6%)	3 (60.0%)	6 (100.0%)	0.034
Foreign	1 (4.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (6.4%)	2 (40.0%)	0 (0.0%)	

that the frequency of genotype 4 was rapidly increasing in our country because of migration as a result of the war in Syria and that its surveillance was important because genotype 4 was more resistant to treatment (23,24). In our study, we detected genotype 4 at a rate of 3.5%, similar to the literature data.

Treatment may fail in patients infected with mixed genotypes. In various studies conducted in our country, the prevalence of mixed genotype has been reported as 0-8.6% (7,25,26).

In our study, the mixed genotype rate was 4.2%, which was in line with the literature. In a study conducted in our hospital in 2019, the most common genotype was genotype 1b at a rate of 58.9%, and it was observed that there was no change in the most dominant genotype in our hospital (18).

Study Limitations

The retrospective nature of our study is one of the limitations due to the inability to identify risk factors and modes of transmission.

Conclusion

In conclusion, the detection of HCV genotypes is important in determining appropriate antiviral therapy, its duration, and monitoring prognosis. Because the genotype distribution in Turkey varies by region, more molecular epidemiological studies on HCV genotypes are required.

Table 6. Percentage of genotype distribution (%) in some studies conducted in Turkey within the last five years

Study	Year	Genotype 1	Genotype 1a	Genotype 1b	Genotype 2	Genotype 3	Genotype 4	Genotype 5	Genotype 6	Mixed
Kuru et al. (23)	2020	-	4.2	85.8	0.6	3	11	-	-	-
Sarı et al. (26)	2020	12.3	12.5	53.7	5.3	11.8	3.6	0.4	-	-
Ağca et al. (27)	2021	5.8	6.1	72.8	2	9.2	2.5	0.1	-	1.5
Özkaya et al. (28)	2021	3.4	3.7	82.8	1.8	6.7	0.9	-	-	0.6
Alacam et al. (29)	2022	2.6	13.2	56.2	6.7	14	8.8	1.3	0.2	8.6
Arıcı et al. (30)	2022	7.5	10.6	59.3	2.6	15.3	2.1	-	-	2.6
Bozlak et al. (11)	2023	-	8.5	71	12	12 (3a)	6	-	-	-
Cırıt et al. (31)	2023	51.5	-	-	1.3	21.4	20	4.6	-	1.23
Our Study	2024	15.3	4.9	34.7	4.9	32.6	3.5	-	-	4.2

Ethics

Ethics Committee Approval: This retrospective study was approved by the Ethics Committee on Non-Medicine and Non-Medical Device Research of Necmettin Erbakan University (decision number: 2024/4963, date: 17.05.2024).

Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

Concept: E.K., A.C., M.Ö., Design: E.K., A.C., M.Ö., Data Collection or Processing: B.E., Analysis or Interpretation: B.E., E.K., A.C., M.Ö., Literature Search: B.E., E.K., A.C., M.Ö., Writing: B.E., M.Ö.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declare no financial support.

References

1. Dilbaz N, Kuloğlu M, Evren EC, Paltun SC, Bilici R, Noyan CO, Kulaksizoglu B, Karabulut V, Umut G, Unubol B, Ucbilek E. HCV genotype distribution among people who inject drug in Turkey: findings from multicenter and cross-sectional study. Subst Abuse. 2023;17:11782218231157340.
2. Guntipalli P, Pakala R, Kumari Gara S, Ahmed F, Bhatnagar A, Endaya Coronel MK, Razzack AA, Solimando AG, Thompson A, Andrews K, Enebong Nya G, Ahmad S, Ranaldo R, Cozzolongo R, Shahini E. Worldwide prevalence, genotype distribution and management of hepatitis C. Acta Gastroenterol Belg. 2021;84:637-656.
3. World Health Orfanization. Global hepatitis report 2024. Action for access in low- and middle-income countries. 2024;1:17-19. Available from: https://www.who.int/publications/i/item/9789240091672
4. Tiryaki Y, Çetin Duran A, Özçolpan OO. Distribution of hepatitis C virus genotypes in Aydın province. Viral Hepat J. 2018;24:70-74.
5. Ergünay K, Abacıoğlu H. Clinical impact of hepatitis C virus genomic variations. Mikrobiyol Bul. 2015;49:625-635.(Turkısh).
6. Sanlidağ T, Akçali S, Ozbakkaloğlu B, Ertekin D, Akduman E. Manisa bölgesinde hepatit C virus genotiplerinin dağilimi [Distribution of hepatitis C virus genotypes in Manisa region, Turkey]. Mikrobiyol Bul. 2009;43:613-618. (Turkısh).
7. Selek MB, Baylan O, Karagöz E, Özyurt M. Changes in hepatitis C virus genotype distribution in chronic hepatitis C infection patients. Indian J Med Microbiol. 2018;36:416-421.
8. Robaeys G, Bielen R, Azar DG, Razavi H, Nevens F. Global genotype distribution of hepatitis C viral infection among people who inject drugs. J Hepatol. 2016;65:1094-1103.
9. 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;22:7824-7840.
10. 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.
11. Balkan Bozlak ÇEB, Gümüs A, Yılmaz A. Hepatitis C genotype assay and sequence analysis at a university hospital in Eastern Türkiye. Kafkas J Med Sci. 2023;13:279-286.
12. Çekin Y, Gür N, Çekin AH, Altuğlu İ, Yazan Sertöz R. Antalya Eğitim ve Araştırma Hastanesinde kronik hepatit C hastalarının genotip dağılımının araştırılması [Investigation of hepatitis C virus genotype distribution in patients with chronic hepatitis C infections in Antalya Training and Research Hospital, Turkey]. Mikrobiyol Bul. 2014;48:484-490.
13. Li HC, Lo SY. Hepatitis C virus: virology, diagnosis and treatment. World J Hepatol. 2015;7:1377-89.
14. Wilson EM, Rosenthal ES, Kattakuzhy S, Tang L, Kottilil S. Clinical laboratory testing in the era of directly acting antiviral therapies for hepatitis C. Clin Microbiol Rev. 2017;30:23-42.
15. Caliskan A, Kirisci O, Ozkaya E, Ozden S, Tumer S, Caglar S, Guler SA, Senol H. Distribution and predominance of genotype 3 in hepatitis C virus carriers in the province of kahramanmaras, Turkey. Hepat Mon. 2015;15:e25142.
16. Gökahmetoğlu S, Atalay MA, Kılınç A. Determination of the hepatitis C virus genotypes with "pyrosequencing" method. Erciyes Medical Journal. 2011;33:99-102.
17. Aktaş E, Ogedey ED, Külah C, Beğendik Cömert F. Zonguldak bölgesinde hepatit C virüsü genotipleri [Hepatitis C virus genotypes in a province of western Black-Sea region, Turkey]. Mikrobiyol Bul. 2010;44:647-650.
18. Gülseren YD, Esenkaya Taşbent F, Özdemir M, Feyzioğlu B. Hepatitis C genotypes in patients with chronic hepatitis C ınfection: a three-year evaluation. FLORA. 2020;25:347-353.(Turkısh).
19. 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;24:12-17.
20. Sağlik İ, Mutlu D, Öngut G, İnan D, Öğünç D, Can Sarinoğlu R, Özhak Baysan B, Gültekin M, Çolak D. Akdeniz Üniversitesi Hastanesinde kronik hepatit C enfeksiyonu olan hastalarda hepatit C virüs genotipleri: Beş yıllık sonuçların değerlendirilmesi [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;48:429-437.
21. Öz S, Köroğlu M, Özbek A, Demiray T, Karabay O, Trak G, Altındiş M. Hepatitis C virus genotype distribution in Sakarya province; three-year retrospective study. OTSBD. Aralık 2019;4:444-453.(Turkısh).
22. Bulut ME, Topalca US, Murat A, Teke L, Canalp HZ, Ocal M, Bayraktar B. HCV genotype distribution of patients with chronic hepatitis C in Istanbul. Sisli Etfal Hastan Tip Bul. 2021;55:86-92.
23. Kuru C, Hamidi AA. Genotype distribution of the hepatitis C virus and demographic features of patients in the province of Karabük. Viral Hepat J. 2020;26:163-166.
24. Altindis M, Dal T, Akyar I, Karatuna O, Gökahmetoğlu S, Tezcan Ulger S, Kulah C, Uzun B, Sener AG, Özdemir M, Aydoğan S, Kuskucu MA, Midilli K, Otlu B, Celen MK, Buruk Kurtulus, Guducuoğlu. Six-year distribution pattern of hepatitis C virus in Turkey: a multicentre study. Biotechnol Biotec Eq. 2015;30:335-340.
25. Kulah C, Altindis M, Akyar I, Gokahmetoglu S, Sayiner A, Kaleli I, Fidan I, Altuglu I, Aydin F, Topkaya A, Us T, Findik D, Ozdemir M, Oztürk E, Ulger ST, Karsligil T, Cekin Y, Aksaray S, Uzunoglu E, Aktas O, Uslu H, Cetinkol Y, Gureser AS, Ece G, Toptan H, Koroglu M, Comert F. The prevalence of mixed genotype infections in turkish patients with hepatitis C: a multicentered assessment. Clin Lab. 2019;65.
26. Sarı ND, Karatas A, İnci A, Yörük G. Evaluation of hepatitis C virus genotype distribution in domestic and foreign patients. Türkiye Klinikleri J Med Sci. 2020;40:148-153.
27. Ağca H, Ener B, Sağlık İ, Yılmaz E, Kazak E. Retrospective evaluation of hepatitis C Virus genotypes. Turk Mikrobiyol Cem Derg. 2021;51:303-308.(Turkısh).
28. Özkaya E, Buruk CK, Aydın F, Kaklıkkaya N, Baran I, Tosun İ. Distribution of hepatitis C virus genotypes: 18 years of experience in an academic center. Viral Hepat J. 2021;27:118-123.
29. Alacam S, Bakir A, Karatas A. Hepatitis C virus genotypes and viremia in a tertiary hospital in Istanbul, Turkey. J Infect Dev Ctries. 2022;16:668-674.
30. Arıcı N, Kansak N, Adaleti R, Aksaray S, Ankaralı H. Distribution of HCV genotypes in local and ınternational chronic hepatitis C patients: a six-year evaluation. ANKEM Derg. 2022;36:101-107.(Turkısh).
31. Cırıt OS, Demir Y, Yıldırım MS, Alpaslan B, Avcıoglu F, Doğan Y, Astam P. Genotype distribution of hepatitis C virus in the province of Gaziantep, a 10-year evaluation. Acta Microbiol Immunol Hung. 2023;70:348-352.

Research Article

DOI: 10.4274/vhd.galenos.2024.2024-5-2
Viral Hepat J 2024;30(3):70-76

Long-Term Outcomes of Patients with Chronic Hepatitis C Treated with Direct-Acting Antivirals in Turkey

Türkiye'de Direkt Etkili Antivirallerle Tedavi Edilen Kronik Hepatit C Hastalarının Uzun Dönem Sonuçları

Esra Zerdali[1], Mediha Bozkurt[2], İnci Yılmaz Nakir[1], Filiz Pehlivanoğlu[1]

[1]University of Health Sciences Turkey, Haseki Training and Research Hospital, Clinic of Infectious Diseases and Clinical Microbiology, İstanbul, Turkey
[2]Tatvan State Hospital, Clinic of Infectious Diseases and Clinical Microbiology, Bitlis, Turkey

ABSTRACT

Objectives: Direct-acting antivirals (DAA) improve clinical outcomes in chronic hepatitis C (CHC). Data about the long-term outcomes of patients with CHC treated with DAAs in Turkey. We aimed to analyze the characteristics and outcomes of patients with CHC who attended the 3-year follow-up visit after completing DAA therapy and to present their implications for clinical management and public health.

Materials and Methods: This single-center, single-arm, retrospective study included adult CHC patients treated with DAA ± ribavirin and attended the year 3 follow-up visit after completing treatment. Data on patient characteristics, laboratory parameters, recurrent/relapsing hepatitis C virus (HCV) infection, cirrhosis, and hepatocellular carcinoma (HCC) were collected from the hospital medical records and descriptively analyzed.

Results: Sixty-eight patients (55.9% women), including 15 patients (22.1%) of foreign origin, were included in the study. Forty-six patients (67.6%) had a known route of HCV transmission, and 27 (58.7%) were infected through blood transfusion and/or surgical intervention. Most participants (57.4%) were infected with HCV genotype (GT) 1b: all patients of European origin with GT1b, two-thirds of Syrian participants with GT4, and half of Asians with GT3. Three patients had cirrhosis (4.4%; all compensated) at baseline. No patient developed relapse, reinfection, cirrhosis, decompensation, or HCC.

Conclusion: Sustained virologic response, absence of new cases of cirrhosis, decompensation, or HCC during follow-up support the

ÖZ

Amaç: Direkt etkili antiviraller (DAA), kronik hepatit C (KHC) tedavi sonuçları iyileştirmiştir. Türkiye'de DAA'larla tedavi edilen KHC hastalarının uzun dönem takibine ilişkin veriler sınırlıdır. DAA tedavisini tamamladıktan sonra 3. yıl takibine gelen KHC hastalarının özelliklerini ve sonuçlarını analiz etmeyi ve bunların tedavi yönetimine ve halk sağlığına etkilerini sunmayı amaçladık.

Gereç ve Yöntemler: Bu tek merkezli, tek kollu, retrospektif çalışmaya DAA ± ribavirin ile tedavi edilen ve tedavi tamamlandıktan sonra 3. yıl takibine gelen yetişkin KHC hastaları dahil edildi. Hastaların özellikleri, laboratuvar parametreleri, nüks veya yeni gelişen hepatit C virüsü (HCV) enfeksiyonu, siroz ve hepatoselüler karsinom (HCC) gelişimi verileri hastane tıbbi kayıtlarından toplanmış ve tanımlayıcı olarak analiz edilmiştir.

Bulgular: Çalışmaya 15'i (%22,1) yabancı kökenli olmak üzere 68 hasta (%55,9'u kadın) dahil edildi. Kırk altı hastada (%67,6) HCV'nin bulaşma yolu biliniyordu ve bunların 27'si (%58,7) kan transfüzyonu ve/veya cerrahi müdahale yoluyla enfekte olmuştu. Katılımcıların çoğu (%57,4) HCV genotip (GT) 1b ile enfekteydi: Avrupa kökenli hastaların tümü GT 1b, Suriyeli katılımcıların üçte ikisi GT 4 ve Asyalıların yarısı GT 3 ile enfekteydi. Başlangıçta üç hastada siroz vardı (%4,4; hepsi kompanse sirozdu). Hiçbir hastada nüks, yeniden enfeksiyon, siroz, dekompansasyon veya HCC gelişmedi.

Sonuç: Kalıcı virolojik yanıt, takip sırasında yeni siroz, dekompansasyon veya HCC vakalarının görülmemesi DAA'ların uzun vadeli klinik etkinliğini desteklemektedir. Hepatit C önleme

Cite this article as: Zerdali E, Bozkurt M, Yılmaz Nakir İ, Pehlivanoğlu F. Long-term outcomes of patients with chronic hepatitis C treated with direct-acting antivirals in Turkey. Viral Hepat J. 2024;30(3):70-76

Address for Correspondence: Esra Zerdali, MD, University of Health Sciences Turkey, Haseki Training and Research Hospital, Clinic of Infectious Diseases and Clinical Microbiology, İstanbul, Turkey
E-mail: esrayerlikaya@gmail.com **ORCID ID:** orcid.org/0000-0002-7023-6639
Received: 27.05.2024 **Accepted:** 17.12.2024 **Publication Date:** 30.12.2024

long-term clinical effectiveness of DAAs. Hepatitis C Prevention and Control strategies should include post-treatment follow-up of patients at high risk of progression, HCC, recurrence, and relapse, and individuals who could potentially spread the infection.
Keywords: Hepatitis C, hepatocellular carcinoma, cirrhosis, death, sustained virologic response

ve kontrol stratejileri, ilerleme, HCC, nüks ve yeniden enfeksiyon riski yüksek olan hastaların ve enfeksiyonu yayma potansiyeli olan kişilerin tedavi sonrası takibini içermelidir.
Anahtar Kelimeler: Hepatit C, hepatoselüler karsinom, siroz, ölüm, kalıcı virolojik yanıt

Introduction

Chronic hepatitis C (CHC) affects millions of people worldwide and predisposes individuals to clinical conditions with significant morbidity and mortality, such as liver cirrhosis and hepatocellular carcinoma (HCC) (1).

To eliminate hepatitis C as a public health threat by 2030, the World Health Organization (WHO) aims to reduce new hepatitis C infections by 75% (from 20 to 5 per 100,000) and associated deaths by 60% (from 5 to 2 per 100,000) between 2020 and 2030 (2). Given that there is not yet an effective hepatitis C virus (HCV) vaccine and acute hepatitis C silently progresses to chronicity in 70% of cases, it is crucial to identify and effectively treat patients with CHC to achieve these targets (1,2).

The introduction of direct-acting antivirals (DAAs) in the 2010s marked a major milestone in the treatment of CHC (3). The oral route of administration, short duration of treatment (8-12 weeks in most cases), specific mechanisms of action targeting proteins essential for HCV replication, availability of pangenotypic regimens, achievement of better clinical outcomes with an acceptable safety and tolerability profile are the key advantages of DAAs over pegylated interferon-ribavirin therapy, which was the standard of care for hepatitis C in the 2000s (3,4).

Sustained virological response (SVR) rates exceed 95% with current DAA regimens and are above 85% even in challenging clinical conditions such as decompensated cirrhosis and HCC (5,6,7,8,9,10). The achievement of SVR is considered cure in patients with non-cirrhosis (5,6) and independently predicts reduced mortality, decompensation, HCC occurrence, and recurrence in CHC (11).

The European Association for the Study of the Liver (EASL) and the American Association for the Study of the Liver Diseases: The Infectious Diseases Society of America recommends regular post-treatment follow-up, even if SVR is achieved, for individuals with liver cirrhosis or predisposition to liver disease (obesity, diabetes mellitus and excessive alcohol intake) and those with ongoing risk behaviors for HCV reinfection and transmission, such as people who inject drugs (PWIDs) and men who have sex with men (MSMs) (5,6).

To eliminate hepatitis C, the 5-year National Viral Hepatitis Prevention and Control Program was created by the Turkish Ministry of Health in 2018, and in the same year, a road map for hepatitis C was developed in cooperation with the Viral Hepatitis Society and the Turkish Association for the Study of the Liver (12,13,14).

In Turkey, the first DAA therapy for CHC was approved in 2015, and DAAs have been reimbursed by the Social Security Institution since June 2016 (15). Real-life studies from Turkey have reported SVR12/24 rates of 85% to 100% with various DAA regimens in CHC, but data on long-term follow-up are limited (16,17,18,19,20, 21,22,23,24,25).

This article presents a descriptive analysis of the characteristics and clinical outcomes of patients with CHC who were followed-up for 3 years after completing DAA therapy and their implications for clinical management and public health.

Materials and Methods

This real-world study was based on a retrospective review of hospital medical records of patients treated for chronic HCV infection at the Infectious Diseases Clinic of the Haseki Training and Research Hospital between June 1, 2016 and January 31, 2020. The inclusion criteria were age ≥18 years, treatment with DAA ± ribavirin, adherence to the treatment regimen, and attendance at the follow-up visit 3 years after the completion of DAA treatment.

Information on patients' demographics [age, sex, body mass index (BMI), country of origin], comorbidities (diabetes mellitus, hypertension, heart disease, chronic renal failure, thyroid disease, cirrhosis, and co-infection with [hepatitis B virus (HBV)/human immunodeficiency virus (HIV)], HCV genotypes (GT), hepatitis activity index (HAI), and fibrosis (F) score before DAA treatment (if a liver biopsy was made), route of HCV transmission, and DAA regimens were recorded. In addition, data on HCV-RNA, blood counts [leukocytes, erythrocytes and platelets (PLT)], coagulation, prothrombin time, international normalized ratio, and blood biochemistry [urea, creatinine, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma-glutamyl transferase, total protein, albumin, total bilirubin, alpha-fetoprotein (AFP)] at the onset of DAA therapy and at post-treatment weeks 12 and years 3 were collected.

The study was approved by the Ethics Committee of Clinical Research Ethics Committee of Haseki Training and Research Hospital, University of Health Sciences Turkey (decision no: 105-2021, date: 27.10.2021) and conducted in accordance with The Declaration of Helsinki.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics version 25 (SPSS Inc., Chicago, IL, USA). Continuous variables were expressed as means and standard deviations, categorical variables as numbers and percentages, and non-normally distributed variables as median (minimum-maximum) values. The normality of the data was checked using the Kolmogorov-Smirnov or Shapiro-Wilk tests. The Friedman and Wilcoxon tests were used to compare continuous variables. Results were assessed on a bilateral basis at a 95% confidence level, with a significance level of <0.05.

Results

At the time of analysis, 77 out of the 143 patients treated with DAAs for CHC had at least 3 years since treatment completion. The study included 68 (55.9% women) patients who attended year 3 visits after the end of DAA therapy. The remaining nine patients died: two due to a heart attack and the third due to postoperative bleeding. The exact causes of death were not available for six patients, but none of these patients had cirrhosis or HCC at the last follow-up, and the deaths were not related to CHC complications. Table 1 presents the key demographic and clinical characteristics of the study population.

Table 1. Patients' characteristics

Characteristics (n=68)		**n (%)* or mean ± SD**
Sex	Female	38 (55.9)
	Male	30 (44.1)
Age, years		52±15
Country of origin	Türkiye	53 (77.9)
	Others**	15 (22.1)
BMI, kg/m²		26.9±5.1
	Obese (>30 kg/m²)	20 (29.4)
HBV	Inactive carrier	2 (2.9)
	Chronic hepatitis	2 (2.9)
	Immune (natural infection)	11 (16.2)
Anti-HIV (+)		2 (2.9)
HCV genotype	1	54 (79.4)
	Subtype 1a	12 (17.6)
	Subtype 1b	39 (57.4)
	Subtype not determined	3 (4.4)
	3	8 (11.8)
	4	5 (7.4)
	5	1 (1.5)
Cirrhosis	Compensated (Child-Pugh A)	3 (4.4)
Liver biopsy		43 (63.2)
	Hepatic activity index	6.2±2.2
	Fibrosis score	1.7±1.0
Route of transmission	Transfusion	11 (16.2)
	Surgery	8 (11.8)
	Surgery + transfusion	8 (11.8)
	IV drug use	6 (8.8)
	Other medical intervention	4 (5.9)
	Intrafamilial Dental procedure	4 (5.9) 3 (4.4)
	Sexual relation***	2 (2.9)
	Not known	22 (32.4)
Comorbidities	Hypertension	16 (23.5)
	Diabetes mellitus	12 (17.6)
	Renal disease****	6 (8.8)
	Cardiac disease	6 (8.8)
	Thyroid disease	6 (8.8)

*presented as % in the total study population
**Syria (n=6), Turkmenistan (n=2), Afghanistan (n=1), Azerbaijan (n=1), China (n=1), Uzbekistan (n=1), Ukraine (n=1), Moldova (n=1), Romania (n=1)
***includes 1 man having sex with men
****5 patients on hemodialysis
BMI: Body mass index, HBV: Hepatitis C virus, HBsAg: Hepatitis B surface antigen, HCV: Hepatitis C virus, HIV: Human immunodeficiency virus, IV: Intravenous, SD: Standard deviation

HCV GT1 was detected in 79.4% (n=54) of patients, the majority of whom (n=39; accounting for 57.4% of the whole study population) were infected with subtype 1b. The study population included 15 foreign nationals (22.1%), six from Syria and Asia, and three from Eastern Europe. Four out of six patients from Syria (66.7%) were infected with GT4, and the other two patients (33.3%) were infected with GT1a. Asian patients were equally infected with GT1b and GT3. All patients from Europe were infected with GT1b.

Two-thirds of the patients (67.6%; n=46) had a known route of HCV transmission, and 27 (58.7%) were infected through blood transfusion and/or surgical intervention. Seven foreign nationals (46.7%) and two Turkish patients (3.8%) reported HCV transmission through surgery or transfusion outside Turkey: Ukraine (n=1), Bulgaria (n=1), Uzbekistan (n=1), Turkmenistan (n=1), and Syria (n=5).

IV drug users (n=6) were equally infected with HCV GT1 and GT3. There were four patients (5.9%) co-infected with HBV (inactive carrier/chronic), two of whom were already on treatment at the time of onset of DAA therapy.

A liver biopsy was performed in 63.2% (n=43) of the study participants before starting DAAs. The mean HAI and F score in these patients were 6.2±2.2 and 1.7±1.0, respectively. Three patients (4.4%) had cirrhosis (all Child-Pugh A); none of them progressed during follow-up.

Overall, 39 patients (57.4%) had at least one comorbid condition. Hypertension was the most common comorbidity (23.5%; n=16) followed by diabetes mellitus (17.6%; n=12).

Table 2 summarizes the key treatment characteristics of the study. Overall, 23.5% (n=16) of patients were treatment-experienced, and relapse was the main reason for switching to DAAs in these patients (93.8%; n=15). The antiviral regimen used before DAAs in all patients except one was pegylated interferon plus ribavirin. Paritaprevir/ritonavir/ombitasvir-dasabuvir was the most frequently used (44.1%) DAA regimen.

The median serum viral load was 4500000 (31.880-41.100.000) IU/mL at baseline, which was significantly reduced and became undetectable at the first month of DAA treatment. All patients achieved SVR12, and the response was maintained at post-DAA year 3. The results of serial laboratory assessments at baseline and post-treatment assessment time-points are shown in Table 3. Liver function tests improved significantly compared with baseline at both 12 weeks and 3 years after treatment. There were no clinically significant changes in other laboratory parameters.

Discussion

In this retrospective study evaluating the clinical outcomes of patients with CHC over a 3-year period after completing

DAA therapy, virologic response was maintained in all patients, regardless of GT and treatment regimen. No new cirrhosis cases occurred during follow-up, and no patient developed relapse, reinfection, decompensated cirrhosis, or HCC.

The long-term maintenance of a 100% virologic response rate in the current study confirmed the suitability of SVR12 as a marker for predicting cure in CHC. Most studies have shown that SVR12/24 rates are lower in patients with decompensated cirrhosis or HCC (5,6,7,8,9,10). The achievement and maintenance of virologic response in all patients in the current study may be explained by the low proportion of patients with cirrhosis (4.4%; all compensated) and the absence of patients with HCC in our patient population. In a recent retrospective study in which all patients without cirrhosis achieved SVR12, Ebik et al. (23) found a 94.1% SVR rate in patients with cirrhosis, 53% of whom were classified as Child-Pugh B and C. In contrast, there are real-life studies from Turkey that reported that baseline cirrhotic status did not have a significant impact on achieving SVR with DAA treatment despite the inclusion of higher percentages of patients with cirrhosis compared with our study (34% and 58%; 42% of whom were decompensated in both studies) (18,19,20,21,22). Several factors, including study population characteristics and treatment regimens, are likely to play a role in the inconsistency of results on the cirrhosis-SVR relationship in real-life studies.

Decompensation and HCC are important clinical outcomes that determine prognosis in patients with CHC and cirrhosis. A systematic review and meta-analysis of 39 studies evaluating the impact of various DAA combinations on disease progression revealed that the risks of decompensation, HCC occurrence, and recurrence were significantly lower in patients with CHC who achieved SVR than in those who did not (11). Furthermore, estimates in another meta-analysis showed that despite achieving SVR with IFN-free DAA regimens, the incidence of HCC was approximately four-fold higher in patients with cirrhosis than in those with F3 fibrosis (26). To date, few studies have investigated the development of HCC in DAA users in Turkey

Table 2. Treatment characteristics

Characteristics (n=68)		**n (%)***
Prior treatment for HCV		16 (23.5)
	Peg – IFN + RBV	15 (22.1)
	TVR + Peg – IFN + RBV	1 (1.5)
Reason for switching to DAA	Relapse	15 (93.8)
	Non-response	1 (6.3)
DAA regimen	PRoD	30 (44.1)
	LDV/SOF	16 (23.5)
	PRoD + RBV	9 (13.2)
	SOF + RBV	5 (7.4)
	GLE/PIB	3 (4.4)
	LDV/SOF + RBV	3 (4.4)
	OMV/PTR/r + RBV	1 (1.5)
	SOF	1 (1.5)
Duration of DAA therapy (weeks), mean ± SD		13.4±4.9

*All patients were reported as n (%) unless otherwise specified
HCV: Hepatitis C virus, DAA: Direct acting antiviral, GLE: Glecaprevir, LDV: Ledipasvir, OMV: Ombitasvir, PIB: Pibrentasvir, PRoD: Paritaprevir/ritonavir/ombitasvir-dasabuvir, PTR: Paritaprevir, r: Ritonavir, RBV: Ribavirin, SD: Standard deviation, SOF: Sofosbuvir, TVR: Telaprevir

Table 3. Laboratory assessments throughout the observation period*

Laboratory parameters	**Before starting DAA therapy**	**12 weeks after completing DAA therapy**	**3 years after completing DAA therapy**	**p**	**p1**	**p2**	**p3**
HCV-RNA (IU/mL)	450000 (31880-41100000)	0	0	<0.001	<0.001	11	<0.001
AST (U/L)	37 (11-190)	19 (7-194)	18 (9-102)	<0.001	<0.001	0.002	<0.001
ALT (U/L)	37 (10-374)	14 (4-138)	14 (5-63)	<0.001	<0.001	0.373	<0.001
GGT (U/L)	35 (6-3023)	18 (7-1388)	19 (6-1021)	<0.001	<0.001	0.127	<0.001
Total bilirubin (mg/dL)	0.7 (0.2-1.8)	0.6 (0.2-1.6)	0.5 (0.2-1.9)	<0.001	0.113	0.003	<0.001
ALP (IU/L)	79 (44-286)	72 (45-157)	70 (32-238)	0.010	0.397	0.016	0.001
Albumin (g/dL)	4.2 (2.8-5.0)	4.2 (3.3- 4.8)	4.4 (3.3- 5.1)	<0.001	0.496	<0.001	0.004
AFP (ng/mL)	3.7 (1.1-24)	2.8 (1.1-9)	2.7 (0.9-7.8)	<0.001	<0.001	0.004	<0.001
Urea (mg/dL)	30 (10-171)	30 (11-210)	30 (9-195)	0.238	0.338	0.871	0.351
Creatinine (mg/dL)	0.6 (0.3-8.6)	0.6 (0.4-7.1)	0.7 (0.48-9.6)	<0.001	0.911	<0.001	0.002
HCT (%)	41.0 (11.6-52.7)	40.8 (14.2- 50.0)	41.0 (27.0- 50.0)	0.169	0.117	0.730	0.128
WBC (x10^3/mL)	6.96 (2.91-14.00)	6.78 (3.88-13.61)	6.80 (1.30-13.50)	<0.001	0.844	<0.001	<0.001
PLT (x10^3/mL)	226 (79-541)	246 (74- 483)	255 (88-1710)	0.087	0.135	0.708	0.013
PT (sec)	11.8 (10.5-46.7)	11.6 (9.8-17.1)	12 (8.5-33.4)	0.030	0.016	0.004	0.120
INR	0.9 (0.7-3.8)	0.9 (0.8-1.3)	1 (0.7-2.8)	<0.001	0.412	<0.001	<0.001

*Values are presented as median (minimum-maximum)
p: Across the 3 time-points, p1: Before starting DAA therapy vs 12 weeks after completing DAA therapy, p2: 12 weeks after completing DAA therapy vs 3 years after completing DAA therapy, p3: Before starting DAA therapy vs 3 years after completing DAA therapy
AFP: Alpha fetoprotein, ALP: Alkaline phosphatase, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, GGT: Gamma glutamyl transferase, HCT: Hematocrit, HCV-RNA: Hepatitis C virus ribonucleic acid, INR: International normalized ratio, PLT: Platelet, PT: Prothrombin time, WBC: White blood cell

(23,27). In a study evaluating the incidence of HCC in patients who achieved SVR with DAAs, HCC occurred in 5.7% of patients during a median follow-up of 29 months (6-66 months). In that study, only Child-Pugh B and C patients developed HCC (>six-fold more frequent in Child-Pugh C), and 40% of these cases were recurrences (23). Similarly, HCC occurred in 5.6% of patients during a median follow-up of 43±16.2 months (all in patients with cirrhosis; information on decompensation not reported) in a study investigating the biochemical determinants of HCC development in CHC patients who achieved SVR24 with DAAs. The authors reported that serum AFP and albumin levels before, at the end of, and 24 weeks after treatment, and the PLT count at 24 weeks after treatment were predictors of HCC development (27). The low percentage of patients with cirrhosis compared with those studies (4.4% vs 47.6% and 33.5%) and the absence of patients classified as Child-Pugh B or C may explain why we did not observe any HCC cases in our study population. The mean fibrosis score of 43 patients (63.2%) who underwent biopsy at baseline was 1.7±1.0. Since HCV-RNA levels became undetectable after the first month of DAA treatment and subsequent clinical, laboratory, and ultrasound evaluations did not indicate progression, decompensation, or HCC, no patient underwent liver biopsy during follow-up. Three patients with compensated cirrhosis at baseline remained clinically stable throughout the study period.

The findings on HCV GT distribution and transmission routes in the present study are noteworthy as they may have implications for the prevention and effective management of HCV infections in Turkey. Consistent with global HCV data (28), the most common HCV GT in our study population was GT1 (79.4%; 57.4% GT1b), followed by GT3 (11.8%) and GT4 (7.4%). In a recent large-scale study involving centers from different geographical regions of Turkey, GT3 was the second most frequently encountered HCV GT (3.6%) after GT1, while GT4 ranked fourth (1.3%) (29). GT3 is the leading GT in South Asia and the second most common GT in Central Asia, while GT4 is an "endemic" GT predominant in Africa, accounting for 65% of HCV cases in North Africa and the Middle East and 83% of those in Central Sub-Saharan Africa (28). Consistent with our findings, real-life studies in Turkey have shown that GT3 and GT4 have become more prevalent in Turkey in recent years (17,22,29,30,31,32,33). This can be explained, at least in part, by increased migration to Turkey from countries where these HCV subtypes are predominant (17,30,31), as shown by the latest available national migration statistics (34). In the current study, both GT3 (11.8%) and GT4 (7.4%) were more frequent than recently reported data (29,30,31,32,33) except for a study from southern Turkey, which reported a frequency of 28.6% for GT3 (17). Patients of foreign nationality accounted for >20% of the study population and were from Asia, the Middle East, and Central and Eastern Europe. Syria was the most common foreign country (40%) and two-thirds of Syrian patients were infected with GT4. Similarly, in a recent large-scale study conducted in Southern Turkey, GT4 was the most common GT among Syrian refugees (48.8%), who made up 7.8% of the study population (17). Some groups of foreign origin, such as irregular migrants and asylum seekers, experience problems in accessing treatment services. Hepatitis C poses a significant threat to the prevention and control of infection (35). In addition, there are barriers to treatment for those who have acquired the infection in their home country. Overcoming these problems is expected to contribute to reducing the prevalence of hepatitis C in Turkey by improving treatment and follow-up rates.

PWIDs, a group with a high probability of being infected with GT3 (17,36,37), accounted for 8.8% of our study population, and half of them were infected with GT3. Consistent with our findings, several studies reported GT3 as the most common GT among PWIDs (17,36,37). Sarıgül Yıldırım et al. (36) reported that GT3 was almost 9 times more prevalent in PWIDs than in non-PWIDs. In another large-scale study conducted in Turkey, GT3 was detected in 61.5% of GT PWIDs (17). Consistently, GT3 was the most frequently detected GT among PWIDs receiving substance abuse treatment in specialized centers in Turkey (37). The clinical significance of GT3 is based on its association with poorer prognosis due to high rates of hepatosteatosis, rapid progression to cirrhosis, high rates of HCC (38), and increased risk of treatment failure due to the inherent presence of resistance-associated substitutions (RAS) to non-structural protein 5A (NS5A) inhibitors (39). According to official data, injected drug use in Turkey has increased in recent years (40). This may further increase the prevalence of HCV GT3 infected people in the coming years.

The high-risk of re-infection in PWIDs and MSMs due to ongoing high-risk behavior should also be considered in the follow-up of patients who have cleared HCV. To reduce the risk of relapse, recurrence, or transmission to healthy individuals, these individuals should be carefully monitored even if SVR is achieved (5,6). In our practice, we comprehensively inform PWIDs about harm reduction and behavior change through constructive communication from the start of treatment. After achieving SVR12, annual follow-ups with HCV RNA testing are performed to ensure prompt and effective management of the infection, if necessary, and to prevent further spread of the virus.

Consistent with the findings of a national study investigating the risk factors for HCV transmission (30), we observed that surgical and other medical interventions, including blood transfusions and dental procedures, were the main routes of HCV transmission. Many patients in the present study had a history of potential exposure to the virus before 1996 when HCV screening became mandatory prior to blood donation and medical/surgical interventions in Turkey. In addition, two-thirds of the migrant patients reported HCV transmission through surgery and/or blood transfusion before migrating to Turkey. These findings emphasize the importance of taking measures to eliminate the risks associated with unsafe medical practices. This is of particular concern for patients with limited or no health insurance. It is of great importance for individuals and public health to identify these vulnerable individuals and ensure that they are appropriately treated and followed up.

In this study, 24 patients (35.3%) had at least one condition requiring post-treatment surveillance according to the EASL guideline recommendations. The two most common conditions were obesity and diabetes mellitus. In recent real-life studies in Turkey, diabetes was reported in 19-40% of HCV-infected patients (19,27). Referral of patients to relevant healthcare professionals for effective diabetes management, including diet and exercise

recommendations, is important and should not be ignored during and after antiviral therapy to prevent future liver damage.

Study Limitations

The major limitations of this study are the small sample size and retrospective design. The study population characteristics did not allow a comparison between patients with and without cirrhosis. Furthermore, the study was conducted at a single center, which may have affected the generalizability of the findings. However, our findings are valuable because, to our knowledge, this is the first study in Turkey to report long-term follow-up outcomes in patients who completed DAA therapy for CHC.

Conclusion

This study, examining the 3-year follow-up results after the end of antiviral therapy, demonstrated the long-term benefit of DAA therapy in terms of maintaining virological response and preventing adverse outcomes in CHC. The follow-up strategy should consider the sociodemographic and clinical characteristics of patients. To achieve the WHO's target of eliminating HCV as a public health priority by 2030, it would be useful to expand the scope of the National Viral Hepatitis Prevention and Control Program and the HCV roadmap to include post-treatment follow-up of patients at risk of progressive liver damage, HCC, relapse, and recurrent infection.

Ethics

Ethics Committee Approval: The study was approved by the Ethics Committee of Clinical Research Ethics Committee of Haseki Training and Research Hospital, University of Health Sciences Turkey (decision no: 105-2021, date: 27.10.2021) and conducted in accordance with The Declaration of Helsinki.

Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

Concept: E.Z., Design: E.Z., Data Collection or Processing: E.Z., M.B., İ.Y.N., Analysis or Interpretation: E.Z., M.B., İ.Y.N., Literature Search: E.Z., M.B., İ.Y.N., F.P., Writing: E.Z., F.P.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

References

1. World Health Organization. Hepatitis C fact sheet (updated June 24, 2022). Available from: https://www.who.int/news-room/fact-sheets/detail/hepatitis-c (Accessed on September 28, 2023).
2. World Health Organization. Global health sector strategies on, respectively, HIV, viral hepatitis and sexually transmitted infections for the period 2022-2030. Available from: https://www.who.int/publications/i/item/9789240053779 (Accessed on September 28, 2023).
3. Manns MP, Maasoumy B. Breakthroughs in hepatitis C research: from discovery to cure. Nat Rev Gastroenterol Hepatol. 2022;19:533-550.
4. Dietz C, Maasoumy B. Direct-acting antiviral agents for hepatitis C virus infection-from drug discovery to successful implementation in clinical practice. Viruses. 2022;14:1325.
5. European Association for the Study of the Liver. EASL recommendations on treatment of hepatitis C: final update of the series. J Hepatol. 2020;73:1170-1218.
6. Bhattacharya D, Aronsohn A, Price J, Lo Re V. Hepatitis C guidance 2023 update: AASLD-IDSA recommendations for testing, managing, and treating hepatitis C virus infection. Clin Infect Dis. 2023:ciad319.
7. Krassenburg LAP, Maan R, Ramji A, Manns MP, Cornberg M, Wedemeyer H, de Knegt RJ, Hansen BE, Janssen HLA, de Man RA, Feld JJ, van der Meer AJ. Clinical outcomes following DAA therapy in patients with HCV-related cirrhosis depend on disease severity. J Hepatol. 2021;74:1053-1063.
8. Tahata Y, Hikita H, Mochida S, Enomoto N, Kawada N, Kurosaki M, Ido A, Miki D, Yoshiji H, Takikawa Y, Sakamori R, Hiasa Y, Nakao K, Kato N, Ueno Y, Yatsuhashi H, Itoh Y, Tateishi R, Suda G, Takami T, Nakamoto Y, Asahina Y, Matsuura K, Yamashita T, Kanto T, Akuta N, Terai S, Shimizu M, Sobue S, Miyaki T, Moriuchi A, Yamada R, Kodama T, Tatsumi T, Yamada T, Takehara T. Liver-related events after direct-acting antiviral therapy in patients with hepatitis C virus-associated cirrhosis. J Gastroenterol. 2022;57:120-132.
9. Zhang W, Zhang J, Tang S, Liu Y, Du X, Qiu L, Liu M, Yu H, Pan CQ. Efficacy and safety of sofosbuvir-based regimens in hepatitis C patients with decompensated cirrhosis: a systematic review and meta-analysis. J Clin Transl Hepatol. 2023;11:144-155.
10. He S, Lockart I, Alavi M, Danta M, Hajarizadeh B, Dore GJ. Systematic review with meta-analysis: effectiveness of direct-acting antiviral treatment for hepatitis C in patients with hepatocellular carcinoma. Aliment Pharmacol Ther. 2020;51:34-52.
11. Sahakyan Y, Lee-Kim V, Bremner KE, Bielecki JM, Krahn MD. Impact of direct-acting antiviral regimens on mortality and morbidity outcomes in patients with chronic hepatitis C: systematic review and meta-analysis. J Viral Hepat. 2021;28:739-754.
12. T.C. Sağlık Bakanlığı. Türkiye viral hepatit önleme ve kontrol programı. (In Turkish) (The Turkish viral hepatitis prevention and control program). 2018. Available from: https://hsgm.saglik.gov.tr/depo/Yayinlarimiz/Programlar/Turkiye_Viral_Hepatit_Onleme_ve_Kontrol_Programi_2018-2023.pdf (Accessed on September 28, 2023).
13. Akarca US, Aygen B, Bodur H, Güner HR, İdilman R, Kaymakoğlu S, Lazarus J, Örmeci N, Razavi H, Robbins S, Tözün AN. Türkiye'de Hepatit C eliminasyonu yol haritası önerileri ve çalıştay raporu. (In Turkish) (Hepatitis C elimination in Turkey, roadmap recommendations and workshop reports). 2018. Presented at 11th National Viral Hepatitis Symposium. June 14-16, 2019.
14. Akarca US, Baykam N, Güner R, Günşar F, İdilman R, Kaymakoğlu S, Köksal İ, Tabak F, Yamazhan T. Eliminating viral hepatitis in Turkey: achievements and challenges. Viral Hepat J. 2022;28:47-54
15. Idilman R, Razavi H, Robbins-Scott S, Akarca US, Örmeci N, Kaymakoglu S, Aygen B, Tozun N, Güner R, Bodur H, Lazarus JV. A micro-elimination approach to addressing hepatitis C in Turkey. BMC Health Serv Res. 2020;20:249.
16. Özer Çakır Ö. Kronik hepatit C tedavisinde direkt etkili antiviral ajanların tedavi yanıtının gerçek yaşam verileri: tek merkez çalışması. (In Turkish). (The real-world outcomes of virologic response of treatment with direct acting antiviral agents of chronic hepatitis C: single center study). Sakarya Tıp Dergisi. 2019;9:455-463.
17. Suntur BM, Kaya H, Eker HBŞ, 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.
18. Yamazhan T, Turan İ, Ersöz G, Günşar F, Pullukçu H, Danış N, Ünal NG, Vardar R, Oruç N, Tekin F, Taşbakan M, Sipahi OR, Akarca US. Real-life experience of ledipasvir and sofosbuvir single-tablet regimen among chronic hepatitis C patients in Turkey. Turk J Gastroenterol. 2020;31:239-245.
19. Değertekin B, Demir M, Akarca US, Kani HT, Üçbilek E, Yıldırım E, Güzelbulut F, Balkan A, Vatansever S, Danış N, Demircan M, Soylu A, Yaras S, Kartal A, Kefeli A, Gündüz F, Yalçın K, Erarslan E, Aladağ M, Harputluoğlu M, Özakyol A, Temel T, Akarsu M, Sümer H, Akın M,

Albayrak B, Sen İ, Alkım H, Uyanıkoğlu A, Irak K, Öztaşkın S, Uğurlu ÇB, Güneş Ş, Gürel S, Nuriyev K, İnci İ, Kaçar S, Dinçer D, Doğanay L, Göktürk HS, Mert A, Coşar AM, Dursun H, Atalay R, Akbulut S, Balkan Y, Koklu H, Şimşek H, Özdoğan O, Çoban M. Real-world efficacy and safety of ledipasvir + sofosbuvir and ombitasvir/paritaprevir/ritonavir ± dasabuvir combination therapies for chronic hepatitis C: a Turkish experience. Turk J Gastroenterol. 2020;31:883-893.

20. Çölkesen F, Tarakçı A, Kacar F, Eroğlu E, Özdemir Armağan Ş. Real life data for glecaprevir/pibrentasvir. a single-centre study. Arch Curr Med Res. 2021;2:14-18.
21. Demirtürk N, Aygen B, Çelik İ, Mıstık R, Akhan S, Barut Ş, Ural O, Batırel A, Şimşek F, Ersöz G, İnan D, Kınıklı S, Türker N, Bilgin H, Gürbüz Y, Tülek N, Tarakçı H, Yıldız O, Türkoğlu E, Kamalak Güzel D, Şimşek S, Tuna N, Aktuğ Demir N, Çağatay A, Çetinkaya RA, Karakeçili F, Hakyemez İN, Tuncer Ertem G, Örmen B, Korkmaz P, Yıldız U, Kuruüzüm Z, Şener A, Arslan Özel S, Öztürk S, Suer K, Çelen MK, Konya P, Asan A, Saltoğlu N, Doğan N. Real-world data from Turkey: is sofosbuvir/ledipasvir with or without ribavirin treatment for chronic hepatitis C Really effective? Turk J Gastroenterol. 2021;32:155-163.
22. Gok Sargin Z, Dusunceli I. Distribution of chronic hepatitis C genotype and evaluation of clinical factors affecting direct-acting antiviral treatment responses in the Western Black Sea Region, Turkey. Eur Rev Med Pharmacol Sci. 2022;26:7256-7262.
23. Ebik B, Aygan M, Tuncel ET, Kacmaz H, Ekin N, Arpa M, Yalcin K. Development of hepatocellular carcinoma in patients with chronic hepatitis C who had sustained viral response following direct-acting antiviral therapy. Hepatol Forum. 2022;3:82-87.
24. Altınkaya E, Aktaş A. Efficacy of oral combination antiviral therapy in genotype 4 hepatitis C ınfection and the importance of rapid virological response. Trop J Pharm Res. 2022;21:151-157.
25. Zerdalı E, Yılmaz Nakir İ, Pehlivanoğlu F. Evaluation of direct-acting antiviral agents and clinical responses in chronic hepatitis C patients. Viral Hepat J. 2022;28:79-84.
26. Lockart I, Yeo MGH, Hajarizadeh B, Dore GJ, Danta M. HCC incidence after hepatitis C cure among patients with advanced fibrosis or cirrhosis: A meta-analysis. Hepatology. 2022;76:139-154.
27. Sargin ZG, Dusunceli I. Biochemical predictors of hepatocellular cancer development after one year in patients who achieved HCV clearance by direct-acting antiviral treatment. Eur Rev Med Pharmacol Sci. 2022;26:8459-8466.
28. 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;22:7824-7840.
29. Tabak F, Şirin G, Demir M, Aladağ M, Sümer Ş, Kurtaran B, Tosun S, Yamazhan T, Bozkurt İ, Gürbüz Y, Batırel A, Şenateş E, Kandemir FÖ, Topal F, Doğanay HL, Sezgin O, Mıstık R, Köse Ş, Yılmaz Y, İnan D, Köksal İ, Parlak E, Akdoğan M, Güner R. Demographic characteristics and transmission risk factors of patients with hepatitis C Virus in Turkey: the EPI-C, a multicenter and cross-sectional trial. Viral Hepat J. 2021;27:109-117.
30. Altindis M, Dal T, Akyar I, Karatuna O, Gökahmetoğlu S, Tezcan Ulger S, Kulah C, Uzun B, Sener AG, Özdemir M, Aydoğan S, Kuskucu MA, Midilli K, Otlu B, Celen MK, Buruk K, Guducuoğlu H. Six-year distribution pattern of hepatitis C virus in Turkey: a multicentre study. Biotechnology & Biotechnological Equipment, 2016;30:335-340.
31. Çetin Duran A, Kaya Çetinkaya Ö, 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;31:128-135.
32. Özkaya E, Buruk CK, Aydın F, Kaklıkkaya N, Baran I, Tosun İ. Distribution of hepatitis C virus genotypes: 18-year experience in an academic center. Viral Hepat J. 2021;27:118-123.
33. Bulut ME, Topalca US, Murat A, Teke L, Canalp HZ, Ocal M, Bayraktar B. HCV genotype distribution of patients with chronic hepatitis C in Istanbul. Sisli Etfal Hastan Tip Bul. 2021;55:86-92.
34. TÜİK. International migration statistics. 2021. Available from: https://data.tuik.gov.tr/Bulten/Index?p=Uluslararasi-Goc-Istatistikleri-2021-45814 (Accessed on September 28, 2023).
35. Genç HD. Non-nationals' access to health services in Turkey: an assessment of preliminary findings. Presented at The Migration Conference, Bari, Italy, June 18-20, 2019.
36. Sarıgül Yıldırım F, Üser Ü, Sarı ND, Kurtaran B, Önlen Y, Şenateş E, Gündüz A, Zerdali E, Karsen H, Batırel A, Karaali R, Güner R, Yamazhan T, Köse Ş, Erben N, İnce N, Köksal İ, Çuvalcı Öztoprak N, Yörük G, Kömür S, Bal T, Kaya S, Bozkurt İ, Günal Ö, Yıldız İE, İnan D, Barut Ş, Namıduru M, Tosun S, Türker K, Şener A, Hızel K, Baykam N, Duygu F, Bodur H, Can G, Gül HC, Sağmak Tartar A, Çelebi G, Sünnetçioğlu M, Karabay O, Kumbasar Karaosmanoğlu H, Sırmatel F, Tabak F. In a real-life setting, direct-acting antivirals to people who inject drugs with chronic hepatitis C in Turkey. Turk J Gastroenterol. 2022;33:971-978.
37. Dilbaz N, Kuloğlu M, Evren EC, Paltun SC, Bilici R, Noyan CO, Kulaksizoglu B, Karabulut V, Umut G, Unubol B, Ucbilek E. HCV genotype distribution among people who inject drug in Turkey: findings from multicenter and cross-sectional study. Subst Abuse. 2023;17:11782218231157340.
38. Chan A, Patel K, Naggie S. Genotype 3 infection: the last stand of hepatitis C virus. Drugs. 2017;77:131-144.
39. Smith D, Magri A, Bonsall D, Ip CLC, Trebes A, Brown A, Piazza P, Bowden R, Nguyen D, Ansari MA, Simmonds P, Barnes E. Resistance analysis of genotype 3 hepatitis C virus indicates subtypes inherently resistant to nonstructural protein 5A inhibitors. Hepatology. 2019;69:1861-1872.
40. Turkiye National Drug Report 2022. Available from: https://www.narkotik.pol.tr/kurumlar/narkotik.pol.tr/TUB%C4%B0M/Ulusal%20Yay%C4%B1nlar/Turkiye-National-Drug-Report-2022.pdf (Accessed on September 28, 2023).

2024 Referee Index