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AIM AND SCOPE

Viral Hepatitis Journal (Formerly Viral Hepatit Dergisi) is the regular publishing organ of the Viral Hepatitis Society. This periodical journal covers diagnosis, treatment, epidemiology, prevention and information of hepatitis.

Viral Hepatitis Journal is an open-access journal published 3 times per year (April, August and December). In addition, the special issues are published in some periods. It is a periodic national/international journal, published in English language with abstract and title published also in Turkish language and its editorial policies are based on independent peer-review principles.

The aim of Viral Hepatitis Journal is to continuously publish original research papers of the highest scientific and clinical values specifically on hepatitis, on an international level. Additionally, reviews on basic developments in education, editorial short notes, case reports, original views, letters from a wide range of medical personal containing experiences and comments as well as social subjects are published.

For general practitioners giving first line medical service who are interested in hepatitis, specialists in internal medicine, gastroenterology, microbiology, family physician, public health and hepatology, 'things that must be known' subjects will ensure to involve in Viral Hepatitis Journal.

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Viral Hepatitis Journal is a scientific journal that publishes retrospective, prospective or experimental research articles, review articles, case reports, editorial comment/discussion, letter to the editor, surgical technique, differential diagnosis, medical book reviews, questions-answers and also current issues of medical agenda from all fields of medicine and aims to reach all national/international institutions and individuals.

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In the international index and database, the name of the journal has been registered as Viral Hepatitis Journal and abbreviated as Viral Hepat J.

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The author(s) undertake(s) all scientific responsibility for the manuscript. All the authors must actively participate in the study. The author(s) guarantee(s) that the manuscript itself or any substantially similar content of the manuscript has not been published or is being considered for publication elsewhere. If the manuscript had been presented in a meeting before; the name, date and the province of the meeting should be noted.

Experimental, clinical and drug studies requiring approval by an ethics committee must be submitted to the Viral Hepatitis Journal with an ethics committee approval report confirming that the study was conducted in accordance with international agreements and the Declaration of Helsinki (revised in 2013) (https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/). The approval of the ethics committee and the fact that informed consent was given by the patients should be indicated in the Materials and Methods section (including approval number). All papers reporting experiments using animals must include a statement in the Material and Methods section giving assurance that all animals have received humane care in compliance with the "Guide for the Care and Use of Laboratory Animals" (www.nap.edu/catalog/5140.html) and indicating approval by the institutional ethical review board.

The content of the submitted manuscripts should conform to the criteria stated in "Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals" published by International Committee of Medical Journal Editors and updated in 2016 (available at http://www.icmje.org/).

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The articles sent to be published in the journal shouldn't have been published anywhere else previously or submitted and accepted to be published. However, a complete report that follows publication of a preliminary report, such as an abstract can be submitted. If authors intend to discard any part of the manuscript, a written application should be sent to the Editor.

In case of retraction of the text by author(s) for any reason again needs a written and signed application explaining the reasons.

The name of the institution where the authors work and the name of the institution or the department in which the study has been conducted should not be mentioned in the submitted manuscript.

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The authors should keep a copy of the submitted manuscripts and other documents.

If the whole or a part of the submitted manuscript needs to be published somewhere else, Editorial Office must be informed accordingly.

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The result can be acceptance, minor revision, major revision, rejection in the current form, or rejection. Accepted manuscripts are forwarded for publication; in this stage, all information and data are checked and controlled properly; the proof of the article to be published by the journal are forwarded to the writers for proof reading and corrections.

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The Editorial Policies and General Guidelines for manuscript preparation specified below are based on "Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals (ICMJE Recommendations)" by the International Committee of Medical Journal Editors (2016, archived at http://www.icmje.org/).

Preparation of research articles and systematic reviews meta-analyses must comply with study design guidelines: CONSORT statement for randomized controlled trials (Moher D, Schultz KF, Altman D, for the CONSORT Group. The CONSORT statement revised recommendations for improving the quality of reports of parallel group randomized trials. JAMA 2001; 285: 1987-91) (http://www.consort-statement.org/),

PRISMA for preferred reporting items for systematic reviews and meta-analyses (Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 2009; 6(7): e1000097.) (http://www.prisma-statement.org/),

STARD checklist for the reporting of studies of diagnostic accuracy (Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig LM, et al, for the STARD Group. Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative. Ann Intern Med 2003;138:40-4.) (http://www.stard-statement.org/),

STROBE statement—checklist of items that should be included in reports of observational studies (http://www.strobe-statement.org/),

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Authors are encouraged to follow the following principles before submitting their article:

• Research articles and article collections should not exceed 15 pages including the text, figures, tables and references, while short announcements and case report presentations should not be longer than 5 pages.

Short Announcements

i. Turkish title, English title, author(s)' name(s) and institution(s) (Turkish and English)
ii. Turkish and English Abstract (max 300 words)
iii. Turkish and English Keywords
iv. Introduction (max 300 words)
v. Materials and Methods (max 400 words)
vi. Results (max 400 words)
vii. Discussion (max 700 words)
viii. Referances (should not exceed 15), all words 2000 not exceed.

• Author number for review articles should not exceed three.

• Author number for case report presentations should not exceed four.

• Articles should be written with double line space in 10 font size and right, left, upper and lower margins should all be 2.5 cm. Writing style should be Arial.

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Manuscripts should be written with Microsoft Word and the main text should not exceed 2000 words.

Abbreviations: Abbreviations should be defined at first mention and used consistently thereafter. Internationally accepted abbreviations should be used; refer to scientific writing guides as necessary.

Cover Letter: Cover letter should include statements about manuscript category designation, single-journal submission affirmation, conflict of interest statement, sources of outside funding, equipments (if so), approval for language for articles in English and approval for statistical analysis for original research articles.

Title Page: Title should be concise and informative (in Turkish and English). The title page should include a list of all contributing authors and all of their affiliations. Positions of authors and names of departments and institutions to which they are attached and the province should be written. Supply full correspondence details for the corresponding author, including phone, mobile phone, fax number and e-mail address.

ARTICLE SECTIONS

The text file should include the title in Turkish, keywords, the title in English, keywords in English, the text of the article, references, tables (only one table for one page) and figure

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legends (if any), respectively. Within the text file, the names of the authors, any information about the institutions, the figures and images should be excluded.

Abstract: Turkish and English abstracts should be given together with the article title. It should be divided into four sections in the following order: Objectives, Materials and Methods, Results and Conclusion. Abstracts should not exceed 250 words. Abstracts for case reports should be unstructured and shorter (average 100-150 words; without structural divisions in Turkish and English).

Objectives: The aim of the study should be clearly stated.

Materials and Methods: **The study and standard criteria used should be defined; it should also** be indicated whether the study is randomized or not, whether it is retrospective or prospective, and the statistical methods applied should be indicated, if applicable.

Results: The detailed results of the study should be given and the statistical significance level should be indicated.

Conclusion: Should summarize the results of the study, the clinical applicability of the results should be defined, and the favorable and unfavorable aspects should be declared.

Keywords:

• They should be minimally 3 and maximally 6 and should be written in Turkish and English.

• The words should be separated by semicolon (;) from each other.

• English keywords should be appropriate to "Medical Subject Headings (MESH)" (www.nlm.nih.gov/mesh/MBrowser.html).

• Turkish keywords should be appropriate to "Turkey Science Terms" (www.bilimterimleri.com).

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Introduction: Should consist of a brief explanation of the topic and indicate the objective of the study, supported by information from the literature.

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Results: The results of the study should be stated, with tables/figures given in numerical order; the results should be evaluated according to the statistical analysis methods applied. See General Guidelines for details about the preparation of visual material.

Discussion: The study results should be discussed in terms of their favorable and unfavorable aspects and they should be compared with the literature.

Study Limitations: Limitations of the study should be detailed. In addition, an evaluation of the implications of the obtained findings/results for future research should be outlined.

Conclusion: The conclusion of the study should be highlighted.

Acknowledgements: Any technical or financial support or editorial contributions (statistical analysis, English/Turkish evaluation) towards the study should appear at the end of the article. Only acknowledge persons and institutions who have made substantial contributions to the study, but was not a writer of the paper.

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Case reports should present cases which are rarely seen, feature novelty in diagnosis and treatment, and contribute to our current knowledge. The first page should include the title in Turkish and English, an unstructured summary not exceeding 150 words, and keywords. The main text should consist of introduction, case report, discussion, acknowledgment, conclusion and references. The entire text should not exceed 5 pages (A4, formatted as specified above).

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Review articles can address any aspect of viral hepatitis Review articles must provide critical analyses of contemporary evidence and provide directions of or future research. Most review articles are commissioned, but other review submissions are also welcome. Before sending a review, discussion with the editor is recommended.

Reviews articles analyze topics in depth, independently and objectively. The first chapter should include the title in Turkish and English, an unstructured summary and keywords. Source of all citations should be indicated. The entire text should not exceed 25 pages (A4, formatted as specified above).

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Letters to the Editor should be short commentaries related to current developments in viral hepatitis and their scientific and social aspects, or may be submitted to ask questions or offer further contributions in response to work that has been published in the Viral Hepatitis Journal. Letters do not include a title or an abstract; they should not exceed 1000 words and can have up to 5 references.

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Follow the styles shown in examples below (please give attention to punctuation):

In reference section of the article, there should be no writing in languages other than English. The text language of the article should be indicated in parenthesis at the end of each reference (e.g. Yoldaş O, Bulut A, Altındiş M. The Current Approach of Hepatitis A Infections. Viral Hepatitis J 2012;18:81-86. (Turkish).

Format for journal articles; initials of author's names and surnames, titles of article, journal name, date, volume, number, and inclusive pages, must be indicated.

Example: Tabak F, Ozdemir F, Tabak O, Erer B, Tahan V, Ozaras R. Autoimmune hepatitis induced by the prolonged hepatitis A virus infection. Ann Hepatol. 2008;7:177-179.

Format for books; initials of author's names and surnames, chapter title, editor's name, book title, edition, city, publisher, date and pages.

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Format for on-line-only publications; DOI is the only acceptable on-line reference.

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Explanations about figures, pictures, tables and graphics must be placed at the end of the article.

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Conflict of interest: If any of the writers have a relationship based on self-interest, this should be explained.

Acknowledgment: Only acknowledge persons and institutions who have made substantial contributions to the study, but was not a writer of the paper.

All manuscripts submitted to the Viral Hepatitis Journal are screened for plagiarism using the Crossref Similarity Check powered by "iThenticate" software. Results indicating plagiarism may result in manuscripts being returned or rejected.

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• Title Page

• Article sections

• Turkish and English titles

• Abstract (250 words) (Turkish and English)

• Keywords (minimum 3; maximum 6)

• Article divided into appropriate sections

• Complete and accurate references and citations

• List of references styled according to "journal requirements"

• All figures (with legends) and tables (with titles) cited.

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Research Article

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Demographic Characteristics and Transmission Risk Factors of Patients with Hepatitis C Virus in Turkey: The EPI-C, A Multicenter and Cross-sectional Trial

Türkiye'de Hepatit C Hastalarının Demografik Karakteristikleri ve Bulaşma Risk Faktörleri: Çok Merkezli ve Kesitsel EPI-C Çalışması

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ABSTRACT

Objectives: To describe the prevalence of risk factors in patients infected with hepatitis C virus (HCV).

Materials and Methods: Patients who were aged >18 years visiting outpatient clinics and diagnosed as having HCV infection were enrolled in this cross-sectional, multicenter study conducted in 71 cities. Patient data on socio-demographic and clinical characteristics and pre-defined risk factors were collected.

Results: Among 1,018 patients, 53.0% were women. The mean age was 57.2±14.3 years and 34.8% had been diagnosed as having HCV infection >10 years before enrollment. Almost half of the patients (45.5%) were diagnosed during their regular check-up visits, and only 16.8% were diagnosed because of signs or symptoms of HCV. Genotype 1 and sub-genotype 1 b were detected in 87.9% and 73.7% of the patients, respectively. At least one risk factor was present in 94.8% of the patients. The most frequently reported risk factor was major dental procedures (79.2%), followed by major surgical operations (56.9%) and minor surgical interventions (42.3%).

Conclusion: Our results revealed that most of the patients with HCV infection underwent major dental procedures.

Keywords: Hepatitis C, demography, risk factors, Turkey

ÖZ

Amaç: Bu çalışmanın amacı hepatit C virüsü (HCV) ile enfekte hastalarda risk faktörlerinin prevalansını belirlemektir.

Gereç ve Yöntemler: Poliklinik ziyaretlerinde HCV tanısı almış, 18 yaşından büyük hastalar, 71 farklı ilde yürütülmüş olan bu çok merkezli, kesitsel çalışmaya dahil edilmiştir. Hastaların sosyo-demografik ve klinik karakteristikleri ile önceden belirlenmiş olan risk faktörleri ile ilgili veriler toplanmıştır.

Bulgular: Bu çalışmaya dahil edilen toplam 1.018 hastanın %53,0'ı kadındı. Ortalama yaş 57,2±14,3 yıl idi ve hastaların %34,8'i için HCV enfeksiyonu tanı süresi >10 yıl idi. Hastaların neredeyse yarısı (%45,5) rutin check-up sırasında teşhis edildi ve sadece %16,8'i HCV ile ilişkili bir belirti veya semptom nedeniyle teşhis edildi. Olguların %87,9 ve %73,7'sinde sırasıyla genotip 1 ve alt genotip 1-b tespit edildi. Hastaların %94,8'inde en az bir risk faktörü mevcuttu. En sık bildirilen risk faktörü majör dental işlemler (%79,2) idi, bunu %56,9'luk oranla majör cerrahi operasyonlar ve %42,3'lük oranla minör cerrahi müdahaleler izledi.

Sonuç: Sonuçlarımız, HCV enfeksiyonu olan hastaların çoğunun majör dental işlemler gördüğünü göstermektedir.

Anahtar Kelimeler: Hepatit C, demografi, risk faktörleri, Türkiye

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.

Introduction

Hepatitis C virus (HCV) can cause both acute and chronic hepatitis, ranging in severity from a mild to a serious, lifelong illness (1). According to recent estimates, more than 71 million people around the world have been infected with HCV, and the mortality rate is 350,000 deaths/year. Despite the high prevalence of the disease, most people infected with this virus are unaware of their infection (2).

Risk factors may contribute to the prevalence of HCV in different proportions within countries because they vary according to each society's cultural values and lifestyles, as well as geographic and demographic differences (3). Thus, risk factors for HCV infection should be defined according to the current demographic characteristics and prioritized according to national characteristics. Even though HCV-associated risk factors were previously investigated in Turkey, most of these studies were single-center studies and might not reflect national data (4,5). A multicenter study was conducted in Turkey; however, this retrospective study evaluated sustained virologic response rates achieved by dual therapy in treatment-naïve patients with HCV and did not evaluate HCV-related risk factors (6). Here, we describe the prevalence of risk factors in patients infected with HCV across Turkey.

Challenges arose stemming from the absence of national epidemiologic studies on HCV in Turkey and the lack of robust data on the regional distribution of the disease based on the geographic definitions within the country. This study was planned to contribute data regarding the regional distribution of HCV infection and provide additional data to the current national database for patients infected with HCV and the joint activities of societies and associations linked to liver diseases in Turkey because there has been no similar study performed in Turkey.

Materials and Methods

Study Subjects and Clinical Protocol

The study was initiated on May 2nd, 2017, after obtaining Ethics Committee İstanbul University-Cerrahpaşa, Cerrahpaşa Faculty of Medicine approval in conformation with the Declaration of Helsinki and conducted between June 5th and December 28th, 2017 (approval number: D-02, date: 12.07.2016). Infectious disease speciality and gastroenterology sites were selected based on their patient population and their ability to appropriately conduct the study. Patients who were aged 18 years or older at the time of enrollment, diagnosed as having HCV through anti-HCV and HCV-RNA-positive test results, and attending routine visits to their healthcare providers were enrolled. All patients signed an informed consent form before their inclusion in the study. The patients' socio-demographic status, HCV signs and symptoms, laboratory findings (anti-HCV antibody positivity, HCV-RNA level, and HCV genotype), duration of HCV infection, HCV risk factors (e.g., history of surgical operations, organ transplantation, blood transfusion) and comorbidities [e.g., human immunodeficiency virus (HIV), hepatitis B virus (HBV), diabetes] were recorded. The investigators chose HCV risk factors from the definition of populations with high HCV prevalence, which was provided by the World Health Organization (WHO) [April 2014 version of the current guideline (1)], and classified these factors according to the local population characteristics. Interventions such as angiography, piercing, tattooing, and circumcision were defined as minor surgical operations (4), and other surgical interventions such as intra-abdominal and intrathoracic surgery were considered as major surgical operations (7). Tooth extractions, implants, endodontic

surgery (e.g. root canal surgery) and periodontal therapy were considered as major dental procedures (8). The primary endpoint of the study was the prevalence of specified risk factors present in patients with HCV.

Study Design

This cross-sectional, multicenter study was performed in a single visit in different geographic regions of Turkey to describe characteristics of an HCV-infected population and to evaluate the prevalence of risk factors. Each site enrolled patients consecutively until the number of patients allocated to that site was reached. Among the 81 cities located in Turkey, at least one patient was enrolled from 71 cities. Patients who attended routine clinic visits were consecutively included in the study.

Statistical Analysis

The sample size calculation was based on the frequency of patient-reported risk factors in patients with HCV. Considering the frequency of dental interventions at 68% (5), with a precision of 3% and 95% confidence interval, at least 929 patients needed to be included in the study, thus the inclusion of 1,000 patients was deemed appropriate.

Data from all clinical assessments were summarized using mean, standard deviation (SD), and percentages. For comparison of categorical data for two or more groups, a chi-square test was used. The normality assumption was tested for all continuous variables using the Kolmogorov-Smirnov test. Continuous variables were analyzed using Student's t-test or the Mann-Whitney U test in condition normal/abnormal distribution. Missing values were not interpolated, and no sensitivity analysis was planned. All analyses were performed using the SPSS for Windows, Version 16.0. Chicago, SPSS Inc.

Results

A total of 1018 patients were enrolled in the study. Female patients constituted 53.0% of the study population (n=540). The patients' age ranged between 18 to 91 years, and the mean age (± SD) at inclusion was 57.2±14.3 years. Most of the patients were living with their parents or family members (58.3%); 10.2% were living alone. Almost half of the study population were primary school graduates (Table 1).

Almost one-third of the patients [n=354 (34.8%)] had received an HCV diagnosis ≥10 years before the date of data collection, and 20.7% (n=211) were diagnosed from 5 to 10 years before that date. Almost half of the patients [n=474 (45.5%)] were diagnosed during a regular check-up, and only 16.8% (n=175) were diagnosed during a visit due to signs and/or symptoms of HCV. Among those, the most frequently observed signs and symptoms were clinical symptoms, such as fever, fatigue, and decreased appetite [n=104 (59.4%)], and extra-hepatic findings [n=53 (30.3%)].

All patients enrolled in the study were HCV antibody-positive and HCV-RNA-positive. Genotype 1 had a high distribution ratio [n=895 (87.9%)], and HCV subtype 1-b was the most frequent subtype in the study population [n=750 (73.7%); Table 2].

The most frequently reported comorbidities were diabetes mellitus [n=195 (19.2%)] and chronic renal failure requiring dialysis [n=95 (9.3%)]. Other concomitant diseases included chronic obstructive pulmonary disease and HBV (Table 1).

Table 1. Socio-demographic data

Patient demographics	**n=1018**
Sex	
Female	540 (53.0)
Male	478 (47.0)
Age†, years	
Mean ± SD	57.2±14.3
Min.-max.	18-91
Patient residence	
City/town	829 (81.4)
Village/rural area	186 (18.3)
Closed community areas (incarcerated, hospices, military units)	2 (0.2)
Other	1 (0.1)
Living situation	
With parents/family members	593 (58.3)
With spouse/partner (with or without children)	315 (30.9)
Alone	104 (10.2)
With others (e.g. dormitory, retirement home, jails)	6 (0.6)
Education levels	
Higher education, >18 years education	23 (2.3)
University, 14-18 years education	94 (9.2)
Secondary school, 6-13 years education	298 (29.3)
Primary school, 1-5 years education	448 (44.0)
No formal education	151 (14.8)
Unknown	4 (0.4)
Primary occupation	
Student	13 (1.3)
Employed/self-employed	212 (20.8)
Retired	337 (33.1)
Unemployed; not working for pay	456 (44.8)
Family income, monthly	
No income	111 (10.9)
<1500 TL	327 (32.1)
1500-3000 TL	387 (38.0)
>3000 TL	142 (13.9)
No answer	51 (5.0)
Comorbidity	
HIV	2 (0.2)
Hepatitis B infection	47 (4.6)
Chronic renal failure requiring dialysis	95 (9.3)
Diabetes	195 (19.2)
Disorder requiring immunomodulatory treatment	32 (3.1)
Blood disorder requiring regular blood transfusion	27 (2.7)
Chronic obstructive pulmonary disease	72 (7.1)

Data are n (%) unless otherwise stated. †: Age at the date of signing informed consent. SD: Standard deviation, Min.: Minimum, max.: Maximum, HIV: Human immunodeficiency virus

At least one risk factor was present in 94.8% of the patients. Although more than half of the patients [n=579 (56.9%)] had a major surgical operation as an HCV risk factor, only 4.2% (n=43) had undergone organ transplantation. Additionally, minor surgical operations were reported in 42.3% (n=431) of the study population, and 37.2% (n=379) received a blood transfusion or blood products (Figure 1).

The secondary endpoint of the study was to determine the frequency of pre-specified risk factors according to the age and sex of the patients and duration of infection. The majority of the patients [n=741 (72.8%)] were aged 35 to 69 years. Within this group, most of the patients underwent a major dental procedure (81.9%) or had a major surgical intervention (58.3%). IV/intranasal illicit drug abuse was detected as a significant risk factor in patients aged 18 to 34 years [n=44 (45.4%)], higher than in the other age groups ($p<0.05$). Risk factors such as organ transplant, receiving a blood transfusion or blood products, having a sexual partner with HBV, HCV, or HIV were more frequent in the 35 to 69 years' age group ($p<0.05$). For transmission of HCV, major surgical operations and major dental procedures were determined as significant risk factors for patients aged >70 years ($p<0.05$; Table 3).

Risk factors such as major surgical/dental procedures and a sexual partner with HBV were more common in female patients, whereas minor surgical interventions, organ transplant, sharing personal hygiene equipment (e.g. toothbrush, razor), intravenous/intranasal illicit drug use, and having multiple sexual partners were more frequently seen in male patients ($p<0.05$). No sex difference was detected for risk factors such as receiving a blood transfusion or blood products, encountering any blood or bodily fluids, sexual partner with HCV, and sexual partner with HIV ($p>0.05$; Table 4).

The duration of infection was <5 years for the majority of the patients. Major surgical operations, organ transplant,

Table 2. Genotype and sub-genotype distributions of the patients

	Patients, n (%)
Genotype	
1	895 (87.9)
2	19 (1.9)
3	36 (3.5)
4	13 (1.3)
5	1 (0.1)
Unknown	54 (5.3)
Sub-genotype	
1-b	750 (73.7)
1-a	79 (7.8)
1-unknown	64 (6.3)
1-c	1 (0.1)
1-d	1 (0.1)
3-unknown	18 (1.8)
3-a	17 (1.7)
3-b	1 (0.1)
2-unknown	12 (1.2)
2-a	5 (0.5)
2-b	2 (0.2)
4-unknown	9 (0.9)
4-a	2 (0.2)
4-c	1 (0.1)
4-d	1 (0.1)
5-x	1 (0.1)

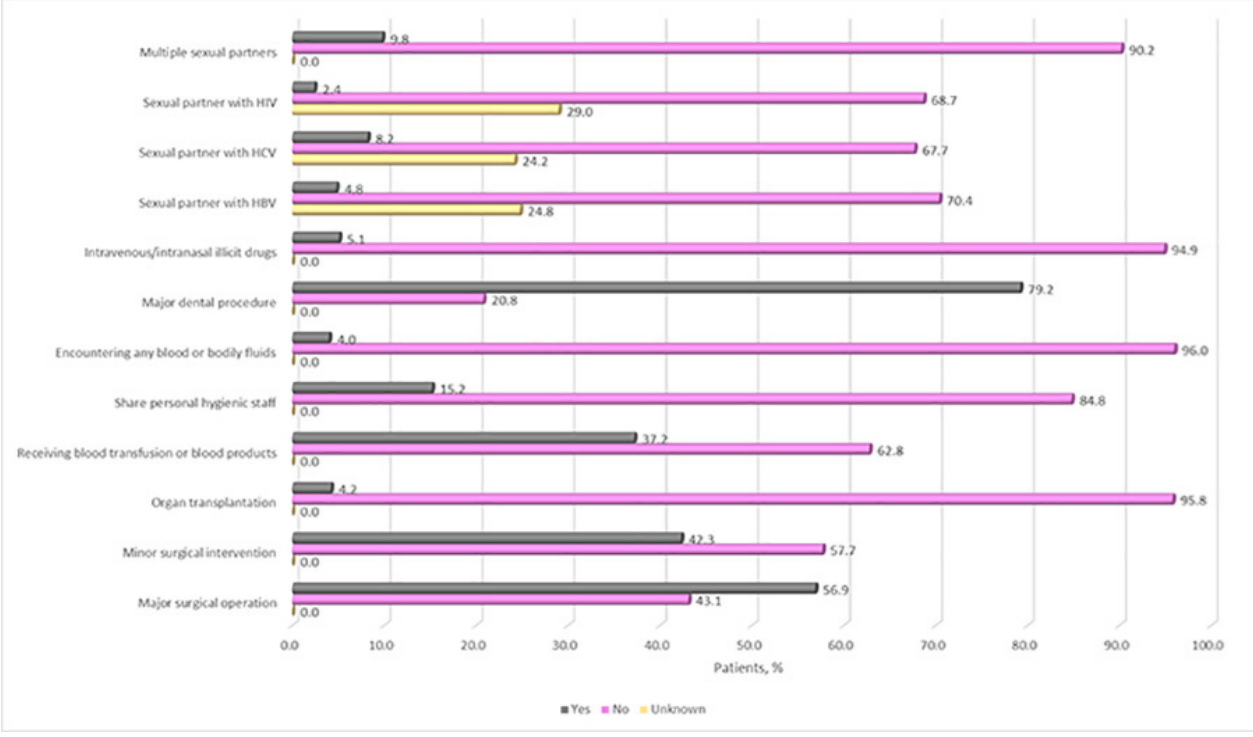

Figure 1. Pre-determined risk factors and distribution of their prevalence
HIV: Human immunodeficiency virus, HCV: Hepatitis C virus, HBV: Hepatitis B virus

Table 3. HCV risk factor by age group

	All patients (n=1018)			Age 18-34 years (n=97)			Age 35-69 years (n=741)			Age >70 years (n=180)			p††
Risk Factor, n (%)	**Yes**	**No**	**UNK**	**Yes**	**No**	**UNK**	**Yes**	**No**	**UNK**	**Yes**	**No**	**UNK**	
Major surgical operation	579 (56.9)	439 (43.12)	-	38 (39.2)	59 (60.8)	-	432 (58.3)	309 (41.7)	-	109 (60.6)	71 (39.4)	-	**0.001**
Minor surgical intervention†	431 (42.3)	587 (57.7)	-	49 (50.5)	48 (49.5)	-	308 (41.6)	433 (58.4)	-	74 (41.1)	106 (58.9)	-	0.229
Organ transplant	43 (4.2)	975 (95.8)	-	-	97 (100.0)	-	41 (5.5)	700 (94.5)	-	2 (1.1)	178 (98.9)	-	**0.003**
Receiving a blood transfusion or blood products	379 (37.2)	639 (62.8)	-	16 (16.5)	81 (83.5)	-	296 (39.9)	445 (60.1)	-	67 (37.2)	113 (62.8)	-	**<0.001**
Share personal hygiene equipment (e.g. toothbrush, razor)	155 (15.2)	863 (84.8)	-	20 (20.6)	77 (79.4)	-	108 (14.6)	633 (85.4)	-	27 (15.0)	153 (85.0)	-	0.296
Encountering any blood or bodily fluids	41 (4.0)	977 (96.0)	-	4 (4.1)	93 (95.9)	-	32 (4.3)	709 (95.7)	-	5 (2.8)	175 (97.2)	-	0.640
Major dental procedure†	806 (79.2)	212 (20.8)	-	45 (46.4)	52 (53.6)	-	607 (81.9)	134 (18.1)	-	154 (85.6)	26 (14.4)	-	**<0.001**
Intravenous/ intranasal illicit drugs	52 (5.1)	966 (94.9)	-	44 (45.4)	53 (54.6)	-	8 (1.1)	733 (98.9)	-	-	180 (100.0)	-	**<0.001**
Sexual partner with HBV	49 (4.8)	717 (70.4)	252 (24.8)	-	53 (54.6)	44 (45.4)	42 (5.7)	537 (72.5)	162 (21.9)	7 (3.9)	127 (70.6)	46 (25.6)	**<0.001**
Sexual partner with HCV	83 (8.2)	689 (67.7)	246 (24.2)	4 (4.1)	50 (51.5)	43 (44.3)	66 (8.9)	519 (70.0)	156 (21.1)	13 (7.2)	120 (66.7)	47 (26.1)	**<0.001**
Sexual partner with HIV	24 (2.4)	699 (68.7)	295 (29.0)	-	53 (29.4)	44 (45.4)	20 (2.7)	523 (70.6)	198 (26.7)	4 (2.2)	123 (68.3)	53 (29.4)	**0.003**

HBV: Hepatitis B virus, HCV: Hepatitis C virus, HIV: Human immunodeficiency virus, UNK: Unknown,†: Some patients experienced >1 event; ††: chi-square test

Table 4. Risk factors for HCV by sex

Risk factor, n (%)	All patients (n=1018)			Female patients (n=540)			Male patients (n=478)			p††
	Yes	No	UNK	Yes	No	UNK	Yes	No	UNK	
Major surgical procedure	579 (56.9)	439 (43.1)	-	324 (60.0)	216 (40.0)	-	255 (53.3)	223 (46.7)	-	**0.036**
Minor surgical intervention†	431 (42.3)	587 (57.7)	-	196 (36.3)	344 (63.7)	-	235 (49.2)	243 (50.8)	-	**<0.001**
Organ transplant	43 (4.2)	975 (95.8)	-	14 (2.6)	526 (97.4)	-	29 (6.1)	449 (93.9)	-	**0.007**
Receiving a blood transfusion or blood products	379 (37.2)	639 (62.8)	-	203 (37.6)	337 (62.4)	-	176 (36.8)	302 (63.2)	-	0.846
Share personal hygiene equipment (e.g. toothbrush, razor)	155 (15.2)	863 (84.8)	-	70 (13.0)	470 (87.0)	-	85 (17.8)	393 (82.2)	-	**0.036**
Encountering any blood or bodily fluids	41 (4.0)	977 (96.0)	-	27 (5.0)	513 (95.0)	-	14 (2.9)	464 (97.1)	-	0.110
Major dental procedure†	806 (79.2)	212 (20.8)	-	443 (82.0)	97 (18.0)	-	363 (75.9)	115 (24.1)	-	**0.020**
Intravenous/intranasal illicit drugs	52 (5.1)	966 (94.9)	-	8 (1.5)	532 (98.5)	-	44 (9.2)	434 (90.8)	-	**<0.001**
Sexual partner with HBV	49 (4.8)	717 (70.4)	252 (24.8)	34 (6.3)	390 (72.2)	116 (21.5)	15 (3.1)	327 (68.4)	136 (28.5)	**0.005**
Sexual partner with HCV	83 (8.2)	689 (67.7)	246 (24.2)	46 (8.5)	377 (69.8)	117 (21.7)	37 (7.7)	312 (65.3)	129 (27.0)	0.140
Sexual partner with HIV	24 (2.4)	699 (68.7)	295 (29.0)	14 (2.6)	386 (71.5)	140 (25.9)	10 (2.1)	313 (65.5)	155 (32.4)	0.071
Multiple sexual partners	100 (9.8)	918 (90.2)	-	11 (2.0)	529 (98.0)	-	89 (18.6)	529 (98.0)	-	**<0.001**

HBV: Hepatitis B virus, HCV: Hepatitis C virus, HIV: Human immunodeficiency virus, UNK: Unknown, †: Some patients experienced more >1 event; ††: Chi-square test.

receiving a blood transfusion or blood products, encountering blood or bodily fluids, major dental procedures, and having a sexual partner with HBV, HCV, or HIV were risk factors more frequently detected in the patient group with disease duration of >10 years ($p<0.05$). The use of intravenous/intranasal illicit drugs and having multiple sex partners were more frequent in patients with a shorter duration of disease (<5 years; $p<0.05$, Table 5).

Discussion

This study was designed to explore the prevalence of pre-determined risk factors of HCV transmission. For the determination of risk factors, the guideline for HCV screening published by the WHO (1) was taken into consideration because population groups with high HCV prevalence were defined in this guideline. According to the guideline, persons who had undergone interventions such as dental and surgical procedures, blood transfusions, piercing/tattooing or persons who inject drugs were in the high-risk group. Furthermore, the guideline mentioned that these risk factors might vary substantially depending on the geographic region; therefore, recommended risk factors were adopted according to the local setting. As a result, having a surgical procedure (major/minor), organ transplantation, blood transfusion, major dental procedures, sharing personal hygienic equipment (e.g. toothbrush, razor) at home, encountering blood/body fluids, using IV/intranasal illicit drugs, having a sexual partner with HCV/HCV/HIV, and having multiple sexual partners were selected as pre-determined risk factors in this study.

In our study, patients who were HCV-positive had experienced a major surgical operation in 6 of 10 cases, whereas in Poland and Italy, this rate was 38.2% and 35.6%, respectively (9). Major dental procedures, which constitute a possible route of HCV transmission, were experienced by 79.2% of patients in our study, which was similar to the frequency reported in Poland (79.4%). A high percentage of patients (85.7%) who live in Hatay Province, Turkey, reported major dental procedures as a risk factor, but Italian patients experienced dental procedures less frequently (24.5%) (10,11). The proportion of patients who experienced a dental and/or surgical procedure was even lower in Greece (10.1%) (12). One possible reason for the higher proportion of Turkish patients who had surgical and/or dental procedures before receiving an HCV diagnosis compared with other European countries may be attributed to the differences between health systems and policies, economic situations, and the awareness of infectious diseases. On the other hand, age is a confounding factor for major dental procedures because many oral diseases and conditions are associated with aging. A study conducted in Turkey showed that dental health worsened with increased age (13); therefore, the history of a major dental intervention cannot be considered as a risk factor alone. In Europe, the risk of HCV transmission via medical procedures is lower than the risk of other factors (14), which could be attributed to the greater level of awareness of the medical staff in European countries compared with Turkey.

Table 5. Risk factors for HCV by duration of infection

	Total Duration of HCV Infection												
	All patients (n=1018)			<5 years (n=453)			5-10 years (n=211)			>10 years (n=354)			p††
Risk Factor, n (%)	Yes	No	UNK	Yes	No	UNK	Yes	No	UNK	Yes	No	UNK	
Major surgical operation	579 (56.9)	439 (43.1)	-	230 (50.8)	223 (49.2)	-	114 (54.0)	97 (46.0)	-	235 (66.4)	119 (33.6)	-	**<0.001**
Minor surgical intervention†	431 (42.3)	587 (57.7)	-	265 (58.5)	188 (41.5)	-	125 (59.2)	86 (40.8)	-	157 (44.4)	197 (55.6)	-	0.627
Organ transplant	43 (4.2)	975 (95.8)	-	4 (0.9)	449 (99.1)	-	13 (6.2)	198 (93.8)	-	26 (7.3)	328 (92.7)	-	**<0.001**
Receiving blood transfusion or blood products	379 (37.2)	639 (62.8)	-	126 (27.8)	327 (72.2)	-	76 (36.0)	135 (64.0)		177 (50.0)	177 (50.0)	-	**<0.001**
Share personal hygienic staff (such as toothbrush, razor, etc.)	155 (15.2)	863 (84.8)	-	74 (16.3)	379 (83.7)	-	33 (15.6)	178 (84.4)	-	48 (13.6)	306 (86.4)	-	0.543
Encountering any blood or bodily fluids	41 (4.0)	977 (96.0)	-	16 (3.5)	437 (96.5)	-	4 (1.9)	207 (98.1)	-	21 (5.9)	333 (94.1)	-	**0.048**
Major dental procedure†	806 (79.2)	212 (20.8)	-	119 (26.3)	334 (73.7)	-	39 (18.5)	172 (81.5)		300 (84.7)	54 (15.3)		**<0.001**
Intravenous/ intranasal illicit drugs	52 (5.1)	966 (94.9)	-	47 (10.4)	406 (89.6)	-	2 (0.9)	209 (99.1)	-	3 (0.8)	351 (99.2)	-	**<0.001**
Sexual partner with HBV	49 (4.8)	717 (70.4)	252 (24.8)	16 (3.5)	293 (64.7)	144 (31.8)	10 (4.7)	153 (72.5)	48 (22.7)	23 (6.5)	271 (76.6)	60 (16.9)	**<0.001**
Sexual partner with HCV	83 (8.2)	689 (67.7)	246 (24.2)	25 (5.5)	290 (64.0)	138 (30.5)	18 (8.5)	144 (68.2)	49 (23.2)	40 (11.3)	255 (72.0)	59 (16.7)	**<0.001**
Sexual partner with HIV	24 (2.4)	699 (68.7)	295 (29.0)	9 (2.0)	290 (64.0)	154 (34.0)	5 (2.4)	147 (69.7)	59 (28.0)	10 (2.8)	262 (74.0)	82 (23.2)	**0.020**
Multiple sexual partners	100 (9.8)	918 (90.2)	-	56 (12.4)	397 (87.6)	-	19 (9.0)	192 (91.0)	-	25 (7.1)	329 (92.9)	-	**0.039**

HBV: Hepatitis B virus, HCV: Hepatitis C virus, HIV: Human immunodeficiency virus, UNK: Unknown, †Some patients experienced more >1 event; ††: chi-square test

An epidemiologic study conducted in Greece found that blood transfusion was the most common method of HCV transmission (14). In the study by Savvas et al. (15) in 2005, 38.5% of patients with HCV had a history of blood transfusion; the percentage in our study was similar (37.2%).

Although HCV can be sexually transmitted, its risk is lower than the risk of other sexually transmitted diseases, such as HBV and HIV. This risk can be decreased from 1% to 0.6% or less per year through monogamous relationships. Although the exact prevalence of sexually transmitted HCV between men and women is not known (16), HCV transmission is more frequent in men who have sex with men, especially those who are HIV-positive (17,18). In this study, 9.8% of patients (8.7% male) had multiple sexual partners, and 8.2% deliberately ensured that their sexual partner was not HCV positive. The rate of having HCV-, HBV-, or HIV-positive sexual partners was lower among younger patients than in older patients. Six male patients (0.6%) reported having a homosexual relationship.

It has been reported that, worldwide, HCV is transmitted primarily among people who inject drugs (17,19,20), and Gigi et al. (21) reported that 11% of HCV-positive patients were IV drug users. Additionally, Raptopoulou et al. (12), in their study of patients with HCV IV, reported that drug users were most frequently male. In our national, observational study, IV/intranasal illicit drug abuse was observed in 5.1% of the study population. Although the prevalence of IV drug abuse in Turkey was lower than in other European countries, it was reported both in the report of European Monitoring Centre for Drugs and Drug Addiction and the Turkish National Drug Report of the Turkish National Police Counter Narcotics Department that IV drug abuse had significantly increased in Turkey (22,23). IV/intranasal illicit drug use was observed mostly among young people compared with other age groups and among male patients compared with female patients in this study. Therefore, to properly define and treat adequately, following-up is especially important in this group of patients.

Patients with chronic HCV infection usually need to change their lifestyle to improve their quality of life. Providing educational programs and counselling services tailored for patients with HCV, especially for those with lower levels of education, may play an important role in the patients changing their lifestyles (24). The impact of HCV on lifestyle was investigated in a study conducted in Italy and the results revealed that 29.5% of the patients were living alone and the majority of the patients (53.3%) attended primary school only (24). A similar Korean study in patients with HCV reported that 23.2% of the patients lived alone and most patients (82.4%) had not received secondary school education (25). In our study, a lower percentage of patients lived alone when compared with the studies conducted in Italy (10.2% vs 29.5%) and Korea (10.2% vs 23.2%). In terms of education levels, we consider our study results to be similar to the results obtained in Italy and Korea.

In a multicenter retrospective study of 1,214 Turkish patients with HCV, 947 were genetically identified, and among those, genotype 1 was the most frequently observed (6). In line with this finding, in our study, the prevalent genotype of the HCV infection was genotype 1-b, which is similar to global study outcomes published elsewhere (10). Correlatively, genotype 1 was one of the most commonly observed genotypes in European countries (9). According to the results of a study conducted in Italy, the predominant genotype was genotype 1 (63.6%), followed by genotype 2 (29.4%). Specifically, genotype 1-b (50.7%) was the most frequent subtype among those with genotype 1 in the Italian study population (9). Even though the results of the Italian study revealed a sex difference in genotype 1-b (females: 56.4%, males 44.7%) and genotype 3 (males: 9.7%, females:2.9%), we found no sex difference in our study population.

The most frequent comorbidity was diabetes, which is strongly associated with HCV (17). According to the 2016 WHO guidelines, the rates of co-infection of HCV with HIV are high owing to the similarity of their transmission routes, but only two patients were HIV-positive in this study. Additionally, 4.6% of patients had HBV/HCV co-infection, although this co-infection is commonly observed in other regions such as Asia, sub-Saharan Africa, and South Africa, which are HBV-endemic areas (17).

Study Limitations

One limitation of this study is that, because of the stigmatizing nature of some risk factors, some patients may not have been open to admitting these risk factors. Another limitation may be that, because patients were followed up in special care centers, IV/intranasal illicit drug users were not included in this study. Patient-reported medical history was the only source of information (except HCV-RNA and anti-HCV test results) because this was a non-interventional, exploratory study, The uncertainty of the patients' initial HCV diagnosis dates could be another limitation.

Conclusion

Even though the most frequently reported risk factor was major dental procedures, it is not possible to consider the history of major dental procedures as a risk factor alone because age is a confounding factor and studies have shown that dental health worsens with increased age. Therefore, additional research is warranted to understand if this is a unique risk factor for HCV infection. Increasing awareness of viral hepatitis may help to reduce the prevalence of HCV because transmission of HCV through all pre-determined risk factors is preventable.

Ethics

Ethics Committee Approval: The study was initiated on May 2nd, 2017, after obtaining Ethics Committee İstanbul University-Cerrahpaşa, Cerrahpaşa Faculty of Medicine approval in conformation with the Declaration of Helsinki and conducted between June 5th and December 28th, 2017 (approval number: D-02, date: 12.07.2016).

Informed Consent: All patients signed an informed consent form before their inclusion in the study.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

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Research Article

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Distribution of Hepatitis C Virus Genotypes: 18-Year Experience in an Academic Center

Hepatit C Virüs Genotip Dağılımı: Akademik Bir Merkez, 18 Yıllık Deneyim

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ABSTRACT

Objectives: Hepatitis C virus (HCV) is an important public health problem worldwide. This study examined long-term changes in distribution of HCV genotypes s in HCV-RNA-positive patients in a large population.

Materials and Methods: Following HCV genotype and subtype sequence analysis, the sequence and the reference sequences were compared with the "line probe assay" method or the multiplex amplification methods of 5'UTR and NS5B or only NS5B.

Results: In the study, HCV-RNA positive 670 patients undergoing genotyping were included. Genotype 1 was detected in 603 patients (90.0%), genotype 3 in 45 (6.7%), genotype 2 in 12 (1.8%), genotype 4 in 6 (0.9%), combined genotypes 1 and 2 in 2 (0.3%), and genotypes 1 and 4 in 2 (0.3%). Genotypes 5, 6, 7 and 8 were not observed in this study. The most dominant subtypes by years were genotype 1b (82.8%) and genotype 3a (4.5%). Genotype 1b was detected in 63.2% of patients under <50 years of age and in 89.7% of those ≥50 years of age (p<0.001), while genotype 3 was determined in 2.0% of patients aged ≥50 years of age and in 20.1% of those <50 years of age (p<0.001).

Conclusion: This study revealed that changes occurred in the general distribution of HCV genotype and subtypes by years, and that HCV genotype 1b was seen at the highest rate, especially in patients over 50 years old.

Keywords: Genotype, hepatitis C virus, subtype

ÖZ

Amaç: Hepatit C virüsü (HCV) kronik hepatit, siroz, hepatosellüler karsinom gibi hastalıklara yol açması nedeniyle önemli bir halk sağlığı sorunudur. Bu çalışmada, geniş bir popülasyondaki HCV-RNA pozitif hastalarda, HCV genotip dağılımında uzun bir dönemdeki değişimin incelenmesi amaçlandı.

Gereç ve Yöntemler: HCV genotip ve subtipleri dizi analizi sonrasında dizinin referans dizilerle karşılaştırılması; "line probe assay" yöntemiyle veya 5'UTR ve NS5B'nin veya sadece NS5B'nin multipleks amplifikasyonu yöntemlerinden biriyle gerçekleştirildi.

Bulgular: Çalışmada HCV-RNA pozitif olup genotiplendirme yapılan toplam 670 hasta yer aldı. Hastaların 603'ünde (%90,0) genotip 1, 45'inde (%6,7) genotip 3, 12'sinde (%1,8) genotip 2 ve 6'sında (%0,9) genotip 4, 2'sinde (%0,3) genotip 1 ve 3 ve yine 2'sinde (%0,3) genotip 1 ve 4 birlikteliği tespit edildi. Çalışmada genotip 5, 6, 7 ve 8'e rastlanmadı. Yıllara göre en baskın subtip genotip 1b (%82,8) idi. İkinci en sık saptanan subtip ise genotip 3a (%4,5) idi. Genotip 1b <50 yaş olan hastalarda %63,2 ve ≥50 yaş olan hastalarda %89,7 oranında (p<0,001) saptanırken, ≥50 yaş olan hastalarda genotip 3 %2,0 ve <50 yaş olan hastalarda ise %20,1 oranında saptandı (p<0,001).

Sonuç: Bu çalışmada HCV genotip 1b'nin en yüksek oranda, özellikle de 50 yaş üzerindekilerde görüldüğü ortaya konmuştur.

Anahtar Kelimeler: Genotip, hepatit C virüs, subtip

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Introduction

The hepatitis C virus (HCV), a member of the genus hepacivirus from the family flaviviridae, infects an estimated 130-200 million individuals worldwide (1,2). According to World Health Organization figures for 2018, 71.1 million individuals worldwide are infected with HCV, and approximately 475,000 die from the infection every year (3). Since the prevalence of HCV varies among different regions, countries are grouped in terms of the incidence of HCV infection. Eighty percent of HCV infections are seen in 31 countries. Six countries in particular (China, Pakistan, Nigeria, Egypt, India, and Russia) are host to 50% of all cases (4). HCV is also an important public health problem with a high probability of chronicization and still with no effective vaccine, that leads to severe liver diseases such as hepatocellular carcinoma and cirrhosis (2,5).

There are eight confirmed HCV genotypes and 86 subtypes to date (4). The distribution of HCV genotypes and subtypes exhibits geographic variations. Genotype 1 is responsible for 44% of all HCV infections worldwide, and for 60% of infections in high and middle-income countries (4). Globally, the leading genotypes are 1a, 1b, 2a, 2b, and 3a. Approximately one in three genotype 1 infections are seen in East Asia. Genotype 3 infections are more widespread in lower-middle-income countries than in high-income, upper-middle-income, and lower-income countries and constitute 25% of all HCV infections (4). Approximately 75% of HCV genotype 3 infections are seen in South Asia. Genotype 4 has been detected more widely in Central Africa and the Middle East, and genotypes 2 and 6 in East Asia (1,4). Genotype 5, 7, and 8 represent less than 1% of all HCV infections, with several cases emerging from southern and central sub-Saharan Africa (1,4). HCV genotypes have been shown to vary in terms of disease severity, prognosis, and response to antiviral drugs (4,6). Therefore, HCV genotyping is an important component of pre-treatment diagnostic algorithms, especially as it guides the therapeutic regimen process (7). Knowing the genotypes is the most important factor determining the selection of an effective antiviral agent, the length of treatment, and the expected virological response (6,8). A knowledge of regional HCV genotype distributions is therefore essential for the development of international and domestic HCV infection management strategies.

The purpose of this study was to examine the HCV genotype distribution in the previous 18 years among HCV-RNA-positive patients in a broad population.

Material and Methods

Research Type and Study Group

HCV genotype results from HCV-RNA-positive blood specimens studied at the Karadeniz Technical University Clinical Microbiology Laboratory, Turkey, between 2002 and 2019 were evaluated retrospectively. Patients' demographic data were retrieved from the hospital information system.

Specimens from the patients included in the study were investigated in the academic clinical microbiology laboratory of a 960-bed tertiary university hospital in the Eastern Black Sea region of Turkey. The study population consisted of patients infected with HCV, the great majority living in the region (approximate population 2.9 million individuals per year). Six hundred seventy patients were enrolled in the study, the first specimen being evaluated in case of repeated specimens.

The study was approved by Karadeniz Technical University Faculty of Medicine Clinical Research Ethical Committee (approval number: 2020/169).

HCV-RNA Quantitation

The HCV-RNA load in specimens was determined using bDNA (Branched DNA, HCV 3.0 bDNA assay, Bayer Diagnostics, USA) or one of various real time PCR applications (COBAS® AmpliPrep/COBAS®TaqMan® HCV test, Roche Diagnostics Corporation, USA, Abbott RealTime HCV Assay, Abbott Molecular Inc., USA, and Bosphore® HCV Quantification Kit, Anatolia Geneworks, Turkey).

HCV Genotyping Procedure

Following HCV genotype and subtype "5'untranslated region (5'UTR)" or "non-structural 5B" (NS5B) amplification and sequence analysis, the comparison of the sequence with reference sequences were studied with either using the "line probe assay" method (INNO-LiPA HCV II, Innogenetics, Belgium) or two different commercial Real-Time PCR kits, by the method of multiplex amplification of the 5'UTR and NS5B (Abbott RealTime HCV Genotype II Assay, Abbott Molecular Inc., USA) or only NS5B (HCV Genotyping Kit v1 Bosphore, Geneworks Anatolia, Turkey).

Statistical Analysis

Statistical analysis was performed on SPSS version 21 software (SPSS Inc., Chicago, IL, USA). Non-parametric data not conforming to normal distribution at the Kolmogorov-Smirnov test were compared using the Mann-Whitney U test. The chi-square test and Fisher's exact test were employed in the comparison of categorical variables. P-values <0.05 were regarded as statistically significant.

Results

Three hundred fifty-nine (59.6%) of the 670 patients in the study were men and 311 (46.4%) were women. The patients' mean age was 58.27±16.53 years (minimum-maximum: 4-112). Genotype distributions by gender are shown in Table 1. No significant difference in genotype distribution was observed between the genders (p=0.461).

Table 1. Distributions of HCV genotypes by gender

	HCV genotypes n (%)									
	1			2		3		4*	Mixed	Total n (%)
Gender	1*	1a	1b	2*	2b	3*	3a			
Female	13 (4.2)	9 (2.9)	261 (83.9)	6 (1.9)	-	6 (1.9)	11 (3.5)	3 (1.0)	2 (0.6)	311 (46.4)
Male	10 (2.8)	16 (4.5)	294 (81.9)	5 (1.4)	1 (0.3)	9 (2.5)	19 (5.3)	3 (0.8)	2 (0.6)	359 (53.6)

*Subtyping could not be performed. HCV: Hepatitis C virus

Table 2. Distribution of HCV genotypes by years

	HCV Genotypes n (%)									
	1			2		3		4*	Mixed	Total (n)
Year	1*	1a	1b	2*	2b	3*	3a			
2002	-	-	5 (100.0)	-	-	-	-	-	-	5
2004	-	-	10 (90.9)	-	-	-	1 (9. 1)	-	-	11
2005	-	1 (16.7)	4 (66.7)	-	-	-	1 (16.7)	-	-	6
2006	-	-	4 (100.0)	-	-	-	-	-	-	4
2007	-	1 (50.0)	1 (50.0)	-	-	-	-	-	-	2
2008	-	-	5 (100.0)	-	-	-	-	-	-	5
2009	-	2 (3.9)	49 (96.1)	-	-	-	-	-	-	51
2010	5 (5.8)	1 (1.2)	72 (82.8)	1 (1.2)	-	3 (3.5)	4 (4.6)	-	1 (1.2) (genotip 1b+3a)	87
2011	3 (4.9)	1 (1.6)	55 (90.2)	2 (3.3)	-	-	-	-	-	61
2012	2 (3.2)	2 (3.2)	50 (79.4)	2 (3.2)	-	2 (3.2)	2 (3.2)	2 (3.2)	1 (1.6) (genotip 1b+4)	63
2013	-	4 (5.4)	63 (85.1)	1 (1.4)	-	2 (2.7)	2 (2.7)	1 (1.4)	1 (1.4) (genotip 1b+4)	74
2014	2 (4.4)	2 (4.4)	38 (84.4)	-	-	1 (2.2)	1 (2.2)	1 (2.2)	-	45
2015	-	3 (11.1)	23 (85.2)	-	-	1 (3.7)	-	-	-	27
2016	8 (11.9)	2 (3.0)	52 (77.6)	1 (1.5)	-	4 (6.0)	-	-	-	67
2017	1 (1.9)	-	48 (90.6)	1 (1.9)	-	-	2 (3.8)	-	1 (1.9) (genotip 1b+3a)	53
2018	1 (1.8)	3 (5.3)	43 (75.4)	1 (1.8)	-	-	7 (12.3)	2 (3.5)	-	57
2019	1 (1.9)	3 (5.8)	33 (63.5)	2 (3.9)	1 (1.9)	2 (3.9)	10 (1.9)	-	-	52
Total	23 (3.4)	25 (3.7)	555 (82.8)	11 (1.6)	1 (0.2)	15 (2.2)	30 (4.5)	6 (0.9)	4 (0.6)	670

*Subtyping could not be performed. HCV: Hepatitis C virus

Genotype 1 was determined in 603 patients (90.0%), genotype 3 in 45 (6.7%), genotype 2 in 12 (1.8%), genotype 4 in six (0.9%), combined genotypes 1 and 3 in two (0.3%), and genotypes 1 and 4 in two (0.3%). Genotypes 5, 6, 7, and 8 were not encountered. The most frequently identified subtypes were genotype 1b (82.8%) and genotype 3a (4.5%). Detailed distributions by years of genotypes and subtypes are shown in Table 2.

The mean age of the 603 patients infected with genotype 1 was 59.72±16.29 years (minimum-maximum: 4-112), compared to 45.26±12.51 years (minimum-maximum: 20-77) for patients infected with other genotypes, and the difference was statistically significant ($p<0.001$). The distribution of HCV genotypes by age is shown in Table 3. Genotype 1b was detected in 63.2% of the 174 patients aged under 50 and in 89.7% of the 496 patients aged over 50 ($p<0.001$). While mixed genotypes and genotype 4 were encountered in patients aged ≥50, genotype 3 and its subtypes were more common in patients aged <50 (20.1% of patients <50 compared to 2% of patients ≥50).

Thirty (4.5%) patients were foreign nationals, and these patients' home countries and genotypes are shown in Table 4. The most common genotype in these patients was 1b (50%) followed by genotype 3 (40%) and genotype 2 (10%).

Discussion

This study adds to the existing literature by determining the distribution of HCV genotypes, an important factor in treatment management, and by evaluating changes in genotype distributions by years in an academic center.

High rates of genotype 1b have been reported in European countries, Israel, and Japan, while genotype 1a has more frequently been reported in North America and Northern Europe (9,10). Similarly to other studies from Turkey, the most frequently identified HCV genotype in all years throughout the present study was genotype 1b (82.8%) (Table 5). HCV genotypes in the study population varied significantly with age. The genotype 1b rate among patients under 50 was significantly lower than that among patients over 50 ($p<0.001$). This may be due to a decrease with age in infection rates with HCV genotypes other than genotype 1b.

In Europe, HCV genotype infections are reported to be mostly seen in women, and at advanced ages, and to be associated with blood transfusions, dental treatment, and nosocomial infections (11). The risk factors and modes of transmission among the patients infected with HVC in the present study are unknown. However, patients ranged in age between 20 and 64, and no gender difference was observed. Globally, genotype 2 is more common in West Africa in particular, and in some regions of South America (12). This clustering is thought to be associated with migration patterns linked to the transatlantic slave trade (12). The distribution rates of genotype 2 across the world are highly heterogeneous, ranging between 0.1% and 24.5%. In the present study, HCV genotype 2 was detected in 1.8% (12) of patients, a rate higher than that in Central Europe (0.1%), but significantly lower than those in the Asian Pacific (24.5%), West Africa (23%), Western Europe (10.8%), and worldwide (9.1%) (12).

HCV genotype 3 is the second most common genotype worldwide, after genotype 1, and is particularly dominant in South Asian countries (12,13). A proportional increase was determined

Table 3. Distributions of HCV genotypes by age

		HCV genotypes n (%)									
		1		2			3		4*	Mixed	Total
Age range (years)		1*	1a	1b	2*	2b	3*	3a			
	0-4	-	-	1 (100.0)	-	-	-	-	-	-	1
	5-9	-	-	-	-	-	-	-	-	-	-
	10-14	-	1 (33.3)	2 (66.7)	-	-	-	-	-	-	3
	15-19	1 (50.0)	-	1 (50.0)	-	-	-	-	-	-	2
	20-24	-	2 (20.0)	6 (60.0)	-	1 (10.0)	1 (10.0	-	-	-	10
	25-29	1 (5.0)	2 (10.0)	13 (65.0)	-	-	3 (15.0)	1 (5.0)	-	-	20
	30-34	3 (15.0)	1 (5.0)	8 (40.0)	2 (10.0)	-	3 (15.0)	3 (15.0)	-	-	20
	35-39	3 (10.0)	2 (6.7)	15 (50.0)	1 (3.3)	-	4 (13.3)	5 (16.7)	-	-	30
	40-44	1 (2.6)	2 (5.3)	26 (68.4)	2 (5.3)	-	-	7 (18.4)	-	-	38
	45-49	-	2 (4.0)	38 (76.0)	2 (4.0)	-	2 (4.0)	6 (12.0)	-	-	50
<50 Total		9 (5.2)	12 (6.9)	110 (63.2)	7 (4.0)	1 (0,6)	13 (7.5)	22 (12.6)	-		174
	50-54	1 (1.3)	2 (2.7)	63 (84.0)	2 (2.7)	-	-	3 (4.0)	2 (2.7)	2 (2.7)	75
	55-59	1 (1.1)	2 (2.1)	85 (90.4)	1 (1.1)	-	-	2 (2.1)	2 (2.1)	1 (1.1)	94
	60-64	2 (2.1)	4 (4.2)	85 (89.5)	1 (1.1)	-	1 (1.1)	-	1 (1.1)	1 (1.1)	95
	65-69	5 (6.0)	2 (2.4)	72 (86.7)	-	-	1 (1.2)	2 (2.4)	1 (1.2)	-	83
	70-74	4 (7.0)	1 (1.8)	52 (91.2)	-	-	-	-	-	-	57
	75-79	1 (2.0)	1 (2.0)	48 (94.1)	-	-	-	1 (2.0)	-	-	51
	80-84	-	-	18 (100.0)	-	-	-	-	-	-	18
	85-89	-	-	8 (100.0)	-	-	-	-	-	-	8
	>90	-	1 (6.7)	14 (93.3)	-	-	-	-	-	-	15
≥50 Total		14 (2.8)	13 (2.6)	445 (89.7)	4 (0.8)	0 (0)	2 (0.4)	8 (1.6)	6 (1.2)	4 (0.8)	496
Total		23 (3.4)	25 (3.7)	555 (82.8)	11 (1.6)	1 (0.2)	15 (2.2)	30 (4.5)	6 (0.9)	4 (0.6)	670 (100.00)

*Subtyping could not be performed. HCV: Hepatitis C virus

Table 4. Distribution of HCV genotypes detected in foreign national patients

	HCV genotypes n (%)									
Country of origin	1*	1a	1b	2*	2b	3*	3a	4*	Mixed	Total
Azerbaijan	-	-	-	1 (50.0)	-	1 (50.0)	-	-	-	2
Georgia	-	-	12 (60.0)	-	-	1 (5.0)	7 (35.0)	-	-	20
Iraq	-	-	-	1 (50.0)	-	1 (50.0)	-	-	-	2
Iran	-	-	1 (100.0)	-	-	-	-	-	-	1
Kirgizstan	-	-	-	-	-	-	1 (100.0)	-	-	1
Russia	-	-	1 (50.0)	1 (50.0)	-	-	-	-	-	2
Tajikistan	-	-	1 (100.0)	-	-	-	-	-	-	1
Ukraine	-	-	-	-	-	1 (100.0)	-	-	-	1
Total	-	-	15 (50.0)	3 (10.0)	-	4 (13.3)	8 (26.7)	-	-	30

*Subtyping could not be performed. HCV: Hepatitis C virus

in genotype 3 after 2010 in the present study. The proportion of patients aged under 50 infected with HCV genotype 3 was significantly higher than that of patients aged over 50 (20.1% and 2.0%, respectively, p<0.001). It was most frequently observed in the 35-39 age group (30.0%), and the genotype is more common in males. This variation may be due to reciprocal human mobility such as tourism, education, workforce activities, and marriages, in the community comprising the study population.

Medical procedures without the use of protective measures are the basic risk factor for HCV infection in Middle Eastern and North African countries, and genotype 4 predominates in those countries (65.3%) (12). Genotype 4 is the most frequently seen genotype in Syria, at 59.0% (2,3,13,14). Turkey has a long historical relationship with these countries for reasons such as religious pilgrimages, migration, and tourism (2). In the present study, HCV genotype 4 began being detected after 2012, and this may have

Table 5. Various previous studies of HCV genotypes in Turkey

Study	Year	No. (n)	HCV genotype distribution (%)											
			1	1a	1b	2	2b	3	3a	4	4a	5	6	Mixed
Bozdayi et al. (19)	1997-2000	365	-	11.0	84.0	3.0	-	1.0	-	1.0	-	-	-	-
Cil et al. (18)	2004-2005	22		22.7	72.7			4.5					-	
İba Yilmaz et al. (20)	2008-2010	46	-	-	100.0	-	-	-	-	-	-	-	-	-
Celik et al. (21)	2010	178	-	9.0	88.2	1.1	-	1.7	-	-	-	-	-	-
Karslıgil et al. (22)	2011	51	-	9.8	78.4	7.8	-	2.0	-	2.0	-	-	-	-
Kayman et al. (2)	2010-2011	218	62.4	2.3	60.1	4.6	-	-	-	33	-	-	-	-
Oztürk et al. (23)	2010-2012	315	-	3.5	55.2	14.6	-	26.0	-	0.6	-	-	-	-
	2010-2012	324	-	0.3	86.7	9.3	-	0.9	-	2.8	-	-	-	-
Sağlik et al. (10)	2009-2013	422	83.4	14.7	63.3	3.5	0.9	-	11.1	1.6	-	-	-	0.2
Çekın et al. (5)	2011-2013	148	8.8	12.8	60.8	4.1	-	11.5	-	2	-	-	-	-
Akar et al. (24)	2012-2013	53	96.2	3.8	50.9	1.9	-	-	-	1.9	-	-	-	-
Tezcan et al. (25)	2013	236	3.8	1.7	84.7	2.1			4.2	-	0.8	-	-	-
Altuğlu et al. (26)	2013	535	93.3	12.9	80.4	1.5	-	3.7	-	1.5	.	-	-	-
Caliskan et al. (27)	2010-2014	313	51.7	-	-	1.3	-	46.0	-	1.0	-	-	-	-
Cirit et al. (16)	2011-2015	312	69.6			14.1		3.8		10.3		1.6	-	0.6
Çetin Duran et al. (14)	2015-2016	119	71.4	12.6	58.8	7.6		16.8		3.4		1	-	
Kulah et al. (15)	2007-2016	6.0	1.7	5.5	79.8	3.3	0.8	-	5.7	0.5	-	-	0.2	1.8
	2007-2016	336	23.0	3.6	82.8	1.2	1.2	0.3	3.3	1.5		0.3	0.6	1.8
	2007-2016	675	5.2	17.2	58.4	1.6	1.2	7.3	5.3	1.6		1.2	0.2	5 (0.7)
Kirdar et al. (17)	2011-2016	286	90.2	-	-	2.1	-	5.9	-	1.4	-	-	-	0.35
Calgin and Cetinkol (28)	2016-2018	165	2	5.6	91.4			2.5		0.5			-	

been the effect of the arrival in Turkey of refugees from Syria. HCV genotype 4 was reported in as many as 32.0% of chronic hepatitis C in one study, and since the timing coincided with times of labor force migration, the authors thought that it might have been carried by people moving to these areas (2). In the present study, however, genotype 4 was detected in only eight patients, and a more reliable interpretation when the future distribution is revealed.

The detection of HCV genotype co-presence facilitates ideal patient follow-up and increases the effectiveness of antiviral drug therapies (15). In recent years, mixed type HCV genotype reports have been issued more frequently in Turkey (15,16,17). One multi-center study from Turkey determined a mixed genotype prevalence of 1.3% (15). Genotype 1b and 4 was the most frequently seen mixed genotype combination in that study, while the lowest rates were reported for 2b+2c, 1a+3, 1a+4, 2+3, and 3+4 genotype combinations (15). High mixed genotype rates of 15.6% in Taiwan, 19.0% in the Dominican Republic, and 15.7% in Iraq have been reported. The highest rate of patients with mixed genotypes among European countries was reported in Serbia, at 8.5%. The closest rates to those of the present study were recorded from Venezuela at 0.7%, Mexico at 0.7%, and the United States of America at 0.5% (13).

Analysis of the 30 foreign national patients in this study revealed that the most common genotypes, in descending order, were 1, 3, and 2, and no other genotypes were detected. Approximately 60% of these patients were Georgians, and Georgians also represent the majority of foreign patients with genotypes 1b and 3a. Genotype 1b was observed at a lower rate, 50%, in this patient group compared to the data for Turkey, while rates for genotype 2 and 3 were higher, at 10% and 40%, respectively. Consistent with the present study, analysis of HCV genotype distributions in Russia, Georgia, and the Turkic republics has shown that genotype 1b is dominant, followed by genotype 3, and then by genotype 2 (13). These data indicate that genotype distributions can change over time, in both our own region, in Turkey, and worldwide, especially as travel becomes easier.

Since the HCV is an RNA virus with high genetic variability, no effective vaccine is available. Therapeutic protocols and novel direct-acting antiviral drug studies are based on genotypes and subtypes (3,18).

Study Limitations

The limitation of our study is that the absence of information about the transmission routes due to the retrospective design of the study.

Conclusion

The findings of the present study revealed a time-dependent change in the general distribution of HCV genotypes and subtypes, and that HCV genotype 1b was observed at the highest rate across the years, particularly among patients over 50. Since HCV genotypes can be affected by social and cultural diversity, it is essential that the data be updated at specific intervals. In addition, determining changes in epidemiological data will serve as a useful guide for the development of vaccines and novel antiviral agents.

Ethics

Ethics Committee Approval: The study was approved by Karadeniz Technical University Faculty of Medicine Faculty Clinical Research Ethical Committee (approval number: 2020/169).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: E.Ö., C.K.B., F.A., N.K., I.B., İ.T., Concept: E.Ö., C.K.B., F.A., N.K., I.B., İ.T., Desing: E.Ö., C.K.B., Data Collection or Processing: E.Ö., C.K.B., Analysis or İnterpretation: E.Ö., C.K.B., F.A., N.K., I.B., İ.T., Literature Search: E.Ö., C.K.B., Writing: E.Ö., C.K.B., N.K., I.B., İ.T.

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Research Article

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The Treatment of Ledipasvir/Sofosbuvir in Patients with Chronic Hepatitis C Virus: The Results of Five-year Follow-up

Kronik Hepatit C Virüslü Hastalarda Ledipasvir/Sofosbuvir Tedavisi: Beş Yıllık Takip Sonuçları

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ABSTRACT

Objectives: Chronic hepatitis C virus (HCV) is a fundamental worldwide health challenge. We assessed the treatment outcomes of ledipasvir (LDV) and sofosbuvir (SOF) with and without ribavirin (RBV) for 12 and 24 weeks in pre-treated and treatment-naive patients with chronic HCV.

Materials and Methods: Totally 65 patients were included in the present study. Patients were divided in two groups. In the first group, LDV and SOF with RBV were administered to 12 patients for 12 weeks. In the second group, LDV and SOF without RBV were administered to 53 patients for 24 weeks.

Results: Sustained virological response (SVR) rates were 100% for the both groups included in the study. The adverse events were weakness (15.39%), pruritus (6.15%), myalgia (4.62%) nausea (3.08%), dry mouth (1.54%) and anorexia (1.54%) in all patients. HCV-RNA was also negative in all patients 48 weeks after the beginning of the treatment. At the end of the fifth year of treatment, all the patients still had SVR and no recurrence was detected.

Conclusion: In the treatment of patients with chronic HCV, LDV and SOF with and without RBV were highly effective. SVR rate of 100% was achieved in all pre-treated or treatment naive patients with or without cirrhosis regardless of genotype of HCV.

Keywords: Chronic hepatitis C virus, direct-acting antiviral agents, sofosbuvir, ledipasvir

ÖZ

Amaç: Kronik hepatit C virüsü (HCV), dünya çapında temel bir sağlık sorunudur. Bu çalışmamızda tedavi naif ve deneyimli kronik HCV'li hastalarda 12 ve 24 hafta boyunca ribavirin (RBV) içeren ve içermeyen ledipasvir (LDV) ve sofosbuvir (SOF) tedavi sonuçlarını değerlendirdik.

Gereç ve Yöntemler: Toplamda 65 hasta çalışmaya dahil edildi. Hastalar iki gruba ayrıldı. Birinci grupta 12 hastaya 12 hafta boyunca LDV ve SOF ile RBV verildi. İkinci grupta 53 hastaya 24 hafta boyunca LDV ve SOF uygulandı.

Bulgular: Her iki grupta da kalıcı viral yanıt oranı (SVR) %100 bulundu. Tüm hastalar içinde yan etki olarak halsizlik (%15,39), kaşıntı (%6,15), kas ağrısı (%4,62), bulantı (%3,08), ağız kuruluğu (%1,54) ve iştahsızlık (%1,54) görüldü. Tedavinin başlangıcından 48 hafta sonra tüm hastalarda HCV-RNA hala negatif idi. Beşinci yılın sonunda tüm hastalarda SVR mevcuttu ve nüks saptanmadı.

Sonuç: RBV'li ve RBV'siz LDV ve SOF tedavisi, kronik HCV'li hastaların tedavisinde oldukça etkindir. HCV genotipinden bağımsız olarak, sirozu olan veya olmayan tüm tedavi deneyimli ve naif hastalarda %100 SVR oranlarına ulaşılmıştır.

Anahtar Kelimeler: Kronik hepatit C virüs, direkt etkili antiviral ajanlar, sofosbuvir, ledipasvir

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Introduction

Viral hepatitis has a major global health challenge, affecting 71 million with chronic hepatitis C virus (HCV) infection. It is a root cause of cirrhosis and liver cancer, causing about 1.4 million deaths annually. HCV can be eliminated without treatment within six month in approximately 15-45% of infected people, whereas it can evolve into chronic infection for the remaining 55-85% of infected people (1). HCV is categorized into 6 genotypes (1-6) with various subtypes based on genetic variations (2). Globally, genotype 1 is the most common type of HCV with the rest of the genotypes accounting for more than half of all HCV infections (1).

The sustained virological response (SVR) rate was 40-50% with standard pegylated-interferon (IFN) and ribavirin (RBV) treatment, and the rate increased to 60-80% with the addition of protease inhibitors [telaprevir (TVR)/boceprevir (BOC)] to the treatment in chronic HCV (3). Revolutionary developments have been achieved in the treatment of HCV after the onset of IFN-free regimens comprising of oral direct-acting antiviral (DAA) agents. Among the treatment options, the one with the highest SVR rate and the least adverse events in the minimum treatment period can be adopted as the best option (4). Literature review showed that DAA regimens can provide evident better antiviral efficacy with remarkable SVR rates exceeding 90-95% (5,6,7,8,9,10).

Treatment with sofosbuvir/ledipasvir (SOF/LDV) introduced very potent, well-tolerated, influential and non-IFN-based antiviral regimens for HCV infection applicable for the first time (11). SOF is a uridine nucleotide analogue inhibitor of the HCV-NS5B polymerase (12) and LDV is an inhibitor of the HCV-encoded NS5A protein (13). The combination of SOF/LDV with or without RBV in the treatment of HCV infection has improved the impressively high SVR rates up to 95%, even reaching 100% in some cases (4,14,15, 16,17,18,19,20,21,22,23). According to its high level genetic barrier, the development of resistance to it is at low. Thus, reaccurance of the disease is almost not observed. Additionally, due to its IFN-free form, treatment with SOF/LDV caused less adverse events than IFN based regimens (18,19). A fixed-dose combination tablet consisting SOF united with LDV has been adopted in European Union, United States and other regions all over the world for the treatment of HCV infection (6) which has been adopted by U.S. Food and Drug Administration (24). This combination regimen is recommended by clinical practice guidelines in the European Union and the United States for the treatment-experienced and treatment-naive patients infected by HCV virus (25).

The fixed-dose combination of SOF/LDV has been used for the treatment of HCV recently. The objective of this study was to compare and assess the real-world effectiveness, safetyand long-term outcomesof SOF/LDV with and without RBV in treatment of patients with HCV.

Materials and Methods

Patients

A total of 65 adult patients were enrolled into the current study. All patients were diagnosed, followed and treated at Düzce University Faculty of Medicine and Bolu State Hospital in Turkey from 2015 to 2021. Treatment-experienced or treatment-naïve patients over 18 years of age with chronic HCV infection, with or without cirrhosis, were included in this study. There was no exclusion criteria based on body mass index (BMI) and age. Liver-biopsy was conducted to determine the presence of cirrhosis for twenty-seven patients according to the Ishak score of 5 or 6 (on a scale of 0 to 6 in which higher scores indicate a greater degree of fibrosis). Treatment-experienced patients had previously received combinations of pegile-IFN + RBV ± TVR/BOC, but infection relapsed in all of treatment-experienced patients.

Study Design

This study was an open-label, multi-centre, real-world study and conducted at Düzce University Hospital and Bolu State Hospital in Turkey. All patients orally received a fixed-dose combination tablet comprising of 400 mg of SOF and 90 mg of LDV with or without RBV once daily. Patients below and equal to 75 kg were treated with 1000 mg RBV and those over 75 kg were treated with 1200 mg RBV. Patients were divided into two groups. One group with 53 patients received SOF/LDV for 24 weeks and the other group with 12 patients received SOF/LDV with RBV for 12 weeks.

Laboratory parameters and adverse events were measured, recorded, and assessed before treatment and 2, 4, 12, 24, 36, 48 weeks and 60 monthsafter the beginning of the treatment. Patients were examined in detail and questioned about the possible adverse events of the treatment during the follow-up. The Child-Pugh score system was adopted to determine clinical status on cirrhotic patients. HCV diagnosis was determined as a positive test for anti-HCV antibodies validated by a positive HCV viral load.

Samples for laboratory parameters were acquired during the procedural examination and none were taken during renal crisis, acute liver or under any acute illness. HCV-RNA levels were measured by real-time polymerase chain reaction according to standard methods.

Study Oversight

The study was approved by Ethics Committee Düzce University (approval number: 2019/103, date: 15.05.2019). The research was performed in accordance with the principles of Good Clinical Practice guidelines, the Declaration of Helsinki, and local regulatory requirements. Written informed consent was obtained from each patient. Demographic and clinical characteristics of all patients were recorded, and concomitant treatments clinical assessments and other medical decisions were applied at the discretion in accordance with standard clinical practice. The authors obtained and edited the data, followed up the all processes of the study and conducted the statistical analyses. Data confidentiality was maintained by the authors.

Study Assessments

Measurement of laboratory data on HCV-RNA level, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and serum biomarkers such as bilirubin, albumin, urea (URE), creatinine, hemoglobin (Hb), total leukocyte [white blood cell-(WBC)] platelets count (PLT) and international normalised ratio were included in follow up assessments. All adverse events were recorded.

Study Endpoints

The primary efficacy endpoint was the proportion of the patients achieving SVR at 12 weeks (SVR12). SVR12 was defined as the rate of patients with HCV-RNA concentration in serum

lower than 25 IU/mL 12 weeks after the completion of treatment. The primary safety endpoint was any adverse event leading to discontinuation of the treatment. The secondary efficacy endpoint was the proportion of the patients achieving SVR at 60 months.

Statistical Analysis

The clinical and demographic characteristics of patients were summarized by using mean, range, standard deviation, frequency (count) and relative frequency (percentage). The two groups were compared by conducting the non-parametric Mann-Whitney for the continuous variables and the chi-square test for the categorical variables, since the quantitative variables were non-normally distributed. Serial measurements for pretreatment and end of treatment were compared by performing the Wilcoxon signed-rank test. Statistically significance was considered for p-values less than 0.05 and the confidence intervals set at the 95% level. The SPSS version 25 was used to conducted the statistical analysis.

Results

Baseline Characteristics

Clinical and demographic characteristics of the patients are shown in Table 1 for both groups. Patients were divided into two groups according to the treatment regiments they received. Twelve patients in the first group received LDV and SOF with RBV and 53 patients in the second group received LDV/SOF. The mean age of the patients was 56.4 in the first group, 65.2 in the second group and 63.5 for the overall study population. The range of patients's age were between 22-71 and 25-86 in those groups, respectively. There were 7 (10.77%) females in the first group and 28 (43.08%) females in the second group. The number of males in the groups were 5 (7.69%) and 25 (38.46%), respectively. Most of the patients were infected by HCV genotype 1b, with 7 (10.77%) patients in the first group infected with HCV genotype 1b and 50 patients (76,92%) in the second group infected with HCV genotype 1b. In

Table 1. Clinical and demographic characteristics of the patients

Characteristic	1. Group LDV/SOF + RBV (n=12)	2. Group LDV/SOF (n=53)	Total	p-value
Age	-	-	-	**0.071**
Mean	56.4	65.2	63.5	-
Range	22-71	25-86	22-86	-
Gender	-	-	-	0.730
Female	7 (10.77%)	28 (43.08%)	35 (53.85%)	-
Male	5 (7.69%)	25 (38.46%)	30 (46.15%)	-
Genotype	-	-	-	**0.001**
1a	1 (1.54%)	3 (4.62%)	4 (6.15%)	-
1b	7 (10.77%)	50 (76.92%)	57 (87.69%)	-
2a	2 (3.08%)	-	2 (3.08%)	-
2b	1 (1.54%)	-	1 (1.54%)	-
3	1 (1.54%)	-	1 (1.54%)	-
Fibrosis	-	-	-	**0.132**
0	-	3 (4.62%)	3 (4.62%)	-
1	2 (3.08%)	1 (1.54%)	3 (4.62%)	-
2	-	3 (4.62%)	3 (4.62%)	-
3	2 (3.08%)	6 (9.23%)	8 (12.31%)	-
4	-	3 (4.62%)	3 (4.62%)	-
5	-	6 (9.23%)	6 (9.23%)	-
6	-	1 (1.54%)	1 (1.54%)	-
Cirrhosis		10	10 (15.39%)	-
Previous HCV treatment(s)	-	-	-	**0.553**
Naive	5 (7.69%)	14 (21.54%)	19 (29.23%)	-
IFN + RBV	6 (9.23%)	35 (53.85%)	41 (63.08%)	-
TVR + BOC	1 (1.54%)	4 (6.15%)	5 (7.69%)	-
HCV-RNA	-	-	-	**0.543**
Mean, $\log_{10}$ IU/mL	5.59±0.6	5.69±0.78	5.67±0.75	-
≥5 $\log_{10}$ IU/mL (%)	9 (75%)	44 (83%)	53 (81.54%)	-
Viral load (IU/mL)	862.064±1.037.904	1.797.026±3.725.150	1.624.418±3.412.528	-

LDV: Ledipasvir, SOF: Sofosbuvir; RBV: Ribavirin, HCV: Hepatitis C virus, IFN: Pegile-interferon, TVR: Telaprevir, BOC: Boceprevir, RNA: Ribonucleic acid, IU: International unit, L: Liter

total, 41 patients had received treatment with IFN + RBV regimen and 5 patients had received treatment with TVR or BOC regimen prior to this study. Nineteen patients were treatment-naive in total. The mean baseline HCV-RNA was 5.50 $\log_{10}$ IU/mL in the first group and 5.66 $\log_{10}$ IU/mL in the second group. Only twenty-seven of the patients received liver biopsy prior to the treatment. Results of the liver biopsies showed that nine patients had minimal or no fibrosis (Ishak F0, 1, 2), eight patients had portal fibrosis (Ishak F3), three patient had bridging fibrosis (Ishak F4) and seven patients had cirrhosis (Ishak F5, 6). There were only 10 (15.39%) patients with cirrhosis in the second group. Decompensated liver failure, such as ascites or jaundice, were not found in any of patients. There was no significant difference between the two groups regarding their gender, fibrosis cases, cirrhosis cases, previous HCV treatment, and baseline HCV-RNA levels.

The differences between two groups regarding mean age were found to be statistically insignificant (p=0.07). There were more patients with HCV 1a genotype and 1b genotype in the second group than the first group. The results of the measurements for the laboratory parameters are shown in Table 2. Reduction in ALT and AST for the both group, increase in WBC for the second group, reduction in Hb for the first group, increment in PLT for the both groups, and increment in URE for the second group were found to be statistically significant.

In both groups, ALT and AST values decreased significantly at 12 weeks compared with pretreatment. The mean AST and ALT levels, which were 2-3 times higher before treatment, returned to normal levels at the end of the treatment. Treatment-induced anemia was observed in the RBV group (p=0.015), whereas Hb was not decreased in the LDV + SOF group (p=0.245). Platelet levels were significantly increased in both groups after treatment (respectively p=0.028 and p=0.027). The increase in URE values in LDV + SOF group was not accompanied by elevation of creatine.

Efficacy

HCV-RNA levels in the treatment weeks and rates of SVR are presented in Figure 1. At the end of the fourth treatment week, HCV-RNA levels for 11 (91.67%) of 12 patients in the first group (LDV/SOF + RBV) and 50 (94.34%) of 53 patients in the second group (LDV/SOF) were not able to be detected. At week 12 of treatment, virologic suppression was achieved on all patients in both groups. All patients in both groups, including the 10 with compensated cirrhosis at baseline, had sustained virologic response 12 weeks after the end of treatment. In addition, HCV-RNA was negative in all patients 48 weeks after starting treatment. No significant differences were observed between the two groups, since all patients achieved SVR after the treatment. Also all the patients achieved SVR 60 months after the end of treatment. None of them had relapse.

Safety

The adverse events experienced by the patients are summarized in Table 3. None of the patients experienced any serious adverse events. At least one adverse event was experienced by 16.9% of the patients during the study. The adverse events were weakness (15.39%), pruritus (6.15%), myalgia (4.62%), nausea (3.08%), dry mouth (1.54%) and anorexia (1.54%). Myalgia (p=0.028) and anorexia (p=0.034) were found to be more common in the first group and statistically significant. Weakness effects were more common in the second group and can be accepted statistically

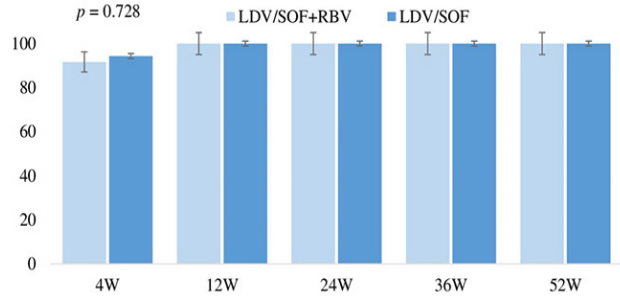

Figure 1. Rates of sustained virological responses. Error bars shows 95% confidence intervals

LDV: Ledipasvir; SOF: Sofosbuvir, RBV: Ribavirin, W: week

Table 2. Laboratory data measurements

	1. Group (LDV + SOF + RBV)			2. Group (LDV + SOF)		
	d0	12W	p-value	d0	24W	p-value
ALT	77	14	**0.008**	54.44	19.97	**0.001**
AST	65	19	**0.008**	57.36	24.94	**0.001**
WBC	6290	7283.33	0.086	6103.33	6967.14	**0.040**
HB	13.72	11.54	**0.015**	12.69	12.6	0.245
PLT	196	255.56	**0.028**	185.65	210.06	**0.027**
URE	34.78	41.89	0.225	35.13	38.85	**0.037**
CRE	0.84	0.92	0.285	0.88	0.78	0.906
BIL	0.83	0.9	0.620	0.72	0.63	0.170
ALB	4.33	4.2	0.344	4.03	4.04	0.882
PT	12.18	12.02	0.528	11.76	11.49	0.210
INR	1.05	1.06	0.317	1.03	1.02	0.134

P-values with statistically significance (<0.005) are in bold. LDV: Ledipasvir, SOF: Sofosbuvir, RBV: Ribavirin, d0: Baseline (at the beginning of treatment), W: Week, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, WBC: White blood cell (total leukocyte), HB: Hemoglobin, PLT: Platelets count, URE: Urea, CRE: Creatinine, BIL: Bilirubin, ALB: Albumin, PT: Prothrombin time, INR: International normalized ratio, n: Number of patients

Table 3. Adverse events

Characteristic	1. Group LDV/SOF + RBV (n=12)	2. Group LDV/SOF (n=53)	Total	p-value
None	8 (12.31%)	46 (70.77%)	54 (83.08%)	0.093
Weakness	4 (6.15%)	6 (9.23%)	10 (15.39%)	0.056
Pruritus	1 (1.54%)	3 (4.62%)	4 (6.15%)	0.728
Myalgia	2 (3.08%)	1 (1.54%)	3 (4.62%)	**0.028**
Nausea	1 (1.54%)	1 (1.54%)	2 (3.08%)	0.243
Dry mouth	-	1 (1.54%)	1 (1.54%)	-
Anorexia	1 (1.54%)	-	1 (1.54%)	-

LDV: Ledipasvir, SOF: Sofosbuvir, RBV: Ribavirin

significant (p=0.056). Discontinuation of the treatment due to adverse events did not occur for any of the patients.

Discussion

The World Health Assembly declared the elimination of viral hepatitis as a public health threat by 2030 in the Global Health Sector Strategy through reducing its incidence by 90% and reducing its mortality by 65%. The global prevalence of HCV was 1% in 2015, ranging between 0.5% (South-East Asia Region) and 2.3% (Eastern Mediterranean Region) depending on the regions. Chronic HCV is considered to be one of the major causes of hepatocellular carcinoma and liver cirrhosis (1). Therefore, every patient with positive HCV-RNA should be treated. There was a complete change in the treatment of HCV with DAAs, which were first introduced in 2011. Nowadays, INF-free therapies are being used in chronic HCV. INF-free therapies are superior in terms of both efficacy and safety when compared to previous therapies.

The aim of the present study was to evaluate the response of patients infected with chronic HCV to treatment with a fixed-dose combination of LDV/SOF with RBV for 12 weeks treatment period or without RBV for 24-week treatment period. The response rates and SVR were compared for two groups received two different regimens. The results of this study showed that a fixed-dose combination of LDV/SOF with RBV during 12 weeks and without RBV during 24 weeks were highly effective treatment regimens for HCV. In the phase studies of LDV + SOF, up to 99% sustained viral response was obtained in different patient groups and for different protocols (18,19,20).

Our results are consistent with the recent studies concluded 100% SVR rates of treatment with LDV/SOF (16,17,21,22). Shousha et al. (17) evaluated the safety and efficacy of generic SOF/LDV for 8 and 12 weeks in 40 naive non-cirrhotic patients with HCV genotype 4. They revealed that 8 weeks of treatment with generic SOF/LDV had SVR12 rates of 100% and SVR12 rates of 95% with 12 weeks of the same regimen.

Liu et al. (16) enrolled 111 patients infected with HCV virus along with HBV infection to their open-label, multicenter and phase 3b study. They administrated a fixed-dose combination of LDV/SOF to all patients, once daily for 12 weeks. They concluded that the combination of LDV/SOF lead to an SVR12 rate of 100% of patients with HCV infection who were co-infected with HBV. Shiha et al. (21) assessed the efficacy and safety of LDV/SOF with and without RBV for 8 and 12 weeks in 255 Egyptian patients infected with HCV virus genotype 4. The results of this study indicated that SVR12 rates were over 90% for all groups. SVR rates of 100% were only found among INF-experienced patients who received 2 weeks of LDV/SOF with RBV.

Mizokami et al. (22) administrated either LDV (90 mg) and SOF (400 mg) or LDV/SOF and RBV orally to 341 patients infected with HCV virus genotype 1a and 1b, once daily for 12 weeks, in their randomized, open-label study. SVR12 rates of 100% were achieved in all 171 patients who received LDV/SOF and SVR12 rates of 98% were achieved in patients who received LDV/SOF with RBV.

Many parameters such as age, sex, cirrhosis, response to previous treatments, BMI, and HCV-RNA levels can affect the success of treatment in INF-based treatments. In our study, all cirrhotic patients had SVR12. 81% of the patients had high viral load (≥5 $\log_{10}$ IU/mL) and treatment was successful in all of them. There was no difference in response to treatment between naive and experienced patients.

Studies on long-term results of SOF-based DAA therapies generally have a short follow-up period. The number of studies with long-term follow-up results is not very high. Some statistical modeling studies show that long-term results are effective and there is a decrease in HCV-related mortality and advanced liver disease. In addition, the treatment was found to be costeffective (26,27). Long-term sustained viral response was observed in the mean 96-week follow-up of 62 patients who had relapsed after liver transplantation and were treated with DAA (28). In the 24-month follow-up period of 120 patients with liver transplantation, it was determined that the treatment was effective and there was improvement in liver tests (29). SOF/LDV therapy is effective and tolerable also in patients with advanced liver disease to HCV. In a study in which 200 patients with advanced liver disease due to HCV were followed for an average of 22 months, SOF/LDV treatment was found to be effective and tolerable. With eradication of HCV, improvement in liver functions was detected and the risk of developing new hepatocellular carcinoma was reduced (30). Although the number of patients was small in our study, the follow-up period was quite long, such as 60 months. At the end of the follow-up period, improvement in liver function tests was observed in all patients. Decompensation and hepatocellular carcinoma did not develop in any of the cirrhotic patients. No patient died.

INF-based treatments were discontinued because of serious adverse effects. This in turn reduced the success rate of the treatment. In our study, no serious adverse effects were observed in any of the patients. The two treatment regimens used in our study were safe and well tolerated. There was no discontinuation caused by any adverse effect. The most common adverse effects

were weakness, rash, myalgia and nausea. One patient had dry mouth and one patient had insomnia. Anemia was observed in the group receiving RBV (p=0.015) but not in the other group. The adverse effects seen in our study were similar with to those seen in the literature and phase studies (18,19,20,21,22).

Study Limitations

There were limitations to the present study. First, the relatively small sample size of the study might affect significance of the statistical tests. Second, the two study groups were not randomized equally. Third, all of the patients infected with HCV virus were selected from justtwohospital and therefore, selection bias could not be avoided.

Conclusion

LDV and SOF regimen is a very effective and reliable treatment for controlling chronic HCV infection in all patient groups. HCV eradication may be possible with systematic and effective treatment of chronic HCV patients.

Ethics

Ethics Committee Approval: The study was approved by Ethics Committee Düzce University (approval number: 2019/103, date: 15.05.2019).

Informed Consent: Written informed consent was obtained from each patient.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: M.P., N.İ., Design: M.P., N.İ., Data Collection or Processing: M.P., N.İ., Analysis or Interpretation: M.P., N.İ., Literature Search: M.P., N.İ., Writing: M.P., N.İ.

Conflict of Interest: The authors of this article declare that they have no conflict of interest.

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Research Article

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Effect of Hepatitis B Virus Genotypes and Viral Load on the Response of Patients Treated with Peginterferon-α-2a

Hepatit B Virüsü Genotiplerinin ve Viral Yükün Peginterferon-α-2a ile Tedavi Edilen Hastaların Yanıtı Üzerindeki Etkisi

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ABSTRACT

Objectives: Present study was aimed to find out the influence of genotype and hepatitis B virus (HBV)-DNA on the treatment response of the patients with chronic HBV.
Materials and Methods: It was a cross-sectional, retrospective study carried out on patients undergoing treatment of chronic HBV. A total of 54 patients with chronic HBV, who were under treatment with peginterferon-α-2a, were included. Effects of genotypes and other factors on virologic response, combined response and hepatitis B surface antigen (HBsAg) clearance were analyzed with logistic regression and chi square test.
Results: Baseline viral load and HBV genotype were found to have significant influence on the patients' response. Patients with genotype A were found to responde more to the treatment than patients with mix genotype infection (A + D). However, this difference was only significant for virologic response. Patients with low (<20,000 IU/mL) baseline viral load showed higher rate of virologic response, combined response and HBsAg clearance than those with high (>20,000 IU/mL) viral load at baseline.
Conclusion: Peginterferon-α-2a therapy is more efficacious in mono-infected HBV patients either with genotype A or D than patients with mix genotypes (A + D). Moreover, patients with low viral load at baseline have a higher response rate than the patients with high viral load at baseline.
Keywords: Hepatitis B virus, HBV genotypes, peginterferon, viral load, treatment response

ÖZ

Amaç: Bu çalışmada, kronik hepatit B virüslü (HBV) hastaların tedavi yanıtı üzerine genotip ve HBV-DNA'nın etkisini ortaya çıkarmak amaçlanmıştır.
Gereç ve Yöntemler: Kronik HBV tedavisi gören hastalar üzerinde yürütülen kesitsel, retrospektif bir çalışmadır. Peginterferon-–2a ile tedavi gören toplam 54 kronik HBV'li hasta incelendi. Genotiplerin ve diğer faktörlerin virolojik yanıt, kombine yanıt ve hepatit B yüzey antijen (HBsAg) klirensi üzerindeki etkileri lojistik regresyon ve ki-kare testi ile analiz edildi.
Bulgular: Başlangıçtaki viral yük ve HBV genotipinin hastaların tedavi yanıtı üzerinde önemli etkiye sahip olduğu bulundu. Genotip A'ya sahip hastaların, miks genotip enfeksiyonu (A + D) olan hastalardan daha fazla yanıt verdiği bulundu. Bununla birlikte, bu fark sadece virolojik yanıt için anlamlıydı. Düşük (<20.000 IU/mL) başlangıç viral yükü olan hastalar, başlangıçta yüksek (>20.000 IU/mL) viral yüke sahip olanlara göre daha yüksek oranda virolojik yanıt, birleşik yanıt ve HBsAg klirensi göstermiştir.
Sonuç: Peginterferon-α-2a tedavisi, genotip A veya D olan mono-enfekte HBV hastalarında karma genotipli (A + D) hastalara göre daha etkilidir. Ayrıca, başlangıçta düşük viral yüke sahip hastalar, başlangıçta yüksek viral yüke sahip hastalardan daha yüksek bir yanıt oranına sahiptir.
Anahtar Kelimeler: Hepatit B virüsü, HBV genotipleri, peginterferon, viral yük, tedavi yanıtı

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Introduction

Chronic Hepatitis B virus (HBV) infection is a leading health problem worldwide. About 400 million people are chronically infected with HBV in world and there are about 9 million HBV carriers in Pakistan (1,2,3). Chronic infection with HBV is also one of the major causes of many liver disease complications like cirrhosis, hepatocellular carcinoma and complete liver failure, which may lead to death (4).

Treatment of chronic HBV infection has generally low response rate and it is also associated with drug resistance and relapse (5). Peginterferon-α-2a, having antiviral activity as well as immunomodulatory function, was reported to have relatively higher response rates as compared to oral agents and conventional interferon in chronic HBV infections (4,5,6).

HBV genotype is established as a strong factor influencing treatment response in chronic HBV infection and contribute in treatment response of patients (5,7,8,9). Comparing genotypes A and D, it is reported that the patients infected with HBV genotype A has higher response rate to interferon α than the patients with genotype D (9). Similarly when the response rates to interferon-α treatment were studied for genotypes B and C, it was found that genotype B infected patients were more sensitive for the treatment than genotype C infected chronic HBV patients (8). Genotype B was reported to have higher rate of hepatitis B surface antigen (HBsAg) clearance than genotype C while infection with genotype A was associated with better rate of HBsAg clearance when compared to the genotypes B, C, D and F infections (10,11).

Most of the previous studies available on the topic have focused genotypes B and C while only a few studies are published who compared genotypes A and D or their combination. The studies involving mix genotyping infections like A + D are needed to know about the dynamics of treatment response in patients with two or more than two genotypes at a time. In Pakistan, where genotype D, A and a mixture of both is prevalent, research has not been performed on this topic. The objective of the present study was to assess the response of peginterferon-α-2a in HBV patients infected with mono-genotypes A, D versus mix genotype infection and to compare hepatitis B e antigen (HBeAg) positive and negative infection for the same treatment.

Materials and Methods

Patients Selection and Outcome Definition

A total of 54 patients received treatment of peginterferon-–2a (180 µg weekly) for 6 months at Pakistan Atomic Energy Commission General Hospital, Islamabad, Pakistan. Virologic response, combined response (virological + biochemical) and HBsAg clearance were determined after 24 weeks. Virologic response was considered as undetectable HBV-DNA in serum, combined response as undetectable level of HBV-DNA and normal alanine aminotransferase (ALT) in serum while HBsAg clearance was defined as undetectable level of HBsAg in serum.

Laboratory Tests

HBV-DNA was extracted and quantified using commercially available extraction kits (AJ Roboscreen, GmbH, Germany) following the manufacturer's protocol. Genotypes of HBV were determined following genotype specific PCR method (12). Quantities of ALT were determined by local laboratory methods and protocols.

Statistical Analysis

Regression analysis and Pearson's chi-square test were used to assess the influence of different viral, biochemical and patient factors including genotype, baseline HBV-DNA, baseline ALT, HBeAg, gender and age on different types of patients' response. Odds ratio (OR) along with confidence interval (CI) was calculated for each factor. The factors found significant in logistic regression analysis were then subjected to Pearson chi-square test to compare the patients' response rates. SPSS, version-16.0 was used for analyses.

Ethical Approval and Patient's Consent

The study was approved by the Ethics Committee of University of the Poonch Rawalakot, Azad Jammu and Kashmir (approval number: UPR/HAEC/2020/M3/C07). All the patients signed a written informed consent.

Results

General Characteristics of Patients

A total of 54 patients completed 6 months of peginterferon-α-2a therapy which included 37 male and 17 female patients with mean age of 33.1±12.6 years. Out of the total 54 patients, 8 were infected with genotype A, 22 with genotype D while 24 of the patients were infected with a combination of both genotypes A and D (mix). Thirty-eight (70.4%) of the patients were HBeAg positive while remaining 16 (29.6%) were negative for HBeAg (Table 1).

Baseline Factors

Out of the 6 factors analyzed in binary logistic regression, only genotype and baseline HBV-DNA were found to be significantly influencing the patients response. All the other factors, i.e. baseline ALT, HBeAg, gender and age had no significant effect on patients response (Table 2).

Table 1. Baseline characteristics of the patients in three genotype groups

Characteristic	Genotype A (n=8)	Genotype D (n=22)	Genotype A + D (n=24)	Overall
HBV-DNA IU/mL (median)	3,153.651	211,400	31,342.846	6,571.235
ALT, U/L (mean ± SD)	90.25±25.2	62.9±24.4	112.6±67.5	93.5±47.5
Age years (mean ± SD)	32.25±14.4	37.4±12.1	29.4±11.6	33.1±12.6
HBeAg positive (n%)	87.5%	63.6%	70.8%	70.4%
Male gender (n%)	62.5%	91%	50%	68.5%

HBV: Hepatitis B virus, ALT: Alanine aminotransferase, SD: Standard deviation, HBeAg: Hepatitis B e antigen

HBV Genotype and Patient's Response

In logistic regression analyses, genotype was found to be significantly influencing the virological response. When patients having single genotype infection were compared with the patients having mix genotype infection, the patients with single genotype infection were found to be significantly more responders (p=0.022) as compared to the patients having mix genotype infection with OR of 3.92 (Table 2). However, combined response and HBsAg clearance were not significantly different between both the patient groups.

In genotype comparisons, genotype A infected patients had a significantly (p=0.020) higher virologic response than the mix infection patients with OR of 3.30 (Table 2). HBsAg clearance and combined response were not different significantly. No difference in any type of response was recorded between either genotype A and D or between genotype D and the mix (A + D) genotype infection (Table 2).

When analyzed with chi-square test, significantly higher rate (p=0.030) of virological response was noted for genotype A infected patients as compared to the patients with mix (A + D) genotype as 75% of the patients with genotype A showed virologic response as compared to 25% of the patients with mix genotype infection (Table 3). There was no difference between both the groups in combined response and HBsAg clearance. Neither genotype A nor the mix genotype showed a significant difference with genotype D infected patients in any type of response rate (Table 3).

Baseline Viral Load and Patient's Response

A total of 10 patients in the cohort had lower than 20,000 IU/mL of HBV-DNA before treatment (baseline) while the remaining 44 had higher than 20,000 IU/mL of baseline viral load. In logistic regression analysis, baseline HBV-DNA (viral load) was found to be significantly affecting patient's virologic response. Low baseline HBV-DNA (<20,000 IU/mL) was found as a strong predictor of both virologic and combine response, as patients with low HBV-DNA had a significantly greater trend of both virological response (OR: 7.15, p=0.044) and combined response (OR: 16.30, p=0.007) as compared to the patients with high (>20,000 IU/mL) baseline HBV-DNA. However, HBsAg clearance was not significantly (p=0.055) higher for low baseline HBV-DNA as compared to high baseline HBV-DNA though an OR of 8.52 (95% CI: 0.95-76.35) was observed (Table 2).

When compared with chi-square test, it was observed that HBV-DNA level at baseline was significantly associated with all three types of patients' responses (Table 4). The patients with low viral load at baseline had significantly higher rates of virologic response (p=0.012), combined response (p=0.005) and HBsAg clearance (p=0.008) as compared to the patients having high viral load at baseline (Table 4).

Discussion

This study reports a higher rate of all types of response rates for genotype A than mix genotype infection (A + D), but it also reports that the response rates between genotype A and genotype D infected patients is not significantly different. The study also compared the single genotype infection and dual genotype infection. Dual genotype infection (A + D) was found to be significantly less responsive as compared to mono-genotype infection specially with genotype A. The results of the current study are in part consistent and in part not consistent with some of the previous reports (9,13,14,15). These studies found a higher response rate of patients with genotype A compared to genotype D patients. We also noted that genotype A is the most sensitive genotype but its response rate is not significantly higher than genotype D, yet it is significantly higher than mix genotype infection. These results indicate that genotype play a role in response of patients to peginterferon-α-2a therapy and are partially supporting the results of another study (5) who reported that the patients with different genotypes have different response rates.

In the current study, single genotype infection was found to be more sensitive as compared to the mix genotype infections. This result is not supported by some of the previously published literature because of the fact that the mix infection with these genotypes (A and D) is less commonly found in the world and less studied. However, it is present in a considerable number of patients

Table 2. Logistic regression analysis for effect of baseline factors on virologic response, combined response and HBsAg clearance

Factor	Comparison	Virologic response		Combined response		HBsAg clearance	
		OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Genotype	Mono v Mix	3.92 (1.21-12.67)	**0.022**	3.00 (0.71-12.66)	0.135	4.60 (0.50-42.37)	0.178
	A v D	3.30 (0.54-20.27)	0.197	0.83 (0.13-5.35)	0.848	3.17 (0.36-27.57)	0.297
	A v Mix	3.00 (1.19-7.56)	**0.020**	1.52 (0.56-4.16)	0.408	2.77 (0.77-9.97)	0.119
	D v Mix	3.00 (0.86-10.42)	0.084	3.27 (0.72-14.73)	0.123	3.63 (0.35-37.83)	0.281
Age	Young v Old	2.93 (0.72-11.89)	0.132	3.91 (0.68-22.54)	0.127	1.02 (0.12-8.59)	0.982
Gender	Male v Female	0.25 (0.05-1.15)	0.075	0.86 (0.15-4.87)	0.862	1.41 (0.09-21.24)	0.800
ALT	Low v High	2.92 (0.69-12.48)	0.060	2.05 (0.44-9.75)	0.364	3.11 (0.38-25.46)	0.290
HBV-DNA	Low v High	7.14 (1.05-48.52)	0.044	16.30 (2.12-125.18)	0.007	8.52 (0.95-76.35)	0.055
HBeAg	Neg v Pos	1.21 (0.29-5.10)	0.787	0.57 (0.09-3.43)	0.540	1.53 (0.16-14.84)	0.714

v: Versus, Low HBV-DNA: <20,000 IU/mL, High HBV-DNA: ≥20,000 IU/mL, Low ALT: Elevated up to 2xULN, high ALT: >2xULN, Neg: Negative, Pos: Positive, Young: <40 years, Old: ≥40 years, Mix: A + D, mono: Genotype A or D only, OR: Odds ratio, CI: Confidence interval, HBsAg: Hepatitis B surface antigen

Table 3. Effect of genotype on rate of virologic response, combined response and HBsAg clearance

Response	Genotype A (n=8)	Genotype D (n=22)	Genotype A + D (n=24)
Virologic response			
n, (%)	6 (75)	11 (50)	6 (25)
p-value	**0.030***	0.126	-
Combined response			
n, (%)	2 (25)	7 (32)	3(12)
p-value	0.578	0.159	-
HBsAg Clearance			
n, (%)	2 (25)	3 (14)	1 (4)
p-value	0.147	0.336	-

*Pearson chi-square test (Fisher's exact test, 2 sided), genotype A versus A + D, HBsAg: Hepatitis B surface antigen

Table 4. Effect of baseline HBV-DNA on rate of virologic, combine and HBsAg response of the patients

Response	Low HBV-DNA (n=10)	High HBV-DNA (n=44)
Virologic response		
n, (%)	8 (80)	15 (28)
p-value		**0.012***
Combined response		
n, (%)	6 (60)	6 (14)
p-value		**0.005***
HBsAg clearance		
n, (%)	4 (40)	2 (4.5)
p-value		**0.008***

*Pearson chi-square test (Fisher's exact test, 2 sided), HBV: Hepatitis B virus, HBsAg: Hepatitis B surface antigen

in Pakistan. This is the first report involving the influence of mix infection with genotypes A and D on treatment response of chronic HBV patients. Further studies may highlight the case more clearly.

Besides genotype, baseline viral load was also recognized as an important factor in virologic response, combine response and HBsAg clearance of the patients in our study. Patients with low baseline viral load showed a significantly higher rate of all three types of responses. This study supports the previous studies in regard of the finding that patients with low HBV-DNA are more likely to respond to therapy than the patients having high baseline HBV-DNA as a lot of previous studies also reported almost similar findings (14,16,17). Similarly, low baseline HBV-DNA was found to be a predictor to peginterferon-α-2a therapy by a study in HBeAg negative patients (5).

Low HBV-DNA at baseline was also found to have association with better response in other therapies like adefovir, lamivudine and telbivudine in many studies (5,18,19,20,21). Our study also confirm the role of baseline HBV-DNA in treatment response from Pakistan which was not known previously. However, for confirmation, the role of genotype in treatment of chronic HBV patients suggested by this study as well as by some previous studies described above may be investigated further with larger data size and all types of antiviral therapies being used.

Conclusion

Genotype A infection and low viral load (HBV-DNA) at baseline are strong predictors of patients' response to peginterferon-α-2a therapy. The study concludes that genotype A infected patients have a better chance of virological response to peginterferon-α-2a therapy than the patients having mix infection with genotypes A and D simultaneously. Patients with <20,000 IU/mL of HBV-DNA at baseline have better rate of virological response, combine response and HBsAg clearance than the patients having >20,000 IU/mL of HBV-DNA at baseline.

Ethics

Ethics Committee Approval: The study was approved by the Ethics Committee of University of the Poonch Rawalakot, Azad Jammu and Kashmir (approval number: UPR/HAEC/2020/M3/C07).

Informed Consent: All the patients signed a written informed consent.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: M.M., M.A.A., Design: M.M. Data Collection or Processing: M.M., M.A.A., M.N.H., Z.A., Analysis or Interpretation: M.M. Literature Search: M.M., M.N.H. Writing: M.M., Z.A.

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Research Article

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Knowledge Level of Physicians in Turkey about Hepatitis C and New Treatments

Türkiye'deki Hekimlerin Hepatit C ve Yeni Tedavileri Konusundaki Bilgi Düzeyi

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ABSTRACT

Objectives: The aim of this study was to evaluate the knowledge level of the physicians in Turkey about the ways of transmission of hepatitis C, prevention, general clinical approach, and new hepatitis C treatment protocols and to increase awareness on this issue.

Materials and Methods: The study was conducted between January 2020 and March 2020 among physicians who were specialists and on specialty training. Three hundred and eight physicians were surveyed on hepatitis C diagnosis, treatment, and clinical approach.

Results: When asked whether hepatitis C was a notifiable disease, 88 physicians (28.6%) answered "no", while 167 physicians (54.2%) answered "yes". While 33.6% of surgical branches had the correct answer that the disease was transmitted by birth in terms of the transmission route, it was found that 20% of physicians answered correctly in non-surgical branches. When the curative treatment of hepatitis C was asked, 70 physicians (22.7%) answered that there was no treatment, while 238 (77.3%) physicians answered that there was a definitive treatment.

Conclusion: With the use of direct-acting antivirals in hepatitis C infection, the chance of cure has increased greatly, and the fact that physicians are not fully aware of this new information may cause deficiencies in the patients' guidance.

Keywords: Chronic viral hepatitis C, antiviral agents, clinical protocols

ÖZ

Amaç: Çalışmanın amacı Türkiye'deki uzmanlık eğitimi alan ya da uzman olan hekimlerin hepatit C'nin bulaş yolları, korunma, genel klinik yaklaşım ve yeni hepatit C tedavi protokolleri konusundaki bilgi düzeylerini değerlendirmek ve bu konudaki farkındalığı artırmaktır.

Gereç ve Yöntemler: Çalışmada, Ocak 2020 ile Mart 2020 tarihleri arasında uzman ve uzmanlık eğitimi alan hekimlere hepatit C tanı, tedavi ve klinik yaklaşımları konularında anket uygulandı. Çalışmadaki 308 hekim dahili ve cerrahi branşlar olarak iki grup altında değerlendirildi.

Bulgular: Hepatit C'nin bildirimi zorunlu bir hastalık olup olmadığı sorulduğunda 88 hekim (%28,6) hayır olarak cevaplarken, 167 hekim (%54,2) evet olarak belirtti. Cerrahi branşların %33,6'sı hastalığın bulaşma yolu açısından doğumla bulaşabildiği doğru cevabını verirken, dahili branş hekimlerinin %20'sinin doğru cevap verdiği görüldü. Hepatit C'nin küratif tedavisi değerlendirildiğinde 70 hekim (%22,7) "tedavisi yok" olarak cevaplarken, "kesin tedavisi var" diyen 238 (%77,3) hekim mevcuttu.

Sonuç: Direk etkili antivirallerin hepatit C enfeksiyonunda kullanılması ile birlikte kür şansı çok artmış olup bu yeni bilgiye hekimlerin tam hakim olmamaları hastaların yönlendirilmesinde eksikliklere neden olabilir.

Anahtar Kelimeler: Kronik viral hepatit C, antiviral ajanlar, klinik protokoller

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Introduction

More than 1.4 million people die every year in the world due to acute hepatitis infection, hepatitis-related liver cancer, and cirrhosis. Unless action is taken, it is believed that by 2040, hepatitis B- and hepatitis C-related deaths will affect a larger population than that of deaths from human immunodeficiency virus, tuberculosis, and malaria (1). Hepatitis C is the main cause of liver cancer and liver transplant. It is known that approximately 400,000 people die of hepatitis C-related causes every year (2). Most of these deaths from hepatitis C could be completely prevented if people were aware of hepatitis C virus (HCV) infection and had access to appropriate treatment (3). For all of these reasons, hepatitis C is one of the most important current health problems. In Turkey, the incidence of hepatitis C is approximately 1% (4).

Hepatitis C is transmitted from an infected mother to the baby during delivery, rarely by unprotected sexual contact with an infected partner, through contacts such as blood and blood products transfusion, solid organ transfusion from an infected donor, intravenous (IV) drug use, unsafe therapeutic injections, and occupational exposures such as needle-sticks (4,5,6). Among these, the most important transmission routes are blood transfusion from a donor who has not been screened, IV drug use, and unsafe therapeutic injections (7,8). Knowing the ways in which it can be transmitted is necessary so as to provide protection. An important step toward the eradication of this disease may lie in having the knowledge that it is possible to treat it with newly developed direct antivirals by healthcare professionals and referring those who have the disease to infectious diseases and gastroenterology physicians so that they can be treated. Nowadays, curative treatment options for hepatitis C disease have been found, and persistent virological responses that exceed 95% have been obtained (9). Treatment protocols are constantly changing today, and when the literature was evaluated, there were insufficient studies that have evaluated the level of knowledge of physicians about hepatitis C transmission routes and new treatments.

The aim in this study was to evaluate the knowledge level of physicians who have been in training or who are specialists in Turkey, about the ways in which hepatitis C can be transmitted, the clinical approach toward the disease, and new treatment protocols.

Materials and Methods

The study was conducted on physicians who were specialists or were receiving specialty training between January and March 2020. There were 21 questions in the questionnaire about hepatitis C disease for the physicians. Multiple-choice questionnaire forms were prepared, which included both correct and incorrect answers for the physicians to choose from. Physicians from internal branches (internal medicine, pediatrics, chest diseases, cardiology) and surgical branches (thoracic surgery, general surgery, cardiovascular surgery, otolaryngology, orthopedics, neurosurgery, gynecology, ophthalmology) were included in the study. A total of 308 physicians, 149 of whom were in internal branches and 159 surgeons from surgical branches, participated in the study. Infectious diseases and gastroenterology physicians were excluded from the study because they were working in a relevant branch, assuming that they had this knowledge. In addition, incomplete questionnaires were excluded from the study.

The demographic characteristics of the physicians, epidemiology of hepatitis C, transmission routes, approaches to diagnosis, and current treatment options were asked about in the survey.

The study was approved by Ethics Committee Yedikule Chest Diseases and Thoracic Surgery Research and Education Hospital (approval number: 2021-109, date: 08.04.2021) and was conducted in accordance with the principles of the Declaration of Helsinki. Authors took informed consent from all participants in this study.

Statistical Analysis

Chi square analysis was applied to show the relationship between the demographic data of the physicians and descriptive statistics and categorical data, and $p<0.05$ was considered as statistically significant. IBM SPSS Statistics for Windows 22.0 (IBM Corp., Armonk, NY, USA) was used for the calculations.

Results

There were 160 female (51.9%) and 148 male (48.1%) physicians included in the study. The average age of the physicians was 36.1±6.1 years, and there were 71 (23.1%) individuals aged 40 and over, and 237 (76.9%) who were under 40 years of age. Moreover, 149 (48.4%) physicians were from surgical branches and 159 (51.6%) were from internal branches. With regard to the specialist physicians, the average number of years that they had spent as a specialist was 9.6±5.7. There were 62 residents and 246 specialist physicians in the study (Table 1).

While 13 of the surgical physicians had not had hepatitis C screening tests before, 136 of the surgery physicians (91.3%) had previously had hepatitis C screening tests. While 23 of the internal physicians had not had hepatitis C screening test before, 136 (85.5%) had previously had a screening test for hepatitis C. There were no statistically significant differences between the groups (p=0.11). When asked whether hepatitis C is a notifiable disease, 88 physicians (28.6%) answered no, 167 physicians (54.2%) answered yes, and 53 (17.2%) answered I do not know. Of the surgical sciences, 38 (25.5%) physicians answered no, 86 (57.7%) answered yes, and 25 (16.8%) did not know. Fifty physicians from internal sciences answered the same question as no (31.4%), 81 physicians (50.9%) answered yes, and 28 physicians (17.6%) answered I do not know. There were no statistically significant differences between the groups (p=0.44).

Table 2 contains details of the questions asked to the physicians in the questionnaire to examine their knowledge of transmission routes.

When the physicians were asked which tests should be ordered in the diagnosis of hepatitis C, the number of physicians who gave HCV-RNA and anti-HCV responses together was 160 (51.9%). While there were 74 physicians (49.7%) who marked the correct diagnostic test options in the surgical departments, this number was 86 (54.1%) in the internal sciences (p=0.43). While 112 (70%) of the physicians who knew the correct diagnostic tests had 10 years or less of specialization, 48 (43.2%) physicians with a specialization duration of more than 10 years answered the question correctly, and a statistically significant difference was found (p=0.02). When the answers of the physicians to the questions about the course of hepatitis C were evaluated, 146 physicians (47.4%) stated that hepatitis C could be treated

Table 1. Demographic characteristics of physicians			
Variables		**n**	**%**
Age (year) (mean ± SD)		**35.1±6.1**	
Gender	Male	148	48.1
	Female	160	51.9
Age (year)	<40	237	76.9
	>40	71	23.1
Branches	Surgical	149	48.4
	İnternal	159	51.6
Specialty duration (mean ± SD) (year)		**9.6±5.7**	
Specialty duration	<10	197	64
	>10	111	36
Residents		62	20.1
Specialist physicians		246	79.9
SD: Standart deviation			

Table 2. Comparison of knowledge of transmission routes						
Variables		**Surgical sciences**		**Internal sciences**		**p-value**
		n	**%**	**n**	**%**	
Transmitted by blood transfusion	True	145	97.3	157	98.7	0.36
	False	4	2.7	2	1.3	
Transmitted by unprotected sexual contact	True	115	77.2	134	84.3	0.11
	False	34	22.8	25	15.7	
It is transmitted by needle stick	True	143	96	151	95	0.67
	False	6	4	8	5	
It is transmitted by blood contact with disintegrated skin	True	133	89.3	136	85.5	0.32
	False	16	10.7	23	14.5	
It is not transmitted via fecal-oral contact	True	131	87.9	140	88.1	0.97
	False	18	12.1	19	11.9	
Transmitted by tattoo	True	121	81.2	128	80.5	0.87
	False	28	18.8	31	19.5	
The disease is not transmitted by sweat and tears	True	121	81.2	137	86.2	0.23
	False	28	18.8	22	13.8	
Transmitted by birth	True	50	33.6	32	20.0	0.008
	False	99	66.4	127	79.9	
Transmitted by dental treatment	True	109	73.2	135	84.9	0.01
	False	40	26.8	24	15.1	
the disease is not transmitted by breastfeeding	True	117	78.5	130	81.8	0.47
	False	32	21.5	29	18.2	
Transmitted by acupuncture	True	86	57.7	77	48.4	0.10
	False	63	42.3	82	51.6	
Transmitted by piercing	True	105	70.5	107	67.3	0.54
	False	44	29.5	52	32.7	
It is not transmitted by shaking hands	True	146	98	155	97.5	1
	False	3	2	4	2.5	
Not transmitted by kissing	True	135	90.6	148	93.1	0.42
	False	14	9.4	11	6.9	
Transmitted by contaminated shaver	True	112	75.2	130	81.8	0.15
	False	37	24.8	29	18.2	
Transmitted by hemodialysis	True	112	81.9	136	85.5	0.38
	False	27	18,1	23	14,5	

spontaneously. When the curative treatment of hepatitis C was questioned, 70 physicians (22.7%) thought that there was no full cure treatment and 238 (77.3%) thought that there was a definitive treatment. While there were 115 (77.2%) surgeons and 123 (77.4%) medical practitioners who knew that there is a curative treatment of hepatitis C (p=0.97); the correct response rate was 73.9% in physicians who had been specialists for 10 years or more, and 79.2% in physicians who had specialized for less than 10 years (p=0.28). When asked if hepatitis C does not cause sudden death, 255 physicians (82.2%) answered correctly. When asked if hepatitis C causes cirrhosis in the long term, 294 physicians (95.5%) answered the question correctly. While the number of physicians who correctly knew that there were oral antiviral treatment options in the treatment was 130 (42.2%), there were 284 physicians (92.2%) who thought that a hepatitis C vaccine was available. Table 3 contains the comparison of the responses of the physicians regarding the diagnosis, treatment, and prognostic approaches of hepatitis C according to their branches.

In the table, it can be seen that 47.7% of the physicians (n=147) answered that they approached all patients similarly when an invasive procedure was going to be performed in HCV positive patients. Moreover, 44.5% (n=137) stated that sent the patient to consult with infectious diseases specialists before the procedure, and 2.99% (n=9) would refer the patient to a higher-level center. While only 3 physicians (1%) stated that they would withdraw from the interventional procedure, 2 physicians (0.6%) stated that they would reevaluate the surgical indication.

Discussion

The aim of this study was to evaluate whether the information about patients with HCV, which can be seen widely in internal and surgical clinics, is up-to-date and accurate in terms of new therapies and patient management. The questions asked in the questionnaire were guiding questions that had selectivity and reliability, and they aimed not only to measure the knowledge level of the physicians on the subject, but to also raise awareness by arousing their curiosity. The questionnaire aimed to distinguish the current level of knowledge and awareness of internal and surgical clinic department physicians. Studies in the UK, Australia, and the USA have suggested that there are gaps in HCV knowledge among clinical physicians, primary care physicians, and other healthcare professionals (10,11,12). In a study conducted by Coppola et al. (10), which examined HCV information among physicians providing primary health care services in the USA, it was revealed that there were mistakes in the HCV information. Although there is currently no HCV vaccine available, 66% of healthcare professionals recommended vaccinating people with an HCV vaccine. In the current study, there were internal physicians (5.7%) and surgical physicians (10.1%) who thought that there was a vaccine for hepatitis C. Studies have shown that many physicians, including primary care physicians, not only medical school students receiving education, have insufficient knowledge about HCV (13). In this study, it was found that the number of surgical physicians (49.7%) and internal physicians (54.1%) who knew the diagnostic tests that are required to diagnose HCV was extremely low.

Table 3. Comparison of Hepatitis C diagnosis, treatment, and prognostic approaches

Variables		Surgical clinics		Internal clinics		p-value
		n	%	n	%	
Diagnostic tests in HCV are anti-HCV and HCV-RNA	True	74	49.7	86	54.1	0.43
	False	75	50.3	73	48.9	
HCV can be curatively treated	True	115	77.2	123	77.4	0.96
	False	34	22.8	36	22.6	
HCV has a treatment	True	125	77.2	123	77.4	0.97
	False	34	22.8	36	22.6	
HCV can recover spontaneously	True	56	37.6	90	56.6	0.001
	False	93	62.4	39	43.4	
Sudden death can occur in HCV	Can not occur	121	81.2	134	84.3	0.47
	Can occur	28	18.8	25	15.7	
HCV causes cirrhosis	True	137	91.9	157	98.8	0.004
	False	12	8.1	2	1.3	
Over 95% of HCV is curative	True	61	40.9	69	43.4	0.66
	False	88	59.1	90	56.6	
There are oral treatment options in the treatment of HCV	True	41	27.5	89	56	<0.001
	False	108	72.4	70	44	
Hepatitis C does not have a vaccine	True	134	89.9	150	94.3	0.14
	False	15	10.1	9	5.7	
Pre-operative screening test should be done	True	140	94	144	90.6	0.26
	False	9	6	15	9,4	

Mencl et al. (11) evaluated the knowledge of universal precautions among emergency medical professionals and stated that 22% of the healthcare professionals thought that hepatitis could be transmitted by air. Most healthcare workers in studies done abroad described the main routes of transmission as blood transfusions, exposure to blood during sexual activity, and sharing needles while injecting drugs (14,15). None of the questions in the survey herein were answered 100% correctly. When asked about the risk of contagion with a needle stick, 6 of the 149 surgical physicians and 8 of the 159 internal physicians said that there was no risk of hepatitis C transmission through needle stick injury.

Bianco et al. (14) stated in their study that they thought that 12% of nurses working in hemodialysis clinics in Italy could transmit HCV by kissing. In addition, 19% of the nurses emphasized that they did not know that tattooing can be a way to transmit HCV. In the current study, it was observed that 81.2% of the surgical clinic physicians and 80.5% of the internal clinic physicians knew that it could be transmitted by tattooing. While the rate of surgeons who thought that HCV could be transmitted by kissing was 9.4% and the rate of physicians in the internal clinics was 6.9%.

In this study, when the information about the transmission routes was examined, it was observed that there were deficiencies in the knowledge that hepatitis C can be transmitted through birth, where 33.6% of the surgeons and 20% of the internal sciences physicians knew that hepatitis C could be transmitted through birth. While the level of knowledge in both branches was actually low (below 50%), it was statistically observed that the level of knowledge in the internal clinics was significantly lower (p=0.008). In this respect, the fact that the surgical clinics have more knowledge than internal clinics may be that the branch of obstetrics, which deals with labor and births, is within the surgical clinics.

In a study of Brazilian dentists, 20% of dentists said there was no risk of HCV transmission during dental treatment, and 13% believed there was an HCV vaccine, showing a lack of clear information about HCV (16). In the current study, there were no dentists participating. When dental treatment and hepatitis C transmission were questioned, 73.2% of the surgeons and 84.9% of the internal physicians gave the correct answer. It was observed that the knowledge of the internal physicians on this issue was significantly higher (p=0.01). Most of the studies carried out showed that there is a lack of knowledge about hepatitis C and that it would be appropriate to develop training programs to update the knowledge of these physicians (11). Another study that looked at the knowledge levels of risk groups and young adults in the community showed that the percentage of correct answers about awareness was 54% and 43%, respectively (17).

Two recent studies, published after the introduction of new interferon-free direct-acting antiviral (DAA) treatments, focused on whether the curability of HCV is known (18). Among health care providers, the specialists (i.e. hepatologists, gastroenterologists, and hepatology nurses) scored higher than the practitioners in questionnaires measuring information about HCV treatment. In the survey, 7 of the 10 primary care physicians were not aware of the new interferon-free DAAs and their mechanism of action (18). In our study, the rate of surgical physicians who knew that hepatitis C had oral treatment with DAAs was 27.5%, while the rate of internal physicians who thought the same was 56%. Moreover, the difference was statistically significant (p<0.001). The rate of surgical physicians who thought that the treatment of hepatitis C was over 95% successful was 40.9%, while the rate of internal physicians who thought the same was 43.4%. The information that patients who have hepatitis C can recover spontaneously was known by 37.6% of those in the surgical branches and 56.6% of those in the internal clinics. The difference between them was statistically significant and drew attention to the lack of knowledge of surgical physicians on this subject.

Study Limitations

The small sample size in the study and the fact that it was a survey study increased the degree of bias.

Conclusion

With the use of DAAs in hepatitis C infection, the chance of cure has increased a great deal, and it may be accepted as natural that this new information is unknown to physicians who do not primarily deal with this issue, except infectious physicians and gastroenterologists. However, since their lack of full knowledge of this issue may cause deficiencies in the referral of patients, it may result in them missing patients who have a chance to be cured, so all physicians should be given in-service information about new information and developments on infectious diseases so that physicians can update themselves. Emphasizing the importance of updating the information learned during medical school education and raising awareness of advancing medical science and emerging treatments and interventions will make a difference in terms of both healthcare providers, patients benefiting from healthcare services and improving public health.

Ethics

Ethics Committee Approval: The study was approved by Ethics Committee Yedikule Chest Diseases and Thoracic Surgery Research and Education Hospital (approval number: 2021-109, date: 08.04.2021).

Informed Consent: Authors took informed consent from all particiant in this study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: A.İ.S., K.K.Y., Design: A.İ.S., K.K.Y., Supervision: A.İ.S., K.K.Y., Resources: A.İ.S., K.K.Y., Materials: A.İ.S., K.K.Y., Data Collection and/or Processing: A.İ.S., K.K.Y., Analysis and/or Interpretation: A.İ.S., K.K.Y., Literature Search: A.İ.S., K.K.Y., Writing Manuscript: A.İ.S., K.K.Y., Critical Review: A.İ.S., K.K.Y.

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Research Article

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Tenofovir Disoproxil Fumarate in the Management of Chronic Hepatitis B Infection in Children

Çocuklarda Kronik Hepatit B Enfeksiyonunda Tenofovir Disoproksil Fumarat Tedavisi

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ABSTRACT

Objectives: The aim of this study was to present real-world data regarding the efficacy and safety of tenofovir disoproxil fumarate (TDF) in pediatric patients with chronic hepatitis B (CHB).

Materials and Methods: In this observational retrospective cohort study, medical records of 10 children with CHB receiving TDF were reviewed.

Results: All patients were positive for hepatitis B e antigen (HBeAg) at baseline. HBV-DNA <400 copies/mL was achieved in 70% of the patients, while 20% had undetectable levels of HBV-DNA at last visit. The median HBV-DNA at baseline was approximately 8 $\log_{10}$ copies/mL and decrease in HBV-DNA levels after 3 months, 12 months and at last visit was approximately 3.2 $\log_{10}$ copies/mL, 5.2 $\log_{10}$ copies/mL and 6.1 $\log_{10}$ copies/mL, respectively. All but 1 had (n=9, 90%) elevated transaminases at baseline and serum alanine aminotransferase (ALT) levels were normalized in an average of 10.1 (3.7; 5-16) months in 7 patients. Three nucleos(t)ide-naïve patients (30%) experienced HBeAg loss and seroconversion in 12 to 18 months. There were no observed serious adverse events. Renal function was maintained well through follow-up in all patients.

Conclusion: Tenofovir monotherapy is effective in terms of virologic and biochemical responses in pediatric patients with CHB. Tenofovir has a favorable safety profile.

Keywords: Antiviral, chronic hepatitis B infection, nucleos(t)ide analog, tenofovir disoproxil fumarate

ÖZ

Amaç: Bu çalışmanın amacı, pediatrik kronik hepatit B (KHB) tedavisinde tenofovir disoproksil fumaratın (TDF) etkinliği ve güvenliği ile ilgili verileri sunmaktır.

Gereç ve Yöntemler: Bu gözlemsel retrospektif kohort çalışmasında, TDF tedavisi alan KHB enfeksiyonu olan 10 çocuğun tıbbi kayıtları geriye dönük olarak incelendi.

Bulgular: Tedavi başlangıcında tüm hastalarda hepatit B e antijen (HBeAg) pozitifti. Son poliklinik kontrolünde, hastaların %70'inde HBV-DNA <400 kopya/mL olarak saptanırken, %20'sinde HBV-DNA negatifti. Başlangıçtaki ortanca HBV-DNA değeri yaklaşık 8 $\log_{10}$ kopya/mL idi ve 3. ay, 12. ay ve son kontrolde HBV-DNA değerlerinde sırasıyla yaklaşık 3,2 log10 kopya/mL, 5,2 $\log_{10}$ kopya/mL ve 6,1 $\log_{10}$ kopya/mL düşüş saptandı. Tedavi başlangıcında biri hariç tüm hastaların (n=9, %90) serum alanin aminotransferaz (ALT) seviyeleri yüksekti ve 7 hastada ortalama 10,1 (3,7; 5-16) ayda ALT seviyesi normale döndü. Daha önce hiç nükleoz(t)it analoğu almayan 3 hastada (%30), 12 ila 18 ayda HBeAg kayboldu ve serokonversiyon görüldü. Hastaların hiçbirinde ciddi yan etki gözlemlenmedi. Hastaların takipleri boyunca böbrek fonksiyonları normal sınırlarda seyretti.

Sonuç: Tenofovir monoterapisi, KHB enfeksiyonu olan pediatrik hastalarda virolojik ve biyokimyasal tedavi hedeflerine ulaşmak açısından etkilidir. Çocuklarda tenofovir tedavisi güvenlidir.

Anahtar Kelimeler: Antiviral, kronik hepatit B enfeksiyonu, nükleoz(t)id analoğu, tenofovir disoproksil fumarat

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Introduction

Hepatitis B infection is caused by the hepatitis B virus (HBV) and can be either acute or chronic. It is estimated that worldwide, 240 million are chronically infected by HBV (1). Chronic hepatitis B (CHB) - defined as persistence of hepatitis B surface antigen (HBsAg) for six months or more - in pediatric patients is a major health problem due to high overall prevalence of the disease globally despite the advances in prevention, diagnosis, and management strategies (2). Chronic HBV infection during childhood has been considered to follow a rather benign course as they are generally in the immune-tolerant phase and the majority of children will not require antiviral therapy. However, early identification and monitoring of children at risk for progression of liver disease remains important due to the risk of developing cirrhosis or hepatocellular carcinoma before adulthood in asymptomatic carriers is non-negligible with the risk of 3-5% and 0.01-0.03%, respectively (3).

Lack of appropriate clinical trials and delay in licensing of new drugs in children are some most important issues regarding the management of pediatric CHB. The therapeutic options for pediatric CHB comprises of five drugs: interferon-alpha (INF-α), lamivudine (LMV), entecavir, adefovir, and tenofovir (4). Tenofovir disoproxil fumarate (TDF) is an oral prodrug of tenofovir with an excellent safety profile. Tenofovir was approved by the US Food and Drug Administration for the treatment of CHB infection in adolescents ≥12 years in March 2010 and in children 2 to <12 years of age weighing ≥10 kilograms in November 2018. The European Medicines Agency has also approved tenofovir for pediatric populations.

Data regarding TDF treatment in children with CHB is promising but limited. Results from a previous clinical trial in adolescents have indicated that tenofovir is an effective and safe treatment option in adolescents older than 12 years old with no observed resistance (5). The results of a phase 3 clinical trial for evaluation of efficacy and safety profiles of TDF in children aged 2 to 12 years with chronic HBV infection revealed higher rates of HBV-DNA suppression and alanine aminotransferase normalization compared to placebo with no resistance at week 48 (6). Recently, TDF monotherapy was reported to be superior to LMV monotherapy in terms of antiviral efficacy in nucleos(t)ide-naïve children and adolescents with CHB (7).

The aim of the present study was to present real-world data regarding the efficacy and safety of tenofovir treatment in pediatric CHB patients.

Materials and Methods

Study Design and Patients

This was an observational retrospective cohort study. All available medical records from 10 CHB patients who were treated with tenofovir at our institution between 2012 and 2018 were retrospectively reviewed. The study was approved by the Non-interventional Ethics Committee of the hospital and conducted in accordance with the principles of the Declaration of Helsinki (approval number: GO 18/575-16, date: 21/06/2018).

The inclusion criteria were as follows: patients <18 years at the time of treatment initiation who were put on tenofovir treatment as a first line therapy or switched from another nucleos(t)ide analog (NA) due to persistent viremia despite adequate treatment for a minimum of 24 weeks before switching to tenofovir, continuation of tenofovir treatment at least 12 months, having available clinical, laboratory and histopathologic data, pretreatment HBV-DNA level >10^4 copies/mL, pre-treatment alanine aminotransferase (ALT) levels more than two times the upper limit of the normal value persisting for >6 months or >3 months without HBV-DNA decrease, pathology revealed histological activity index ≥ grade 4 and/or fibrosis ≥ stage 2 according to the Ishak score or regardless of the ALT level fibrosis ≥ stage 2 according to the Ishak score. Patients with a history of any concurrent liver disease, patients with concomitant hepatitis C infection and immunocompromised patients were excluded. TDF dosage was determined according to the body weight of the patients as recommended by the manufacturer and relevant guidelines. All of the patients in the study were >35 kg in weight and received oral TDF 300 mg once daily.

Clinical data including age at diagnosis, follow up duration to initiation of first treatment, follow up duration to initiation of tenofovir treatment, treatment indication, previous treatment history, type, duration and outcome of previous treatments, reason for switching from another nucleoside analog to tenofovir were recorded. Hemogram, transaminases, liver and kidney function tests, hepatitis B e antigen (HBeAg) and antibody to hepatitis B e antigen (anti-HBe) status, HBV genotype, HBV-DNA, serum alpha-fetoprotein level and hepatobiliary ultrasonography findings were recorded. Liver biopsy was performed prior to initiation of antiviral treatment in all subjects and histologic grading and staging were done with Ishak score by an experienced pathologist. All patients were positive for HBsAg and HBeAg at baseline. Serologic (HBeAg loss and seroconversion to anti-HBe for HBeAg-positive patients), virologic (complete response if HBV-DNA level is undetectable) and biochemical (normalization of ALT levels) responses were evaluated on the follow up of every patient. Any side effect related to tenofovir treatment was noted.

Statistical Analysis

All data were summarized in a descriptive fashion. No statistical testing was performed. Data were presented using descriptive statistics [mean with standard deviation (SD) or median with range for continuous variables, and n (%) for categorical values].

Results

Patient Characteristics

A total of 10 patients treated with tenofovir in our center were enrolled in the study. The demographic and baseline characteristics of the patients are summarized in Table 1. Half of the patients were nucleos(t)ide-naïve before tenofovir treatment and four of them received TDF as the first line CHB therapy. The mean (SD; range) time from the first HBV treatment to initiation of TDF treatment in patients with prior treatment history (n=6, 60%) was 36.6 (19.2;

Table 1. Patient demographics and baseline characteristics	
n (male/female)	10 (7/3)
Age at diagnosis, years, mean ± SD (range)	5.9±3.8 (0.8-13)
Age at the time of first HBV treatment, years, mean ± SD (range)	12.8±3.5 (4.8-16.6)
Age at the time of TDF treatment, years, mean ± SD (range)	14.8±2 (10.4-17.8)
Prior treatment, n (%)	6 (60%)
INF-α (5 M units/m^2)	1
LMV	3
INF-α (5-8 M units/m^2) followed by LMV	2
Baseline HBV-DNA, $\log_{10}$ copies/mL, median (range)	8 (4.3-9.7)
Baseline ALT, U/L, mean ± SD (range)	110.6±58.5 (20-232)
Normal ALT at baseline, n (%)	1 (10%)
Liver biopsy before TDF treatment, n (%)	9 (90%)
Time to normalization of ALT after TDF treatment, months, mean ± SD (range)	10.1±3.7 (5-16)

HBV: Hepatitis B virus, TDF: Tenofovir disoproxil fumarate, LMV: Lamivudine, ALT: Alanine aminotransferase, INF-α: Interferon-alpha; SD: Standard deviation

12-68) months. The mean TDF treatment duration at the time of data collection was 34 (5.6; 24-42) months. All but one had (n=9, 90%) elevated ALT levels at baseline. Hepatobiliary ultrasonography findings were normal in all patients except minimal hepatomegaly which was detected in three patients. Serum alfa-fetoprotein levels were in the normal range in all patients throughout the study period.

Efficacy

Complete virologic response which is defined by undetectable levels of HB-DNA was achieved by only 10% (n=1) of the patients at the end of the first year. When the primary end point of previous TDF trial in adolescents with CHB was used, HBV-DNA <400 copies/mL was achieved by 40% (n=4) of patients by the first year. Among all patients with a mean TDF treatment duration of 34 (5.6; 24-42) months, complete virologic response and HBV-DNA <400 copies/mL were achieved by 20% and 70% of patients at the time of the data collection, respectively. HBV-DNA levels were dramatically decreased with TDF treatment (Figure 1). The median HBV-DNA at baseline was approximately 8 $\log_{10}$ copies/mL in the study group. Decrease in median HBV-DNA after three months, 12 months and at last visit was approximately 3.2 $\log_{10}$ copies/mL, 5.2 $\log_{10}$ copies/mL and 6.1 $\log_{10}$ copies/mL, respectively.

Virologic Breakthrough

Virologic breakthrough, which was defined as an increase in the HBV-DNA level more than 10-fold of patient's HBV-DNA nadir observed during therapy was detected in two patients. The reason for virologic breakthrough was poor adherence to treatment in both patients. After restoration of treatment compliance, rapid decline in patients' HBV-DNA levels was achieved.

Alanine Aminotransferase and Liver Histology

Baseline serum ALT levels were more than two times the upper limit of normal in 90% of patients. A significant decline in ALT levels parallel to the decline in viral load was observed in the study group (Figure 1). The mean ALT levels after six months and at last visit were 40.5 (30.5; 18-122) U/L and 32.4 (21.3; 16-72) U/L, respectively. Serum ALT levels were normalized in seven of the nine patients (78%) with initial hypertransaminasemia in an average of 10.1 (3.7; 5-16) months (Figure 2). Two patients who had mildly elevated transaminases at last follow-up were the ones experiencing virologic breakthrough due to treatment incompliance. The indication of CHB treatment in the only patient with normal ALT was moderate inflammation and fibrosis (histological activity index: 11, fibrosis stage: 3) on liver histology. Eight of nine patients with liver biopsy had only mild inflammation and fibrosis (histological activity index: 1-6, fibrosis stage: 0-2).

Serology

None of the patients experienced HBsAg loss during follow-up. On the other hand, three patients (30%) experienced HBeAg loss and seroconversion in 12 to 18 months after initiation of TDF treatment. All three patients were nucleos(t)ide-naïve with one of the patients had a prior INF exposure.

Safety

There were no observed serious adverse events in any of the patients that could lead to interruption of treatment. One patient experienced transient dizziness and fatigue. Cardiovascular and neurologic evaluation of the patient was normal and her symptoms were resolved without any further intervention.

Serum creatinine and electrolytes were normal in all patients at baseline and during follow-up. Nephrological pathologies were the most common co-morbidities in the study group. Hematuria due to Nutcracker syndrome (n=1), nephrolithiasis (n=1), postural proteinuria (n=1) and nephrotic syndrome due to membranous glomerulopathy (n=1) were baseline pathologies accompanying CHB in four patients (40%). Renal function was maintained well through follow-up in these children.

Discussion

The goal of anti-HBV therapy in children is to improve long-term survival and quality of life by preventing disease progression and its complications. After the approval of NAs with higher efficacy and genotypic barrier to resistance including entecavir and tenofovir, first-line treatment recommendations for adolescents have been changed. Tenofovir DF (for patients older than 12 years of age) or entecavir (for patients >16 years old) are suggested as best therapy

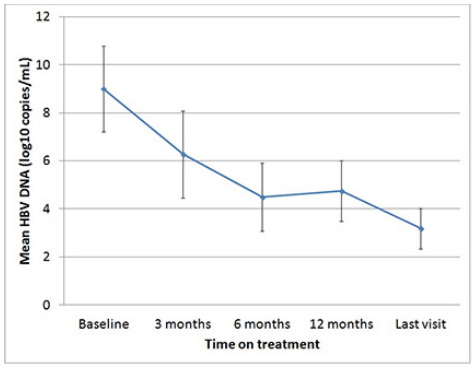

Figure 1. Mean ± standart deviation $\log_{10}$ HBV-DNA (copies/mL) throughout patient follow-up
HBV: Hepatitis B virus

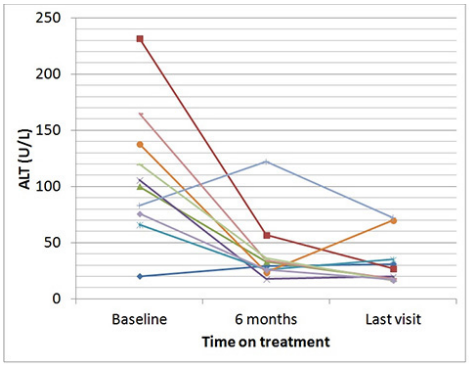

Figure 2. ALT levels of each study patient throughout follow-up
ALT: Alanine aminotransferase

options with strong recommendation and high quality of evidence by European Society of Pediatric Gastroenterology, Hepatology, and Nutrition clinical practice guideline published in 2013 (8). However, data regarding the use of TDF in pediatric CHB patients is limited.

Six of the patients in our study group received first HBV treatment before the age of 12. Five of these patients were non-responders to LMV and switched to TDF in the follow-up. A recent meta-analysis showed that TDF is a more effective rescue therapy than other options in LMV resistant patients (9). Although LMV is not considered to be a first-line treatment for children with CHB due to the low genetic barrier to drug-resistance, it is still the only NA currently approved for younger children. Our center's previous experience with LMV in children with INF refractory CHB showed significant HBV-DNA clearance rate (56.4-64.8%) but ineffective HBeAg seroconversion rates (5.6-12.7%) (10,11). In a large pediatric clinical trial of 52-week LMV treatment for HBeAg-positive children with CHB, mutations associated with drug-resistance was observed in 19% of treated children at 52 weeks (12). In a recent pediatric study comparing TDF with a historical cohort receiving LMV, antiviral resistance was reported 33.3% and 41.7% in the LMV group at 96 and 144 weeks, respectively, while, there was no viral mutation until up to 192 weeks of follow-up in the TDF group (7). High rates of genotypic resistance to older NAs emerged the need for new ones with strong antiviral effects and low resistance rates for the treatment of pediatric CHB. Introduction of TDF and entecavir, being potent NAs with high barrier to resistance, has changed the treatment recommendations in both adults and children (8,13).

A phase 3 placebo-controlled randomized clinical trial, evaluating TDF administered for 72 weeks versus placebo in adolescents aged 12 to 18 years old was published in 2012. Virologic response, defined as HBV-DNA <400 copies/mL, was achieved in 89% of TDF treated adolescent CHB patients at the end of 18 months (0% in placebo group, p<0.001). However, no statistically significant effect on HBeAg clearance was reported in this study (5). Similar results were also reported for children aged 2 to 12 years old (6). Rates of virologic response (77% vs 7%; p<0.001) and ALT normalization (52% vs 18%; p=0.002) were significantly higher in children treated with TDF compared to placebo group, while the rate of HBeAg seroconversion was similar (25% vs 24%) (6). Higher virologic response rates (81.3%, 93.8%, and 100% at 24, 48, and 96 weeks, respectively) were reported recently in TDF treated NA-naïve children (7). Recent adult studies in different CHB populations also reported similar efficacy results in terms of complete virologic response ranging from 62% to 96% (14,15,16,17). However, in our small study group only 70% of the patients achieved HBV DNA <400 copies/mL after an average of three years of treatment with TDF. Although subgroup analyses of TDF treated adolescents with CHB suggested that antiviral efficacy was high regardless of baseline ALT, HBeAg status, age, or prior HBV therapy (5), it can be speculated that some factors including poor compliance, HBV genotype, prior antiviral resistance and HBeAg positivity may be responsible for modest difference in efficacy in our study. In an adult study from Saudi Arabia, a better response to TDF has been reported in HBeAg negative patients when compared to HBeAg positive patients (84.4% vs 21.7%, respectively) (15). All patients in the present study were HBeAg positive at baseline and HBeAg seroconversion was achieved three of the patients. Moreover, the presence of adefovir, but not LMV, resistance was reported to impair TDF efficacy in NA-experienced patients (18). Although none of our patients had a history of adefovir exposure, lack of data regarding genotypic resistance makes any further conclusion impossible about impact of these factors on treatment efficacy in our cohort. Non-adherence to treatment and virologic breakthrough which we documented in two of our patients may be partly responsible for lower complete virologic response rates to TDF treatment in our study group. In adults, nearly 40% of the virologic breakthroughs were found to be correlated with medication non-adherence unrelated to antiviral drug resistance (19).

HBeAg seroconversion rate was reported to be higher in TDF treated adolescents with CHB compared to placebo group (21% vs 15%, respectively) without a statistical significance (5). A higher rate of complete response (HBeAg loss and HBV-DNA <357 IU/mL) with TDF compared to LMV was also reported at week 96, however, again without a statistical significance (41.7% vs 28.6%, p=0.443) (7). The seroconversion rate in our small cohort (30%) was comparable to previous data form children and adults with CHB (5,7,13). HBeAg loss and seroconversion was achieved in three nucleos(t)ide-naïve patients in 12 to 18 months after initiation of TDF treatment.

The current evidence demonstrates that TDF is both a safe and well tolerated choice of treatment in children (20). However, the effect of TDF on renal function and bone mineral density is a major concern in clinical practice. Data regarding the renal safety of

TDF in human immunodeficiency virus-infected children includes conflicting results with some studies reporting significant decline in estimated glomerular filtration rate, increase in serum creatinine, proteinuria and reversible hypophosphatemia (21,22,23) while an excellent renal safety profile has been reported from other cohorts (24,25). All patients in our study cohort were evaluated for renal functions before TDF treatment. Serum creatinine levels were normal at baseline and remained in the normal range during the follow-up period. Two patients had a history of proteinuria before the TDF treatment. Etiologic evaluation of patients revealed postural proteinuria which is a benign condition with excellent prognosis in one patient. The other patient had been diagnosed with HBV-associated membranous glomerulopathy at the age of 10 and on immunosuppressive treatment since then. He was unresponsive to previous treatment with LMV with persistently elevated transaminases and switch of antiviral treatment to TDF was done at the age of 14. Although TDF was reported to cause proteinuria with duration of treatment as an independent predictor (23), no exacerbation of proteinuria and renal impairment related to TDF was observed in both patients. Despite the potential renal toxicity of the drug, TDF treatment in a patient with a history of proteinuria may be used cautiously when there is no other available alternative treatment and close monitoring of renal functions should be provided in the light of present literature. The only TDF trial in children with CHB reported no significant renal complications (5). There were no significant adverse events related to TDF in our study cohort.

Study Limitations

This study was limited by the retrospective design, small number of subjects, lack of bone health assessment and lack of data regarding HBV genotype. However, relatively long-term follow-up period of patients with an average of nearly three years makes the results of this study relevant regarding efficacy, safety and resistance profile of TDF treatment in real life clinical practice.

Conclusion

TDF monotherapy is effective in terms of virologic and biochemical response in pediatric patients with CHB. In the present study, no primary non-response to TDF was observed. Normalization of ALT and at least partial virologic response with ongoing decline in viral load were achieved in all of the patients. TDF has a favorable safety profile even in patients with renal comorbidities including Nephrolithiasis, hematuria and proteinuria and can be used with close follow-up of renal functions in these patients. Although treatment compliance is an important problem in adolescents, tolerability and resistance profile of tenofovir is excellent. However, some important issues about tenofovir treatment including renal toxicity, bone health concerns and very recently reported TDF resistance in CHB patients should be addressed in further pediatric studies.

Ethics

Ethics Committee Approval: This study was approved by the Non-interventional Ethics Committee of the university (approval number: GO 18/575-16, date: 21/06/2018).

Informed Consent: Informed consent was not obtained because of the retrospective nature of the study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: E.G., A.N.K., H.H.G., H.D., İ.N.S.T., Design: E.G., A.N.K., H.H.G., H.D., İ.N.S.T., Data Collection or Processing: E.G., A.N.K., H.H.G., H.D., İ.N.S.T., Analysis or Interpretation: S E.G., A.N.K., H.H.G., H.D., İ.N.S.T., Literature Search: S E.G., A.N.K., H.H.G., H.D., İ.N.S.T., Writing: E.G., H.D., İ.N.S.T., Critical Review: H.D., İ.N.S.T.

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Research Article

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Effect of a Nationwide Universal HBV Vaccination Program and Catch-up Vaccination Campaign on HBV Prevalence in Children

Ülke Çapında Evrensel HBV Aşılama Programının ve Yakalama Aşı Kampanyasının Çocuklarda HBV Prevalansı Üzerindeki Etkisi

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ABSTRACT

Objectives: In infants vaccinated at birth against hepatitis B virus (HBV) in the context of a universal vaccination program, antibody titers may reduce over years, with a need for a booster dose at adolescence. The aim of the study was to evaluate the immunity and carriage status 8-10 years after 3 doses of HBV vaccine administered in infancy.

Materials and Methods: This was a descriptive, cross-sectional, community-based field study and was carried out between 2008 and 2011. Children with an anti-HBs titer ≤9 IU/mL, 10 to 99 IU/mL, and ≥100 IU/mL were categorized as negative, positive, and strongly positive.

Results: A total of 4,256 students born between 1995 and 2004 (age range: 7-12 years) were included in the study. Of the overall study group, 2099 (49.3%) were male and 2157 (50.7%) were female. In 62.3% of the children in group A (born in or before 1999), anti-HBs titers were above the protection limit (≥10 IU/mL), while this rate was 37.3% in group B (born in or after 2000), with a statistically significant difference (p<0.00001, X^2=207.1841).

Conclusion: Three doses of HBV vaccination administered during the universal campaign is adequate with no need for booster doses, unless maternal hepatitis B surface antigen positivity is present.

Keywords: Universal HBV vaccination, children, booster dose

ÖZ

Amaç: Evrensel bir aşılama programı bağlamında doğumda hepatit B virüse (HBV) karşı aşılanan bebeklerde yıllar içinde antikor titreleri azalabilir ve ergenlik döneminde bir takviye dozuna ihtiyaç duyulabilir. Çalışmanın amacı, bebeklik döneminde uygulanan 3 doz HBV aşısından 8-10 yıl sonra bağışıklık ve taşıyıcılık durumunu değerlendirmektir.

Gereç ve Yöntemler: Bu tanımlayıcı, kesitsel, toplum temelli bir saha çalışmasıdır ve 2008 ile 2011 yılları arasında yapılmıştır. Anti-HBs titresi ≤9 IU/mL, 10 ila 99 IU/mL ve ≥100 IU/mL olan çocuklar negatif, pozitif ve güçlü pozitif olarak sınıflandırılmıştır.

Bulgular: Araştırmaya 1995-2004 yılları arasında (yaş aralığı: 7-12 yıl) doğan toplam 4.256 öğrenci dahil edilmiştir. Genel çalışma grubunun 2099'u (%49,3) erkek ve 2157'si (%50,7) kadındı. Grup A'daki (1999 ve öncesi doğumlu) çocukların %62,3'ünde anti-HBs titreleri koruma sınırının üzerindeydi (≥10 IU/mL), grup B'de ise bu rakam (2000 ve sonrası doğumlu) istatistiksel olarak anlamlı bir farkla (p<0,00001, X^2=207.1841) %37,3 idi.

Sonuç: Maternal hepatit B yüzey antijeni pozitifliği olmadıkça, evrensel aşılama programı kapsamında uygulanan üç doz HBV aşısı, rapel dozlara gerek kalmadan yeterlidir.

Anahtar Kelimeler: Evrensel HBV aşılaması, çocuklar, güçlendirici doz

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Introduction

Despite the decrease in the prevalence of hepatitis B virus (HBV) infection due to widespread protection and effective vaccination programs, it remains a significant global public health problem. There are an estimated 250-260 million cases of chronic HBV cases worldwide, and based on a 2015 review, nearly 887 thousand deaths have been recorded due to HBV related disease/complications (1). Since early exposure to HBV is associated with very high rates of chronicity, a recommendation has been made to administer vaccination to all infants starting from birth (2,3). The primary target of a universal HBV vaccination program is to prevent exposure to the virus. Since 1990, universal HBV vaccination has been successfully undertaken, with a worldwide coverage rate of 84% for 3 doses as of 2015 (4).

Several studies have reported very successful outcomes regarding the efficiency of the universal hepatitis B vaccination. In the most successful example of Taiwan, the campaign had been initiated in 1988 with the vaccination of newborns, with a nationwide coverage achieved in 1992. One study conducted 22 years after the initiation of the universal vaccination showed that the hepatitis B surface antigen (HBsAg) positivity rate declined from 5.6% during the pre-vaccination period to less than 1% after the program (5).

In our country, all newborns receive hepatitis B vaccination since 1998 in the context of the Turkish National Vaccination Program, like Thailand (6). Manisa in western Turkey which is located in, hepatitis B carrier in the Aegean Region is estimated 3:47 ratio %; shows mesoendemic propagation feature (7). Although previous studies from Turkey showed a decline in HBsAg positivity rates after the initiation of widespread vaccination program, this infection remains an important health problem in certain endemic regions (4,7). Again, following the initiation of universal HBV vaccination, the Ministry of Health initiated another HBV vaccination campaign involving 8th grade primary school students in 2005-2006 academic year as a transition to an adolescent vaccination program, with a subsequent 3-dose vaccination program (catch-up program) in 2007-2008 involving all primary school students between 3rd and 8th grades as well as high school students, as a coverage for inadequate or missing doses. During this campaign, a 3-dose HBV vaccination was repeated in primary school children born in or before 1999, assuming that there might have been cases who could not adhere to the program, had delayed vaccination, or had no vaccination at all during the nationwide HBV vaccination program initiated in 1998. Also, throughout the campaign, children born in 2000 and later did not receive repeated HBV vaccination.

This study was undertaken to assess the changes in HBV prevalence among children after initiation of the universal HBV vaccination from newborn, and to compare anti-HBs responses between those who never received additional vaccination after infancy and those who received repeated vaccination in the campaign.

Materials and Methods

This was a descriptive, cross-sectional, community-based field study. The target sample population consisted of primary school students in the provincial center of Manisa and Turgutlu district. The data on the current number of students was obtained from Provincial Education Center to determine the schools to be included in the study and to categorize study groups according to socioeconomic and cultural status (as high, intermediate, and low), with stratification based on the year of birth. Since primary education is compulsory in public schools and students are registered to schools based on address; schools are stratified in terms of socioeconomic level according to the region in which they are located. The population of the research was 25,871 students and the sample size consisted of 4,256 students.

The study protocol was approved by the Ethics Committee of İzmir Atatürk Training and Research Hospital (approval number: B41SM4350015-009/263, date: 21.09.2007) as well as the Ministry of Health. Verbal consent was obtained from the participants.

Anti-HBs as an indicator of immunity and HBsAg as an indicator of carriage are the main outcome measures of this study.

During school visits, total of 5 cc of blood sample was obtained from each participant, first to assess anti-HBs using micro-EIA method (Sorin, Italy), and then to further evaluate anti-HBc immunoglobulin G (IgG) and HBsAg in those with an anti-HBs of ≤9 IU/mL. Children with an anti-HBs titer ≤9 IU/mL, 10 to 99 IU/mL, and ≥100 IU/mL were categorized as negative, positive, and strongly positive. HBsAg and anti-HBc IgG were tested in those with an anti-HBs titer of ≤9 IU/mL. At the end of the 3-year study period (2008-2011), the results were analyzed in view of the repeated vaccinations during the catch-up campaign endorsed by the Ministry of Health as well as birth year data.

Statistical Analysis

Analysis of data was done with SPSS version 24.0. Cross tabulation was used to examine the relationships occurring between variables. Associations were tested using chi-square analysis.

Results

A total of 4,256 students born between 1995 and 2004 (age range: 7-12 years) were included in the study. Of the overall study group 2099 (49.3%) were male and 2157 (50.7%) were female. Group A (born in or before 1999, with repeated vaccination during the catch-up campaign) consisted of 3,161 children and group B (born in or after 2000, with no repeated HBV vaccination after the initial 3 doses in infancy) consisted of 1,095 children.

In 62.3% of the children in group A, i.e. among those who received a second series of vaccinations (at least 1 dose or 3 doses) after vaccination at infancy, anti-HBs titers were above the protection limit (≥10 IU/mL), while this figure was 37.3% in group B, i.e. those who had not received further vaccination, with the difference being significant ($p<0.00001$, $c2=207.1841$) (Table 1).

When low and high positivity status for anti-HBs was considered, 62.7% of the children in group B were found to have anti-HBs titers below the protection limit, with a significant difference between the groups, together with a lower proportion (14.4%) of children in group B with strong positivity for anti-HBs (anti-HBs >100 IU/mL) ($p<0.00001$, $c2=265.3519$). However, the two groups were comparable with regard to the proportion of subjects with positivity (10-99 IU/mL) (Table 2).

Antibody levels were evaluated according to the socioeconomic

Table 1. Distribution of anti-HBs positivity titers according to age and vaccination history

HBV vaccination history	≤9 mIU/mL	≥10 mIU/mL	Total
Group A			
Born in or before 1999 (receiving second series of vaccination in addition to infancy %)	1191 (37.7%)	1970 (62.3%)	3161
Group B			
Born in 2000 and later (vaccinated only during infancy %)	687 (62.7%)	408 (37.3%)	1095
Total	1878 (44%)	2378 (56%)	4256

P<0.00001, X^2: 207.184, anti-HBs: anti-Hepatitis B surface antigen, HBV: Hepatitis B virus

level of the students (Table 3). Accordingly, the proportion of non-immunities increases as the socio-economic level deteriorates; according to the socio-economic level, these rates are at the same order high 39.5% (425), medium 40.0% (468), low 49.0% (985). Conversely, the proportion of those with an immunity higher than 100 IU/mlincreases as the socioeconomic level improves; in the same order, these rates were set as low 30.9% (622), medium 31.4% (367) and 37.2% (399) high.

Twenty-three children (0.5%) were found to have HBsAg positivity. The birth year and gender distribution of these cases with HBsAg positivity are shown in Table 4.

Among HBsAg positive children, 17 were born in or before 1998, when universal HBV vaccination was initiated, and only 6 were born after 1999. Again, of these 23 children, 21 had maternal HBsAg positivity, one had paternal HBsAg positivity, and one had sibling HBsAg positivity.

Four-hundred and fifty-six children with anti-HBs titers below the protection level could be reached, and when the anti-HBs testing was repeated 4 to 6 weeks after a single dose HBV vaccination, an anamnestic response was found in 440 (96.4%) of these, with all children having anti-HBs titer of greater than 100 IU/mL. All of the 16 children who failed to develop an anamnestic response after the first follow-up testing achieved an anti-HBs response following the second HBV vaccination.

Discussion

Several studies have established that in subjects who received 3-doses of HBV vaccination during infancy, the immune memory can be maintained for prolonged periods of time, even if anti-HBs loss occurs (9). In Pakistan, a universal HBV vaccination program was initiated in 2002, with a dosing schedule involving vaccinations at 6-10 and 14 weeks of life. Of the 200 children vaccinated using this scheme, 58% were found to have protective anti-HBs levels when the protective properties of the vaccination was assessed in 2014, and in half of the children between ages 8 and 10 years, anti-HBs levels were ≤10 IU/mL. When a single dose vaccination was administered, all children developed anamnestic response (10).

In a Taiwanese study between 2008 and 2012, blood samples were obtained from 887 adolescents born between 1993 and 1997 and vaccinated during infancy, and the proportion of subjects with adequate anti-HBs titer and HBsAg positivity were found to be 34.7% and 0.7%, respectively. A booster dose was given in a total of 501 children with anti-HBs titers below the protection limit, with 94% of these developing anamnestic response 6 months after vaccination (11).

In a multi-center phase 4 study from Germany, blood samples were obtained from children aged between 12-13 years who were vaccinated during infancy using a hexavalent vaccine (HBV + DTPa + IPV + Hib). Among the overall population of 293 children, 60.5% had an anti-HBs titer ≥10 IU/mL, and 97.6% of those without adequate antibody levels developed anamnestic response following a single booster dose (12).

In another study where blood tests were performed among 293 adolescents 15 to 15 years after vaccination, 71.2% were found to have adequate anti-HBs levels, with a mean anti-HBs titer of 26.5 IU/mL (range: 21.4-32.8). One month after a booster dose, the mean anti-HBs titers increased above 100 IU/mL. Thus, the authors suggested that immunity was maintained 15 to 16 years after vaccination, and that a good response was achieved after the booster dosing (13).

In a recent study from Korea, laboratory results in 19.072 individuals were tested between 2000 and 2015. Study participants were divided into two groups. The first group consisted of those born before 2005 who received both the recombinant and plasma-derived vaccines, while the second group included subjects born after 2005 who received only the recombinant vaccine. Overall, 55.8% of the population had anti-HBs positivity, and the corresponding figures in the first and second groups were 53.0% and 78.1%, respectively. In children with loss of anti-HBs titers, anti-HBs was found to develop following a single booster dose. It was concluded that lifelong protection is important for HBV infections and therefore a booster dose may be needed during adolescence (14).

In our country, a significant reduction in cases with acute HBV infection was observed after initiation of the nationwide HBV vaccination program, particularly among pediatric and adolescent populations. In a study looking at the contributions of this vaccination program published in 2012, the reported cases of acute HBV infection between 1990 and 2012 were analyzed and a dramatic decline in acute HBV infections in all patients under 15 years of age was identified during the time period between 1997 and 2014 (8).

Similarly, we also observed statistically significantly higher anti-HBs titers among children who received repeated vaccination approximately 10 years after the 3-dose HBV vaccination during infancy, as compared to those who never received booster doses. Following a single booster dose among children with an anti-HBs tire of ≤10 IU/mL, 96.4% developed an anamnestic response. A booster dose administered upon decline and loss of antibody titers in previously vaccinated individuals was associated with a significant increase in antibody titers, while in those with high initial anti-HBs levels, this results in a more prolonged maintenance of

Table 2. Distribution of anti-HBs titers				
HBV vaccination history	≤9 mIU/mL	10-99 mIU/mL	≥100 mIU/mL	Total
Group A				
Born in or before 1999 (receiving second series of vaccination in addition to infancy %)	1191 (37.7%)	740 (23.4%)	1230 (38.9%)	3161
Group B				
Born in 2000 and later (vaccinated only during infancy %)	687 (62.7%)	250 (22.8%)	158 (14.4%)	1095
Total	1878 (44.1%)	990 (23.2%)	1388 (32.6%)	4256
P<0.00001, X^2: 265.352, anti-HBs: anti-Hepatitis B surface antigen, HBV: Hepatitis B virus				

Table 3. Distribution of anti-HBs titrations by socio-economic status				
Socioeconomic status	**≤9 mIU/mL**	**10-99 mIU/mL**	**≥100 mIU/mL**	**Total**
High	425 (39.5%)	251 (23.3%)	399 (37.2%)	1075 (25.3%)
Medium	468 (40.0%)	334 (28.6%)	367 (31.4%)	1169 (27.5%)
Low	985 (49.0%)	405 (20.1%)	622 (30.9%)	2012 (47.2%)
Total	1878 (44.1%)	990 (23.3%)	1388 (32.6%)	4256 (100%)
P<0.0001, X^2: 51.018, anti-HBs: anti-Hepatitis B surface antigen				

Table 4. Age and gender distribution in children with HBsAg positivity (n=23)			
Year of birth	**Girl (%a)**	**Boy (%a)**	**Total (%b)**
1995	2 (15.4%)	1 (10.0%)	3 (13.0%)
1996	4 (30.8%)	-	4 (17.4%)
1997	-	2 (20.0%)	2 (8.7%)
1998	4 (30.8%)	4 (40.0%)	8 (34.8%)
1999	1 (7.7%)	1 (10.0%)	2 (8.7%)
2000	2 (15.4%)	2 (20.0%)	4 (17.4%)
Total	13 (43.5%)	10 (56.5%)	23 (100.0%)
a: With in gender, b:With in total, HBsAg: Hepatitis B surface antigen			

the titers (15,16). These observations suggest that immunologic memory is formed as a result of the vaccination during infancy, and that the protective effect may last many years.

In 23 (0.5%) of the 4,256 children included in the study, HBsAg positivity was found. Seventeen of these children were born prior to the nationwide vaccination program, while six were born after that. Of these 23 children 21 had maternal, one had paternal, and one another had sibling positivity for HBsAg. In South East Asian countries where HBsAg positivity rates are high, the primary factor for persistence of HBsAg positivity among children (although at low percentage) and unresponsiveness to vaccination was reported to be maternal HBsAg positivity, which, according to the authors, should be the primary focus (17,18).

Taiwan is an hyperendemic country for HBV infections where universal HBV vaccination was started in 1984, followed by 5 different sero-epidemiologic studies at 0, 5, 10, 15, and 20 years after the initiation of the program. These studies showed that the program was remarkably successful, with HBsAg, anti-HBc IgG, and anti-HBs positivity rates of 10.0%, 28.0%, and 24.5%, respectively at the time of study initiation in 1984, decreasing to 0.9%, 7.0%, and 55.9%, respectively in 2009. A more recent study conducted at 25 years after initiation of the program involved 3332 individuals under 30 years of age with inclusion of participants from each of the 5 different study cohorts described above, and showed further reduction in HBsAg positivity rates, with a vaccination failure rate of 86% in the presence of maternal HBsAg positivity (19).

These observations again underscore the importance of screening pregnant women for HBsAg and administration of appropriate immunization (vaccination + hepatitis B immune globulin) at the time of labor in carrier mothers.

The relationship between socio-economic status and immunity levels is thought to be related to the behaviors of students' families using preventive health services, including immunization. With the implementation of the universal HBV vaccination campaign, the increase in the coverage of immunization services provides herd immunity by eliminating the possible negative effects of socio-economic differences.

Study Limitations

The limitations of the study: Participants are not aware of their previous hepatitis B participants are not aware of their previous hepatitis B vaccination status as they were assumed to have been vaccinated in the National Vaccination Program. Another weakness of the study is that it did not include students who were absent from school for any reason.

The strengths of the study are showing the trend effect between weak and strong immunity and socioeconomic levels, as well as demonstrating the effectiveness of Universal HBV Immunization.

Conclusion

Twenty-two years after initiation of a nationwide HBV vaccination program, a significant decrease in HBsAg positivity among primary school children aged between 7 and 14 years has been detected. We may assume that our results can be extrapolated to children in other geographical areas in Turkey as a result widespread vacination. However, in order to maintain this success, same meticulous care should continue for vaccination of newborns. Detection of maternal HBsAg positivity in 21 out of 23 children with HBsAg positivity one more time underlines the importance of administering vaccination and hepatitis B hyper-immunoglobulin to babies born to carrier mothers.

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Ethics

Ethics Committee Approval: The study protocol was approved by the Ethics Committee of İzmir Atatürk Training and Research Hospital (approval number: B41SM4350015-009/263, date: 21.09.2007) as well as the Ministry of Health.

Informed Consent: Verbal consent was obtained from the participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: S.T., Concept: S.T., S.D., Design: S.T., S.D., Data Collection or Processing: S.T., Analysis or Interpretation: S.D., Literature Search: S.T., S.D., E.K., Writing: S.T., S.D., E.K.

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Research Article

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Molecular Analysis of Hepatitis B Virus Reverse Transcriptase Domain for Mutations Associated with Viral Resistance in Pakistani Patients

Pakistanlı Hastalarda Viral Dirençle İlişkili Mutasyonlar İçin Hepatit B Virüsü Ters Transkriptaz Domaininin Moleküler Analizi

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ABSTRACT

Objectives: Current study was designed to screen out the resistant mutations in reverse transcriptase (RT) domain of hepatitis B virus (HBV) genome from non-responder Pakistani patients.

Materials and Methods: A total of 22 patients, receiving different nucleot(s)ide analogues were included in the study. RT domain of the virus from samples of non-responder patients was amplified and sequenced. Sequences were aligned and analyzed for RT domain mutations.

Results: After 18 months, 18 patients were responder and 4 were non-responder. Mean alanine aminotransferase (ALT) and viral load of responder patients decreased significantly as compared to those of non-responder patients. Two of the 4 samples from non-responders were successfully sequenced. Mutations rtY135S, rtI169P, rtV173P, rtL180I, rtA181V, rtT184Y and rtM204V were identified from the sample of patient 1, while rtL80V/rtL80G and rtY135S were identified from the sample of patient 2.

Conclusion: Mutations rtY135S, rtI169P, rtV173P, rtL180I, rtA181V, rtT184Y, rtM204V, rtL80V/rtL80G, and rtY135S are present in genome of HBV circulating in Pakistani patients. These mutations give resistance to virus against lamivudine, telbivudine, adefovir, and partially resistance against entecavir. However, no mutation was found to be associated with the viral resistance against tenofovir.

Keywords: Hepatitis B virus, RT domain, resistant mutations, nucleot(s)ide analogues, HBV genome

ÖZ

Amaç: Mevcut çalışma, yanıtsız Pakistanlı hastalardan hepatit B virüsü (HBV) genomunun ters transkriptaz (RT) domainindeki dirençli mutasyonları taramak için tasarlanmıştır.

Gereç ve Yöntemler: Çalışmaya farklı nükleot(z)id analogları alan toplam 22 hasta dahil edildi. On sekiz ay sonra, 18 hasta yanıt verdi ve 4 hasta yanıt vermedi. Yanıt vermeyen hastaların örneklerinden alınan virüsün RT domaini amplifiye edildi ve dizilendi. Diziler hizalandı ve RT domaini mutasyonları açısından analiz edildi.

Bulgular: Yanıt veren hastaların ortalama alanin aminotransferaz (ALT) ve viral yükü, yanıt vermeyen hastalarla karşılaştırıldığında önemli ölçüde azaldı. Yanıt vermeyen hastalardan alınan 4 örnekten 2'si başarıyla sıralandı. Birinci hastanın örneğinden rtY135S, rtI169P, rtV173P, rtL180I, rtA181V, rtT184Y ve rtM204V mutasyonları belirlenirken ikinci hastanın örneğinden rtL80V/rtL80G ve rtY135S mutasyonları belirlendi.

Sonuç: Pakistanlı hastalarda saptanan HBV genomunda rtY135S, rtI169P, rtV173P, rtL180I, rtA181V, rtT184Y, rtM204V, rtL80V/rtL80G ve rtY135S mutasyonları mevcuttu. Bu mutasyonlar, virüse lamivudin, telbivudin ve adefovire karşı tam, entecavire karşı kısmen direnç sağlamaktaydı. Bununla birlikte, tenofovire karşı viral dirençle ilişkili hiçbir mutasyon bulunmadı.

Anahtar Kelimeler: Hepatit B virüsü, RT alanı, dirençli mutasyonlar, nükleot(z)id analogları, HBV genomu

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Introduction

Hepatitis B virus (HBV), a member of hepadnaviridae family of viruses, is a pathogen of human hepatocytes first recognized in 1960s (1,2). The undesirable effects caused by HBV infection include liver degeneration, liver cirrhosis, hepatocellular carcinoma, and liver failure (3). Approximately 257 million people are chronic carriers of HBV in the world. The annual number of deaths caused by HBV related infections were estimated to be 887000 in 2015 (4). However, the infection rate of HBV has been decreased significantly in developed countries (5,6) but there is no such report from developing and underdeveloped countries, including Pakistan.

Interferon-- and nucleot(s)ide analogues (NAs) are clinically available treatments for HBV. Interferon reduces the hepatitis B surface antigen level from blood alongside immunomodulatory effects but it poses many adverse side effects (7). NAs treatment is easier in use than interferon therapy, though it also has some side effects but fewer (8). Five nucleotide/nucleoside analogues are so far used for treatment of chronic HBV Infection. These are: lamivudine, adefovir dipivoxil, entecavir, telbivudine, and tenofovir disoproxil fumarate. All of these act on the reverse transcriptase (RT) region of the viral genome stopping the production of DNA from pre-genomic RNA (8,9,10).

HBV replication is an error prone process because it has no proofreading activity, leading to high mutation rate in the genome (11,12). Some of these mutations may cause viral resistance against treatment and this antiviral resistance is the greatest stumbling block in HBV treatment (13). Several mutations in the RT domain are considered to be associated with resistance to nucleotide or nucleoside analogues in the treatment of chronic HBV (3,6,14). However, all the mutations occurring in HBV polymerase region are not associated with resistance. A few are well known mutations associated with primary drug resistance to NAs, which are: rtL80G/I, rtI169T/P, rtV173L, rtL180M/I, rtA181T/V/S, rtT184S, rtS202I/G/S, rtM204V/S/I, rtN236T, rtN238D/S/R and rtM250V/I/L.

The objective of current study was to screen out the non-responder patients for resistance mutations in RT domain of HBV from non-responder patients, and to compare some factors of non-responders with responder patients.

Materials and Methods

This was a cross sectional study conducted during August 2020 to March 2021. The surveys were conducted in hospitals to select the patients receiving different nucleotide analogues. A total of 22 chronic HBV patients who completed at least 18 months of NAs treatment were selected with the help of a gastroenterologist. A performa was filled for each patient, which included all the treatment history and other important information. A written informed consent was given to each of the patient and the patients keen to contribute in the study were enrolled. The patients with a positive treatment response were also monitored for breakthrough. Blood samples were collected from all the non-responder patients (Figure 1).

Pre-treatment Factors

Pre-treatment viral factors like genotype, hepatitis B e antigen, and viral load were recorded for all patients before and on every 6 months of treatment. Different host factors like age, gender, body weight, alanine aminotransferase (ALT), dental procedure, previous surgery record, infection age, and previous treatment, history were also recorded.

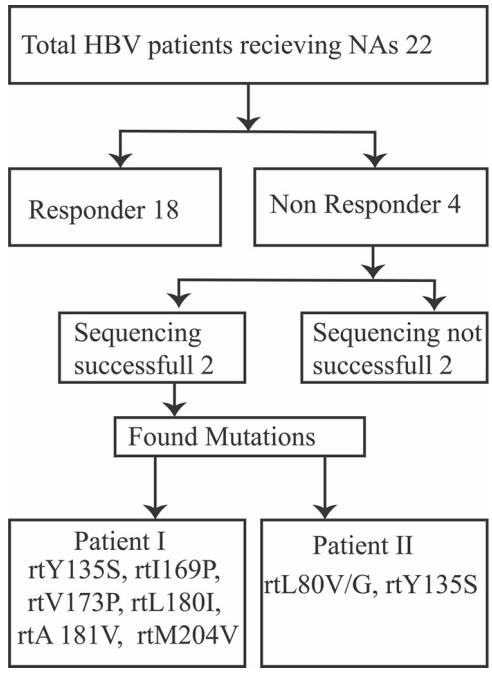

Figure 1. Flow chart of complete study including patients' selection and main findings

HBV: Hepatitis B virus, NAs: Nucleot(s)ide analogues

DNA Extraction and Amplification

Viral DNA was extracted using commercially available kits and a fragment of genome including RT domain was amplified by polymerase chain reaction using previously described primers (15,16). PCR conditions were optimized in a gradient PCR machine and the quantitative measurement of viral load was achieved by a real time PCR machine.

Products Purification

The amplified DNA fragments were purified for sequencing using ethanol precipitation kit protocol (Beckman Coulter, USA).

Sequencing

Sequencing of the purified DNA fragments of RT domain was obtained commercially by sending the DNA to commercial service providers where the sequencing was performed by chain termination method. The sequencing instrument used was "CEQ 8000 XL" analysis system for the sequencing reaction.

Statistical Analysis

The sequences were aligned with wild type HBV sequences and analyzed for resistant mutations in the RT domain. Manual analyses of sequences were also carried out. The mutations were also confirmed by "geno to pheno HBV", the online data base for HBV genome analysis.

Ethical Approval

The study was started after the approval from "humans and animals ethics committee", University of Poonch Rawalakot. An

informed consent was given to each of the patient for reading and signing before his/her enrolment to the study.

Results

Patients and Treatment Details

During the study period, 22 hepatitis B patients receiving treatment were enrolled at Pakistan Atomic Energy Commission General Hospital Islamabad. Out of the 22 enrolled patients, 18 (81.8%) showed response during treatment while 4 (18.2%) did not show any response to treatment and considered non-responder after 18 months of treatment (Figure 1). The mean age of responder patients was calculated to be 36.45±14.89 years while the mean age of non-responder patients was calculated to be 38.50±13.63 years. No significant difference (p=0.660) of mean age between responder and non-responder patients was found (Table 1).

In total, 12 patients were male and 10 were female. Out of the four non-responder patients, 2 were male and 2 were female while 10 of the 18 responder patients were male and eight were female (Table 1).

Viral Load Comparison

The mean pre-treatment viral load of responder patients was found to be 7.24E7±3.56E5 while the mean pre-treatment viral load of non-responder patients was 7.13E7±2.32E3. There was no significant difference (p=0.183) of mean viral load between responder and non-responder patients before the treatment (Table 1).

After 6 months of treatment, the viral load of responder patients was significantly lower as compared to the viral load of non-responder patients (p=0.009). At this stage, the mean viral load of responder patients was calculated to be 9.74E4±3.46E5 while the mean viral load of non-responder patients after 6 months of treatment was 9.02E7±2.42E3 (Table 1).

The mean viral load of responder patients after 12 months of treatment was 7.57E3±3.27E4 while the mean viral load of non-responder patients after 12 months was 3.96E5±1.48E3 (Table 1) and the difference was again significant statistically (p=0.021).

After 18 months of treatment, the responder patients had undetectable or very low viral load in serum but the non-responder patients still had a mean viral load of 4.63E5±6441 copies/mL (Table 1).

ALT

The mean pre-treatment ALT of responder patients was found to be 53.90±31.16 while mean ALT of non-responder was 55.11±14.51. There was no significant difference (p=0.697) of mean ALT between responder and non-responder patients before treatment (Table 1).

The mean ALT of responder patients after six months of treatment was 41.35±20.51 while mean ALT of non-responder patients after six months was 47.61±9.27. There was no significant difference (p=0.705) of mean ALT between responder and non-responders (Table 1).

After 12 months of treatment, the mean ALT of responder patients was 30.55±8.34 while mean ALT of non-responder after twelve months was 44.61±8.13. There was significant difference (p=0.001) of mean ALT between responder and non-responder after 12 months of treatment (Table 1).

The mean ALT of responder patients after 18 months of treatment was 26.50±4.12. While mean ALT of non-responder after eighteen months was 43.83±8.06. There was significant difference (p=0.000) of mean ALT between responder and non-responder patients after 18 months of treatment (Table 1).

Mutational Analysis

The blood samples of all 4 non-responder patients were sent for sequencing but unfortunately, DNA of two samples was not successfully sequenced while the remaining two samples were sequenced successfully. RT mutations, well known for their role in resistance, were found in both of these samples.

Mutational Profile of Patient 1

Patient 1 was male of 49 years who received lamivudine and entecavir treatments. The RT domain of the virus isolated from this patient was detected with rtY135S, rtI169P, rtV173P, rtL180I, rtA181V, rtT184Y and rtM204V mutations. These mutations are known to be associated with viral resistance against lamivudine telbivudine adefovir and entecavir. The patient was non-responder against lamivudine due to compensatory mutations rtL180I,

Table 1. Comparison of responder and non-responder patients in the study

Factor		Responder	Non-responder	Sig.
Age		36.45±14.89	38.50±13.63	0.662
Gender	Male	10 (83.3%)	2 (16.7%)	0.377
	Female	8 (80%)	2 (20%)	
Viral load	Pre treatment	7.24E7±3.56E5	7.13E7±2.32E3	0.183
	After 6 months	9.74E4±3.46E5	9.02E7±2.42E3	**0.009**
	After 12 month	7.57E3±3.27E4	3.96E5±1.48E3	**0.021**
	After 18 month	Undetectable or very low	4.63E5±6441	**0.001**
ALT	Pre treatment	53.90±31.16	55.11±14.51	0.697
	After 6 months	41.35±20.51	47.61±9.27	0.705
	After 12 month	30.55±8.34	44.61±8.13	**0.001**
	After 18 month	26.50±4.12	43.83±8.06	**0.000**

ALT: Alanine aminotransferase, Sig: Signature

rtV173P, rtL180I and rtM204V, while it was non-responder against entecavir due to rtM204V, rtI169P, rtT184Y and rtL180V (Table 2).

Mutational Profile of Patient 2

Patient 2 was a female of 41 years who was treated with lamivudine for 18 months. According to resistance profile, the patient was resistant against lamivudine due to compensatory mutations rtL80V/rtL80G and rtY135S. This mutational profile shows that the patient is not resistant against adefovir, entecavir, and tenofovir (Table 2).

Discussion

The quantitative factors like viral load and ALT significantly decreased during treatment in responder patients while not in non-responders. However, the sample size of the study was low and not enough for comparative analysis of quantitative factors. So, the study was designed to detect the RT mutations responsible for resistance instead of quantitative comparison.

In this study the resistant mutations rtY135S, rtV173P, rtL180I, rtM204V, rtA181V, rtI169P, and rtT184Y were detected which made the patients non-responder against lamivudine, telbivudine, adefovir and entecavir. However, no mutation was found in association with tenofovir.

In a similar type of previous study from Pakistan, almost same mutations were detected from multiple drug resistant patients (16). The mutations reported in that study were: rtL80G, rtY135S, rtI169P, rtV173L, rtL180M, rtA181V, rtT184Y, rtM204V and rtN248H, which were reported to be associated with lamivudine, telbivudine, adefovir and entecavir. Mutation rtN248H was not found in current study, however it was reported in the only previous study from Pakistan. Mutation rtY135S was found in current study which was only reported in the other study from Pakistan (16). It was reported previously from Pakistan that resistance mutations are found frequently on the positions rtL80V/G, rtY135S, rt169P and rt248H while present in low proportion on positions rt184Y and rtL80G.

Another recent study from Iraq reported the mutations rtL80I/V, rtV173L, rtL180M, rtA181S, rtA194T, rtS202I, rtM204V/I, rtN236T and rtM250L/V associated with resistance against lamivudine, telbivudine, adefovir, entecavir and tenofovir (17). However, the mutations rtA194T, rtN236T, and rtM250L/V were not found in our study. Mutation on position rtA194T was generally considered as associated with tenofovir resistance.

The mutations rtM204I and rtL180M, detected in our study, were most frequently found in previous studies from different areas of the world (2,3,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42). In many of the studies (7,9,16,17,20,22,24,25,26,27,28,29,32,39,40,41), these two mutations were reported to have an association with lamivudine while in some other studies (7,9,16,17,31,32,35,37), these were found to be associated with telbivudine resistance as well. These reports confirm that the mutations rtM204I and rtL180M have association with lamivudine and telbivudine resistance. Besides telbivudine and lamivudine resistance, these mutations were also reported to have some association with other NAs like adefovir, entecavir and tenofovir (2,3,18,19,23,30,33,38,42).

The mutations rtV173P and rtL80G, detected in our study, were second most frequent mutations reported in previous studies (2,9,16,17,18,19,26,29,37,43,44). In some studies (9,16,17,26,29,37,43,44), rtV173P was reported to have an association with lamivudine while in some other studies (9,16,17,37,43), it was found to be associated with telbivudine. In some studies (16,17,26,37,43), the mutation rtL80G was reported to have an association with lamivudine and telbivudine resistance. These reports confirm that the mutations rtV173P and rtL180M have association with lamivudine and telbivudine resistance. In some studies (2,18,19), rtV173P was reported to have association to other NAs too.

The mutations rtA181V detected in our study was third most frequent mutation reported in previous studies (7,9,16,17,33,34,37,38,40,41). In all studies, rtA181V was reported to have an association with adefovir resistance while it was reported to cause multiple drug resistance in some studies too. This mutation was also detected in our study from a non-responder patient.

The mutations rtI169P and rtT184Y detected in our study were fourth most frequent mutations reported previously (16,19,29,33,35,39,40). In some studies (16,19,29,39,40), rtT184Y was found to be associated with entecavir resistance while in some other studies, these mutations were shown to have an association with adefovir and lamivudine resistance too (33,35). In three studies, mutation rtI169P was reported to be associated with entecavir resistance too (16,19,29).

In Pakistan, the mutational analysis is not performed before the start of therapy which increases the risk of treatment failure in chronic HBV patients. It is mainly due to lack of facility and lack of awareness. Another fact behind the unavailability of mutation testing is the unavailability of experts who can carry out the mutational screening. Present study confirms that the resistance

Table 2. Resistance mutations profile of the non-responder patients in the study

	Patient 1		Patient 2	
	Detected mutations	**Resistance prediction**	**Detected mutations**	**Resistance prediction**
Lamivudine associated	rtY135S, rtL180I, rtV173P, rtL180I, rtM204V	Resistant	rtI80V, rtL80G, rtLY135S	Resistant
Adefovir associated	rtA181V	Resistant	None	Susceptible
Telbivudine associated	rtI169P, rtT184Y	Resistant	rtI80V, rtL80G	Resistant
Entecavir associated	rtM204V, rtV173P, rtL180I	Partly	None	Susceptible
Tenofovir associated	None	Susceptible	None	Susceptible

mutations are present in the genomes of viruses circulating in the country. So, it is necessary to analyze the RT domain of virus before start of therapy in the patients.

Study Limitations

The study has a small number of non-responder patients that is a limitation of current study.

Conclusion

Mutations rtY135S, rtI169P, rtV173P, rtL180I, rtA181V, rtT184Y, rtM204V, rtL80V/rtL80G, and rtY135S are present in genome of HBV circulating in Pakistani patients. These mutations give resistance to virus against lamivudine, telbivudine, adefovir, and partially resistance against entecavir. However, no mutation was found to be associated with viral resistance against tenofovir

Ethics

Ethics Committee Approval: The study was started after the approval from "humans and animals ethics committee", University of Poonch Rawalakot.

Informed Consent: An informed consent was given to each of the patient for reading and signing before his/her enrolment to the study.

Peer-review: Externally peer-reviewed.

Authorship Contributions:

Concept: M.M. Design: M.M., M.A.A., Data Collection or Processing: S.J., Z.U.R., M.A.A., Analysis or Interpretation: M.M., S.J. Literature Search: S.J., Z.U.R. Writing: M.M., S.J., Z.U.R.

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Glecaprevir/Pibrentasvir Treatment in a Patient with Hemophilia and Mixed Genotype Hepatitis C Infection

Mikst Tip Hepatit C Genotipine Sahip Hemofili Hastasının Glecaprevir + Pibrentasvir ile Tedavisi

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ABSTRACT

Patients who require frequent blood product transfusions, such as patients with hemophilia, are at risk of contracting hepatitis C virus (HCV). The prevalence of mixed HCV genotype infection is higher in this patient group. The patient presented here was a 22-year-old Afghan citizen who had received blood product transfusions since birth due to hemophilia A. He was found to have HCV infection during follow-up in the hematology clinic three years ago, and HCV genotypes 1b, 3, and 4 were detected. Treatment with glecaprevir/pibrentasvir was administered for 8 weeks. The patient reported no side-effects other than headache that resolved when treatment was discontinued. Transaminase values improved from pre-treatment level and were within normal limits at the end of the treatment. The patient showed sustained virologic response at 24 weeks. Pangenotypic direct-acting antivirals, which have just been included in the reimbursement scope in our country, have eliminated the difficulty in choosing drugs in this indication. Single drug administration has led to more successful patient outcomes by increasing treatment compliance.
Keywords: Antiviral drugs, hemophilia A, hepatitis C virus

ÖZ

Hemofili hastalığı gibi sık kan ürünü transfüzyonu gerektiren hastalıklara sahip hastalar hepatit C bulaşı açısından risk altındadır. Bu grup hastalarda mikst hepatit C virüs (HCV) genotip prevalansı daha yüksektir. Olgumuz, 22 yaşında Afganistan uyruklu ve hemofili A hastalığı nedeni ile doğumdan itibaren kan ürünü transfüzyonu yapılan bir hastadır. Üç yıl önce hematoloji kliniğinin takipleri sırasında HCV enfeksiyonu saptandı. HCV genotip 1b, 3 ve 4 tespit edildi. Glekaprevir/pibrentasvir tedavisi 8 hafta verildi. Baş ağrısı dışında bir yan etki gözlenmedi ve tedavinin sonlanması ile yakınması geriledi. Tedavi başlangıcına göre tedavi sonunda transaminaz değerleri normal sınırlarda izlendi. Yirmi dördüncü hafta kalıcı virolojik yanıtı sağlanan hasta başarılı bir şekilde tedavi edildi. Ülkemizde geri ödeme kapsamına henüz giren pangenotipik direkt etkili antiviraller, bu endikasyonda ilaç seçim güçlüğünü ortadan kaldırmıştır. Tek ilaç uygulaması, tedaviye uyumunu artırarak daha başarılı hasta sonuçlarına yol açmıştır.
Anahtar Kelimeler: Antiviral ilaçlar, hemofili A, hepatit C virüsü

Karaşahin Ö, Karaşahin EF. Glecaprevir/Pibrentasvir Treatment in a Patient with Hemophilia and Mixed Genotype Hepatitis C Infection. Viral Hepat J. 2021;27:159-161.

Introduction

Most hemophilia patients who received blood products prior to the implementation of virus inactivation procedures were infected with hepatitis C virus (HCV) (1). Hemophilia A, the most common hemophilia, is caused by an abnormal factor VIII gene located on the X chromosome and affects 1 in 5,000 males (2). According to the literature, hemophilia patients have significantly higher rates of anti-HCV positivity (70-90%) compared to the normal population (3,4). The prevalence of HCV was also reported to increase in parallel with the need for factor concentration, which increases with hemophilia severity (2). Moreover, it has been shown that deaths from chronic liver disease and liver cancer due to viral hepatitis are more common in hemophilia patients than in the general population (1).

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In the management of HCV, pegylated interferon and ribavirin combination therapy has been associated with a high discontinuation rate due to the longer treatment duration and adverse effects (5). The use of direct-acting antivirals (DAAs) has substantially increased treatment success. The approval of DAAs with pangenotypic efficacy has also simplified antiviral treatment selection for patients with mixed HCV genotypes (6). Here we present a patient with hemophilia and mixed genotype HCV infection who was successfully treated with pangenotypic glecaprevir and pibrentasvir (GLE and PIB) therapy.

Case Report

A 22-year-old Afghan man had a history of repeated transfusions due to hemophilia A. During follow-up in the hematology department for hemarthrosis, he was referred to the infectious diseases outpatient clinic when he was found to be anti-HCV positive. At initial presentation, he tested positive for anti-HCV, anti-HBs, and anti-HAV immunoglobulin G (IgG) and negative for anti-human immunodeficiency virus (anti-HIV), hepatitis B surface antigen, and anti-HBc IgG. HCV genotypes 1b, 3, and 4 were detected and HCV-RNA level was 19,212.349 IU/mL. Other initial laboratory values were alpha-fetoprotein 2.94 (0-8.1) ng/mL, alanine aminotransferase (ALT) 155 (0-55) U/L, aspartate aminotransferase (AST) 66 (5-34) U/L, albumin 46.20 (35-50) g/L, creatinine (Cre) 0.96 (0.72-1.25) mg/dL, hemoglobin (Hb) 16.8 (14.1-17.8) g/dL, platelet count (PLT) 221 (152-383) $x10^9$/L, white blood cell count (WBC) 5.08 (3.91-10.90) $x10^9$/L. On abdominal ultrasound, the liver was of normal size with smooth contours and homogeneous echogenicity, and the intrahepatic bile ducts and vascular structures appeared normal. There was no sign of a space-occupying lesion in the parenchyma. Sinus rhythm was normal on electrocardiography.

The patient was HCV treatment naive, non-cirrhotic, and had an AST to platelet ratio index of 0.782. Treatment with pangenotypic GLE/PIB was initiated 3 times a day. In follow-up examination at week 4 of treatment, the patient's only complaint was headache which was started at the first week of the treatment. However, it was not severe enough to warrant discontinuation, and treatment was continued for a total of 8 weeks. After completing treatment, he tested negative for HCV-RNA at 12-week follow-up. At 24-week follow-up, he was still negative for HCV-RNA and his laboratory values were ALT: 23 U/L, AST: 22 U/L, albumin 48.62 g/L, Cre: 0.80 mg/dL, Hb: 17.2 g/dL, PLT: $235x10^9$/L, and WBC: $7.20x10^9$/L. Based on his sustained virologic response (SVR) and transaminase values within normal limits, treatment was considered successful.

Discussion

In this report, we present a 22-year-old hemophilia patient with mixed genotype HCV infection who achieved SVR after treatment with GLE/PIB. Although there is some geographical variability, the prevalence of mixed genotype HCV infection in the general patient population is approximately 2% to 7% (7). Mixed genotype HCV infection is more common in populations with high risk of HCV exposure and may be the result of co-infection or superinfection (8,9). The frequency of mixed genotype HCV infection is higher in hemophilia patients compared to the general population (10,11). In addition, as hemophilia patients can be exposed to several thousand donors with a single factor infusion, their HCV genotypes generally reflect the dominant genotype of the donor population (2). Genotypes 1 and 3 are predominant in the general population of Afghanistan (12). Our patient was an Afghan immigrant with a history of repeated factor transfusion from birth and was found to have mixed HCV genotype (1b, 3, and 4).

There is insufficient data on DAA therapy for mixed genotype HCV infection. In a real-life study conducted in Taiwan, 2.2% of HCV patients who received ledipasvir/sofosbuvir (LDV/SOF) and GLE/PIB between 2017 and 2019 had mixed infections with double or triple combinations of the genotypes 1a, 1b, 2, 3, and 6. Rates of SVR at 12 weeks (SVR12) were found to be 96.6% with LDV/SOF therapy and 100% with GLE/PIB therapy, and both treatments were associated with high SVR12 rates in patients with mixed genotype HCV infections (13). In another study evaluating the real-life results of GLE/PIB therapy, the frequency of mixed genotype infection was 1.4% and all of those patients achieved SVR (14). Similarly, in the present case, our non-cirrhotic, treatment-naive patient with mixed HCV genotype demonstrated SVR at 24 week after 8 weeks of pangenotypic GLE/PIB.

In a study reported from our country, 21 patients, 15 of whom were intravenous drug users, were shown to be infected with two different HCV genotypes. DAA treatment was applied to the patients. Virological response was achieved in all of those evaluated at the end of treatment. It has been emphasized that DAA treatment can be successful in breaking the chain of transmission in the community as well as the treatment of the infected person (15).

Conclusion

Patients with a history of blood product transfusion have a clear risk for mixed genotype HCV infection. This case demonstrates that patients with high risk of mixed genotype HCV infection can be successfully treated without genotyping using pangenotypic DAAs, which were recently included in the reimbursement coverage in our country.

Ethics

Informed Consent: Written informed consent was obtained from the patient included in the study and our study was conducted in accordance with the Declaration of Helsinki Principles.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Ö.K., Concept: Ö.K., Design: Ö.K., Data Collection or Processing: Ö.K., Analysis or Interpretation: E.F.K., Ö.K., Literature Search: E.F.K., Ö.K., Writing: E.F.K., Ö.K.

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