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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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INSTRUCTIONS TO AUTHORS

GENERAL INFORMATION

Viral Hepatitis Journal (Formerly Viral Hepatit Dergisi) is an independent, peer-reviewed international journal published quarterly in April, August, December. The official language of the journal is English.

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.

Viral Hepatitis Journal does not charge any article submission or processing charges. Any processes and submissions about the journal can be made from the website: www.viralhepatitdergisi.org. Past issues of the journal are also available at this website. Manuscripts should submitted online from www.journalagent.com/vhd

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

SCIENTIFIC POLICIES

Scientific and Ethics Responsibility

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 2013) (http://www.wma.net/en/30publications/10policies/b3/). 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). In experimental animal studies, the authors should indicate that the procedures followed were in accordance with animal rights as per the Guide for the Care and Use of Laboratory Animals (http://oacu.od.nih.gov/regs/guide/guide.pdf) and they should obtain animal ethics committee approval.

The content of the submitted manuscripts should conform to the criteria stated in Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication published by International Committee of Medical Journal Editors and updated in October 2008 (available at http://www.icmje.org/).

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 authors should acknowledge and provide information on grants, contracts or other financial support of the study provided by any foundations and institutions or firms.

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.

The corresponding author must give the full corresponding address (including telephone, fax number and e-mail address). Contact information for corresponding author is published in the journal.

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.

Review Process: Upon submission, all manuscripts are reviewed to check for requirements requested by the Journal. Manuscripts that do not comply with these requirements will be sent back to authors without further evaluations. All the papers are first evaluated by the editor; later the papers are sent to advisory board members. If needed, some questions can be asked to the authors to answer; or some defaults may have to be corrected by the authors.

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.

The journal does not charge from the author for published articles and there is no royalty payment to the authors.

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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 (2013, 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/),

MOOSE guidelines for meta-analysis and systemic reviews of observational studies (Stroup DF, Berlin JA, Morton SC, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting Meta-analysis of observational Studies in Epidemiology (MOOSE) group. JAMA 2000; 283: 2008-12).

MANUSCRIPT PREPARATION

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 name and institution(s) (Turkish and Engilish)

ii. Turkish and english Abstract (max 300 words)

iii. Turkish and english Key Words

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.

Manuscripts should have double-line spacing, leaving sufficient margin on both sides.

Manuscripts should be written with Microsoft Word.

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

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

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

Original researches should have the following sections;

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

Materials and Methods: The study plan should be clearly described, indicating whether the study is randomized or not, whether it is retrospective or prospective, the number of trials, the characteristics, and the statistical methods used.

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.

References: Authors are responsible for the accuracy of the references. See General Guidelines for details about the usage and formatting required.

Case Reports

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 key words. 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).

Review Articles

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 key words. Source of all citations should be indicated. The entire text should not exceed 25 pages (A4, formatted as specified above).

Letters to the Editor

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 1,000 words and can have up to 5 references.

References: The authors are required to cite only those references that they can submit to the Journal in the event they are requested to do so. References should be cited in numerical order (in parentheses) in the text and listed in the same numerical order at the end of the manuscript on a separate page or pages. All authors should be listed regardless of number. Journal abbreviations should conform to the style used in the Cumulated Index Medicus (www.icmje.org). Only list the literature that is published, in press (with the name of the publication known) or with a doi number in references. It is preferred that number of references do not exceed 50 for research articles, 100 for reviews and 10 for case reports.

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.

Example: Vissers RJ, Abu-Laban RB. Acute and Chronic Pancreatitis. In: Tintinalli JE, Kelen GD, Stapczynski JS (eds.), Emergency Medicine: A comprehensive Study Guide. 6 st ed. New York: McGraw-Hill Co; 2005; p. 573-577.

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

Figures, Pictures, Table 's and Graphics:

• All figures, pictures, tables and graphics should be cited at the end of the relevant sentence.

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

• Figures, pictures/photographs must be added to the system as separate .jpg or .gif files.

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• All abbrevations used, must be listed in explanation which will be placed at the bottom of each figure, picture, table and graphic.

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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 'iThenticate' software. Results indicating plagiarism may result in manuscripts being returned or rejected.

Checklist for Submitted Articles:

Articles must be complete. They must include the following:

• Cover Letter

• 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.

• "Copyright Form" signed by all authors.

• Manuscripts lacking any of the above elements will be rejected from the production process.

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EDITORIAL

Dear Colleagues,

The Viral Hepatitis Journal, which journal three issues a year and publishes qualitative scientific hepatitis articles at the International level, has come end of the year. In our journal in 2016, 4 reviews, 15 articles, 3 case reports, 5 letter to the editors were published. We thank to all authors, reviewers and others for the contributing.

We would like to remind you that the Viral Hepatitis Journal has been indexed in Emerging Sources Citation Index (SCI-E). Your articles are both indexing with ESCI and can be accessed as open access.
In this issue, there are one review article about "Immunosuppressive Therapy and Hepatitis B Virus Reactivation" and four articles about "Hepatitis B Virus Vaccination Rates among Medical Laboratory Workers: A Multi-centered Assessment", "Seroepidemiology of Hepatitis B Virus Infection in İstanbul: A 20-year Survey" "Real-life Outcomes of Tenofovir Disoproxil Fumarate Monotherapy in Nucleos(t)ide Analogue-naive and Nucleos(t)ide Analogue-experienced Chronic Hepatitis B Patients: A Single-center Experience", "Interleukin 28B *rs12979860* CT, *rs12980275* GA, *rs8099917* GT and TT genotypes are the Predictors of Rapid Viral Response in Hepatitis C Virus-Infected Patients" and "Hepatitis B Virus Carrying Drug-resistance Compensatory Mutations in Chronically Infected Treatment-naive Patients".

Also; "Acute Viral Hepatitis B with a Severe Clinical Course in Pregnancy: A Case Report", "An Important Financial Burden: Unnecessary Test Requests for Viral Hepatitis" and "Human Pegivirus and Its Relationship with HIV?", subjects were included in the letter to the editor in this issue.

We expect your contributions with articles, case reports, reviews, and letters to the editor.

Best wishes

Prof. Dr. Fehmi TABAK **Prof. Dr. Mustafa ALTINDİŞ**

Doi: 10.4274/vhd.48615
Viral Hepat J 2016;22(3):69-73

Immunosuppressive Therapy and Hepatitis B Virus Reactivation

İmmünsüpresif Tedavi ve Hepatit B Virüs Reaktivasyonu

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ABSTRACT

Reactivation of hepatitis B virus (HBV) refers to reappearance of active necroinflammatory disease of the liver in an individual at an inactive hepatitis B surface antigen carrier state or who is known to have resolved HBV infection. Patients receiving immunosuppressive therapy for malignant, autoimmune or chronic rheumatic diseases are at risk for HBV reactivation and a flare of their HBV infection due to loss of HBV immune control. HBV reactivation can be prevented by antiviral prophylaxis. Antiviral drugs given after the initiation of immunosuppressive therapy are less effective for liver injury and should not be preferred.

Keywords: Hepatitis B virus, immunosuppressive therapy, reactivation

ÖZ

Hepatit B virüs (HBV) reaktivasyonu, inaktif hepatit B yüzey antijeni taşıyıcılarında veya iyileşmiş HBV enfeksiyonu olan kişilerde aktif nekroenflamatuvar karaciğer hastalığının yeniden ortaya çıkmasıdır. Malign, otoimmün ve romatizmal hastalıklarda kullanılan immünsupresif tedaviler, HBV immün baskılanmasının kaybına bağlı olarak HBV reaktivasyonu ve alevlenme için risk oluştururlar. HBV reaktivasyonu antiviral profilaksi ile önlenebilir. İmmünsüpresif tedaviye başlandıktan sonra verilen antiviral ilaçlar karaciğer hasarını önlemek için daha az etkili olup tercih edilmemelidir.

Anahtar Kelimeler: Hepatit B virüs, immünsüpresif tedavi, reaktivasyon

Köksal İ. Immunosuppressive Therapy and Hepatitis B Virus Reactivation. Viral Hepat J. 2016;22:69-73.

Introduction

Hepatitis B virus (HBV) infection is a major public health problem worldwide and there are 350 million chronic HBV carriers in the world. On the other hand, approximately 30% of the world population shows serological evidence of current or past infection (1). HBV persists in the body of all patients with infection, even in those with evidence of serological recovery. These individuals when receiving immunosuppressive therapy for malignant, autoimmune, or chronic rheumatic diseases are at risk for HBV reactivation and a flare of their HBV infection due to loss of HBV immune control. This can result in increased serum aminotransferase levels, fulminant hepatic failure, and/or death. In addition, reactivation of HBV can lead to an interruption of immunosuppressive therapy, delaying treatment of the underlying disease (1).

Reactivation Definition

There is no consensus on the definition of HBV reactivation among guidelines. European Association for the Study of the Liver (EASL) and Asian Pacific Association for the Study of the Liver (APASL) chronic hepatitis B guidelines consider hepatitis B surface antigen (HBsAg) seroreversion and rise in HBV DNA levels as diagnostic criteria, whereas the American Association for the Study of Liver Diseases (AASLD) defines reactivation as reappearance of active necroinflammatory disease of the liver in an individual at an inactive HBsAg carrier state or who is known to have resolved hepatitis B.

Recently, a standardized nomenclature was made for the definition of HBV reactivation at the Reactivation of Hepatitis B AASLD meeting held in 2013. Accordingly, reactivation of HBV replication was defined as a marked increase in HBV replication

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(≥2 log increase from baseline levels or a new appearance of HBV DNA to a level of ≥100 IU/mL) in a person with previously stable or undetectable levels. The types of reactivation were described as reverse HBsAg seroconversion (reappearance of HBsAg), or appearance of HBV DNA in serum in the absence of HBsAg. The severity of reactivation, defined by the presence or absence of jaundice and liver failure; and its outcome (return to baseline status or persistence in an activated state, need for liver transplantation or death) should also be reported (2,3,4).

Reactivation Mechanism and Reactivation Phases

HBV reactivation is defined as a loss of HBV immune control in a patient with an inactive or a resolved HBV infection, resulting in a reappearance or increase in viral replication with liver damage occurring either during or following immune reconstitution. In the initial phase of reactivation, there is an increase in HBV DNA levels, usually with an asymptomatic evolution. In the second phase, both alanine aminotransferase (ALT) and HBV DNA are elevated; symptoms are frequently present and they may be severe and death may occur. In the third phase, resolution occur due to recovery of the immune system strength spontaneously or as a result of immunosuppressive therapy suspension or due to administration of antiviral drugs (Figure 1) (1).

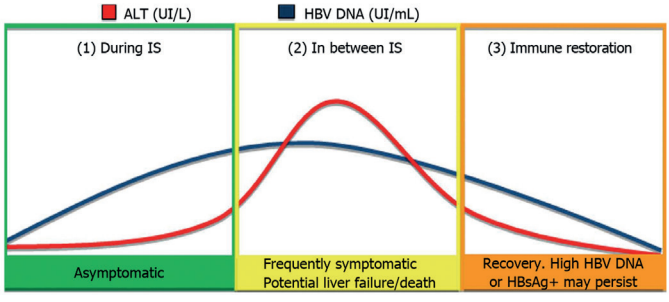

Figure 1. Hepatitis B reactivation phases
IS: Immunosuppression, HBV: Hepatitis B virus, ALT: Alanine aminotransferase, HBsAg: Hepatitis B surface antigen (From Bessone F et al. Hepatitis B reactivation in immunosuppressed patients, World J Hepatol. 2016;8:385-394)

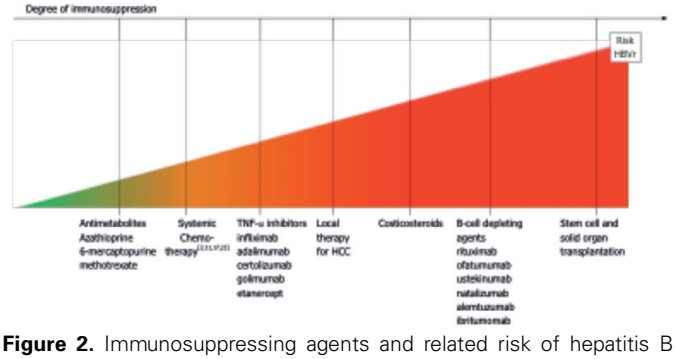

Figure 2. Immunosuppressing agents and related risk of hepatitis B reactivation
HCC: Hepatocellular carcinoma; TNF-α: Tumor necrosis factor-α; HBVr: Hepatitis B virus reactivation (From Bessone F et al. Hepatitis B reactivation in immunosuppressed patients, World J Hepatol. 2016;8:385-394)

Risk Factors for Reactivation

Patients with serologic evidence of HBV infection [HBsAg-positive or anti hepatitis B core antigen (anti-HBc)-positive] are at risk for HBV reactivation if they receive immunosuppressive therapy. Such patients include those being treated for malignancy or autoimmune and chronic rheumatic diseases.

HBsAg-positive patients are at greater risk for reactivation compared to HBsAg-negative patients. If HBsAg-positive patients have hepatitis B e antigen (HBeAg) and/or high baseline levels of HBV DNA (HBV DNA level of >104 IU/mL), they have the highest risk of reactivation. HBsAg-negative, anti-HBc-positive patients have also reactivation risk if they receive immunosuppressive therapy. Reactivation can occur even in anti-HBs-positive patients. However, these patients have a lower risk of HBV reactivation (1).

Reactivation-causing Medications

HBV reactivation risk depends on some factors such as the power and duration of immunosuppression. Some medications, such as corticosteroids and rituximab containing chemotherapeutics, cancer chemotherapy for a variety of hematologic and solid tumors, intra-arterial chemoembolization for hepatocellular carcinoma, and anti-tumor necrosis factor (anti-TNF) therapies for inflammatory bowel disease, and rheumatoid arthritis have been held accountable for HBV reactivation (Figure 2) (1,5,6).

Corticosteroids

In HBsAg-positive patients, HBV reactivation risk increases with corticosteroid therapy with high-dose, rapidly tapered regimens and moderate-dose, prolonged regimens. Reactivation is very rare with low-dose regimens (i.e., <20 mg prednisone per day), even over prolonged periods. Despite the increase in viral replication, serum aminotransferases tend to decline. The opposite occurs once corticosteroids are withdrawn; viral replication decreases while aminotransferases increase. The peak rise in aminotransferase levels typically occurs four to six weeks after withdrawal. HBV reactivation risk increases when corticosteroids are given with other chemotherapy agents.

Chemotherapeutic Agents

HBV reactivation risk is very high among HBsAg-positive patients receiving chemotherapy with a rate of 70%. Reactivation rates are different according to chemotherapeutic agents, their doses and duration. The risk is higher with the use of regimens that include anti-CD20 monoclonal antibodies and/or glucocorticoids (Figure 2).

Drugs for Autoimmune Disorders

Recent studies describe HBV reactivation and flares of hepatitis among patients with autoimmune disorders being treated with a variety of immunosuppressive agents. Anti-TNFs are important drugs for HBV reactivation. In addition to TNF inhibitors, other immunosuppressive drugs, such as methotrexate, abatacept and ustekinumab, may cause HBV reactivation.

Table 1. Recommendations for antiviral prophylaxis in patients receiving cytotoxic or immunosuppressive therapy

AASLD	AGA	EASL
Antiviral prophylaxis recommended for HBV carriers at the start of cancer chemotherapy or of a finite course of immunosuppressive therapy in the following patients: Baseline HBV DNA <2.000 IU/mL level and should continue treatment for 6 months after completion of chemotherapy or immunosuppressive therapy Baseline HBV DNA (>2.000 IU/mL) level and should continue treatment until they reach treatment endpoints as in immunocompetent patients Lamivudine or telbivudine can be used if the anticipated duration of treatment is short (<12 months) and baseline serum HBV DNA not detectable. Tenofovir or entecavir is preferred if longer duration of treatment is anticipated. Interferon should be avoided	Antiviral prophylaxis is recommended for patients with high risk for reactivation: HBsAg+/anti-HBc+ or HBsAg-/anti-HBc+ treated with B cell-depleting agents (e.g., rituximab, ofatumumab) HBsAg+/anti-HBc+ patients treated with either: Anthracycline derivatives (e.g., doxorubicin, epirubicin) Moderate- or high-dose corticosteroids daily for ≥4 weeks Antiviral prophylaxis over monitoring is suggested for patients with moderate risk for reactivation: HBsAg+/anti-HBc+ or HBsAg-/anti-HBc+ patients treated with: TNF inhibitors (e.g., etanercept, adalimumab, certolizumab, infliximab) Cytokine or integrin inhibitors (e.g., abatacept, ustekinumab, natalizumab, vedolizumab) TKIs (e.g., imatinib, nilotinib) HBsAg+/anti-HBc+ patients treated with low-dose corticosteroids for ≥4 weeks HBsAg-/anti-HBc+ patients treated with either: Moderate- or high-dose corticosteroids for ≥4 weeks Anthracycline derivatives (e.g., doxorubicin, epirubicin) Antiviral prophylaxis is not recommended for patients with low risk for reactivation: HBsAg+/anti-HBc+ or HBsAg-/anti-HBc+ patients treated with: Traditional immunosuppressive agents (e.g., azathioprine, mercaptopurine, methotrexate) Intra-articular corticosteroids. Any dose of oral corticosteroids for ≤1 week HBsAg-/anti-HBc+ patients treated with low-dose corticosteroids for ≥4 weeks Antivirals with a high barrier to resistance are preferred over lamivudine Antivirals should be continued for ≥6 months after immunosuppressive therapy discontinuation	HBsAg+ candidates for chemotherapy and immunosuppressive therapy should receive preemptive NA administration during therapy (regardless of HBV DNA levels) for 12 months after cessation of therapy Patients with high HBV DNA level and/or who may receive a lengthy course of immunosuppression, should receive an NA with high antiviral potency and a high barrier to resistance such as entecavir or tenofovir Some experts recommend prophylaxis with amivudine in all HBsAg-/anti-HBc+ patients receiving rituximab and/or combined regimens for hematologic malignancies, if they are anti-HBs- and/or if close monitoring of HBV DNA is not guaranteed NA prophylaxis is recommended for anti-HBc+ patients receiving bone marrow or stem cell transplantation; the optimal duration of prophylaxis for these indications is not known HBsAg- recipients of liver grafts from anti-HBc+ donors should receive prophylaxis with lamivudine and should be continued indefinitely

HBV: Hepatitis B virus, HBsAg: Hepatitis B surface antigen, TNF: Tumor necrosis factor, TKI: Tyrosine kinase inhibitor, AASLD: American Association for the Study of Liver Diseases, AGA: American Gastroenterological Association, EASL: European Association for the Study of the Liver

Anti-tumor Necrosis Factor Agents and Therapeutic Monoclonal Antibodies

TNF inhibitors have been found to be associated with HBV reactivation. TNF inhibitors are used for the treatment of Crohn disease and other intestinal inflammatory disorders, rheumatic diseases, and psoriasis. Among HBsAg-positive patients, the frequency of HBV reactivation has ranged from 0 to 40 percent. The use of concurrent or prior immunosuppressive therapy may contribute to the risk of reactivation. Newer agents, such as therapeutic monoclonal antibodies (rituximab, ofatumumab, and alemtuzumab), may also precipitate HBV reactivation. The US Food and Drug Administration approved the addition of a boxed warning to the prescribing information of rituximab and ofatumumab about this risk and recommends that healthcare professionals:

• Measure HBsAg and anti-HBc levels to determine if a patient is infected with HBV before beginning therapy with rituximab or ofatumumab.

• If screening identifies patients at risk of HBV reactivation due to evidence of previous HBV infection, consult with hepatitis experts prior to use of these agents.

• If treating patients with evidence of previous HBV infection with rituximab or ofatumumab, monitor for clinical and laboratory signs of hepatitis B or HBV reactivation during therapy and for several months thereafter.

• Immediately discontinue rituximab or ofatumumab in patients who develop reactivation of HBV while on therapy and start appropriate treatment for HBV; in addition, any chemotherapy the patient is receiving should be discontinued until the HBV infection is controlled or resolved.

Briefly, all patients receiving anti-TNF agents and therapeutic monoclonal antibodies therapy should be screened for HBV serologic profile.

Serologic tests should include anti-HBc and HBsAg. If there is serological evidence of HBV infection, baseline HBV DNA levels should be measured. Anti-HBs test is not routine because the role of anti-HBs on HBV reactivation is not well known.

Diagnosis and Monitoring of Hepatitis B Virus Reactivation

There is no consensus among guidelines on screening and monitoring recommendations. The consensus guidelines from the AASLD (Management Guidelines), EASL (Management Guidelines), (EASL HBV) and the Centers for Disease Control and Prevention (Management Guidelines) recommend screening for hepatitis B prior to initiating immunosuppressive therapies. The American Gastroenterological Association (AGA), however, recommends screening for HBV only in patients with moderate or high risk for HBV who will receive immunosuppressive therapy and suggests against routine screening in patients at low risk citing cost-effectiveness and false-positive rates as reasons for not screening (2,4,7). If the patient is HBsAg-positive, should be tested for HBeAg and anti-HBe, in addition to HBV DNA levels. If the patient is HBsAg-negative but anti-HBc-positive, hepatitis B reactivation can still occur and close monitoring of HBV DNA and ALT levels during immunosuppressive therapy is warranted. The risks associated with this reactivation can be great and fatal HBV flares in anti-HBc-positive patients who received rituximab-containing chemotherapy for lymphoma. The patients should also be tested for other concurrent infections, such as hepatitis D virus, hepatitis C virus, and HIV. Then, the patients with serologic evidence of HBV infection should be considered for the severity of reactivation risk. Some patients with HBV reactivation are asymptomatic and have normal liver chemistries. Some patients can have a flare of their HBV infection with increased aminotransferase levels and signs and symptoms of liver disease. Patients with detectable HBV DNA should be treated in similar way to HBeAg-positive patients. For patients with undetectable HBV DNA, HBV DNA and ALT should be monitored frequently, every 1-3 months depending on the type of immunosuppressive therapy and comorbidities. In addition, the EASL recommends vaccination of HBV-seronegative patients and notes that higher vaccine doses may be needed in immunocompromised patients.

Level of Reactivation Risk

Very high risk: Patients are at very high risk of reactivation (>20 percent risk of reactivation) if they are HBsAg-positive and are going to receive therapeutic monoclonal antibodies (i.e. rituximab, ofatumumab, obinutuzumab) or undergo hematopoietic cell transplantation. Particularly rituximab is considered an important risk factor for HBV reactivation.

High risk: Patients are considered at high risk for reactivation (11 to 20 percent risk of reactivation) if they are HBsAg-positive and are going to receive high-dose corticosteroids (e.g., ≥20 mg/day for at least four weeks) or therapeutic monoclonal antibodies, alemtuzumab.

Moderate risk: HBsAg-positive patients are at moderate risk of reactivation (1 to 10 percent) if they are going to receive cytotoxic chemotherapy without corticosteroids; anti-TNF therapy; or anti-rejection therapy for solid organ transplants.

Patients who are HBsAg-negative and anti-HBc-positive are at moderate risk for reactivation if they are going to receive therapeutic monoclonal antibodies therapy or undergo hematopoietic cell transplantation.

Low risk: HBsAg-positive patients are at low risk (<1 percent) for reactivation if they receive methotrexate or azathioprine. HBsAg-negative and anti-HBc-positive individuals are at low risk if they receive high-dose corticosteroids (e.g., ≥20 mg/day) or the therapeutic monoclonal antibody, alemtuzumab.

Very low risk: HBV reactivation occurs rarely in HBsAg-negative and anti-HBc-positive patients receiving cytotoxic chemotherapy without corticosteroids; anti-TNF therapy; anti-rejection therapy for solid organ transplants; methotrexate; or azathioprine.

Clinical Findings of Reactivation

Most patients with HBV reactivation are asymptomatic, and there is an increase only in the HBV DNA level. Some patients have increased aminotransferase levels with or without clinical signs and symptoms such as nausea and vomiting. Severe flares can be associated with jaundice, hepatic decompensation, and death; poor outcomes are more likely to occur in cirrhotic patients.

Hepatitis B Virus Prophylaxis

All patients who develop HBV reactivation should be treated. Current guidelines, the AASLD, EASL, and AGA, recommend preemptive or prophylactic therapy before starting chemotherapy or immunosuppressive therapy. Antiviral drugs given after the initiation of immunosuppressive therapy are less effective for liver injury and should not be preferred. The recommendations of the guidelines for prophylaxis are summarized in Table 1 (2,4,7).

Patients with HBV reactivation (with or without a flare) should be treated with entecavir or tenofovir. The duration of treatment depends on the duration and the type of immunosuppressive therapy, the HBV DNA level, and the degree of underlying liver disease.

Tenofovir or entecavir should be chosen for treatment. The decision about which agent to use is based, in part, upon renal function. In patients with reduced kidney function, tenofovir should be avoided. Lamivudine should be given only in short-term course of immunosuppressive treatment since patients receiving lamivudine are at increased risk of developing drug-resistant virus infection. Tenofovir should be preferred for patients who received prior lamivudine therapy. If antiviral therapy is not started, these patients must be monitored closely.

There are no data to guide how long antiviral therapy should be administered before initiating immunosuppressive therapy. Treatment prophylaxis refers to antiviral therapy started before or concurrently as the initiation of immunosuppressive therapy, and before a rise in aminotransferase or HBV DNA levels occurs. However, in patients with a high baseline serum HBV DNA level (e.g., >4 log10 international units/mL), immunosuppressive therapy should be deferred until the HBV DNA level is suppressed to <3 log10 international units/mL.

Duration of Therapy

Although there is no consensus regarding the duration of treatment, the duration of therapy for treatment and prevention is the same and treatment should be maintained for at least 6 months after withdrawal of immunosuppression (AASLD and APASL societies suggest). However, according to the EASL, the recommended duration is 12 months. Treatment should be maintained for at least 12 months after cessation of rituximab (2,3,4).

Antiviral therapy should be continued long-term for hematopoietic stem cell or solid organ transplantation patients since they often remain on chronic immunosuppressive treatment.

Conclusion

Before starting immunosuppressive therapy, the patients should be screened for HBV. All patients who are serologically HBV infection-positive and will receive immunosuppressive treatment should be evaluated for reactivation risk, and prophylaxis should be planned according to the risk factors.

Ethics

Peer-review: External and Internal peer-reviewed.

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Review

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A Review of Hepatitis C in the General Population in Pakistan

Pakistan Toplumunda Hepatit C ile İlgili Bir Gözden Geçirme

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ABSTRACT

To perform a systematic review of the recent scientific literature on hepatitis C infection (2010-2016) for epidemiology, genotypes, co-infection, risk factors and management regime in Pakistan. Present integrated, analyzed updated data, a comprehensive effort to evaluate hepatitis C virus (HCV) disease burden in Pakistan, and to inform public health decision makers. Literature search was performed using PubMed, Google Scholar and Scopus from peer-reviewed journals. Criteria for inclusion and exclusion of pooled data with reference to quality and relevance were set prior to meta-analysis. A total of 129 studies were finally included; more than one study on the same region or group of population was included for comparison and statistical authentication. Hepatitis C is endemic in Pakistan with a 40% raise in the incidence of the disease conferring to resent reports. Data of the last five years from different districts of the country show an abrupt elevation in HCV seroprevalence. HCV genotype 3a remains to be the most prevalent in Pakistan (61.3%). However, in recent years, genotype 1a has raised in the Baluchistan province. A significantly high prevalence in transfusion-transmitted diseases is observed. Management of known risk factor can be a significant parameter to control HCV infection. This review encourages further rigorous research efforts to analyze surveillance of HCV in ruler remote areas of Pakistan. Furthermore, facilitated access to clinical manifestations should be made to identify risk factors, reduce disease burden and to improve the quality of life of hepatitis C carriers.

Keywords: Hepatitis C, epidemiology, prevalence, genotype distribution, co-infection, transmission

ÖZ

Bu çalışmada Pakistan'da hepatit C enfeksiyonunun epidemiyolojisi, genotipleri, ko-enfeksiyonları, risk faktörleri ve yönetimi konusunda son dönem (2010-2016) bilimsel literatürlerin sistematik bir derlemesi yapılmıştır. Pakistan'daki hepatit C virüsü (HCV) hastalık yükünü değerlendirmek ve halk sağlığı uzmanlarını bilgilendirmek için, kapsamlı bir çalışmayla analiz edilen güncellenmiş veriler günümüze entegre edilmiştir. Literatür araştırmaları PubMed, Google Akademik ve Scopus kullanılarak hakemli dergilerden yapılmıştır. Kalite ve uygunluk için referansla toplanan verilerin dahil edilme ve edilmeme kriterleri meta-analiz için önceden belirlenmiştir. Karşılaştırma ve istatistiksel olarak doğrulama için, benzer bölge veya grup üzerinde birden fazla çalışma, sonuçta toplam 129 çalışma dahil edilmiştir. Güncel raporlara göre insidansta %40 artışla hepatit C Pakistan'da endemiktir. Ülkenin farklı kesimlerinden son 5 yıllık veriler, hepatit C seroprevalansında ani bir yükselişi göstermektedir. HCV 3a genotipi Pakistan'da en yaygın genotip olmaya devam etmektedir. Bununla birlikte son yıllarda genotip 1a Balunchistan eyaletinde artış göstermiştir. Transfüzyonla bulaşan hastalıklarda belirgin olarak yüksek bir prevalans gözlenmiştir. Bilinen risk faktörünün yönetimi HCV enfeksiyonunu kontrol etmek için önemli bir parametre olmalıdır. Bu çalışma Pakistan'ın kırsal bölgelerindeki HCV sürveyans analizi için gelecekte yapılacak araştırmaları teşvik edecektir. Ayrıca, risk faktörlerini belirlemek, hastalık yükünü azaltmak ve hepatit C taşıyıcılarının yaşam kalitesini arttırmak için klinik bulguları kolaylaştırmak gerekir.

Anahtar Kelimeler: Hepatit C, epidemiyoloji, prevalans, genotip dağılımı, ko-enfeksiyon, bulaş

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Introduction

Hepatitis C virus (HCV) infection is the most distressing health dilemma worldwide. According to the recent reports of the World Health Organization (WHO), more than 185 million people of the world are infected with HCV (1). Hepatitis C is a predominant cause of chronic liver complications; one third of the infected patients are predicted to develop steatosis, hepatic cirrhosis or hepatocellular carcinoma (2). Despite of all therapeutic interventions, hepatitis C and its associated causes

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are responsible for 350.000 deaths annually (3). Hepatitis C is an asymptomatic infection and the patient remains unaware, but continuous fluctuation in liver enzymes may lead to hepatic injury (4). HCV is more prevalent in developing compared to that in developed countries due to lack of healthcare facilities, poor prognosis and high cost of the available treatments.

Molecular Evolution of Hepatitis C Virus

Choo et al. (4) in 1989, first identified HCV as an enveloped RNA virus. Previously, HCV was named as "non A, non B hepatitis" and was classified as a member of Flaviviridae family (5,6) which also includes Dengue virus. However, the low sequence homology as compared to other flaviviruses eventually led to eastablishment of new hepacivirus family for HCV (7). There are some other hepatotrapic viruses identified as members of hepacivirus family which includes hepatitis A, B, D, E and G (Figure 1) (8).

Genetic sequence of HCV was first characterized in 1989 as highly variable genome with several genotypes which are about 9.6 kb in length (Figure 1) (9). These multiple genomes have high rate of mutation as alteration in sequence that is 1.44x10-3 nucleotide per site per annum. Similarly, an evolutionary rate of 7.4x10-4 nucleotide replacement per site per annum for E1 gene and 4.1x10-4 for the NS5B gene (10) has been identified. Due to this unusual mutational frequency of HCV, development of an effective vaccine is a challenge for scientists and researchers all over the world. How and when HCV was transmitted to human population from Ape species is still a mystery. Moreover, both host and viral factors associated with molecular and cellular mechanism of HCV are not entirely explored. Molecular cloning technique is used for identification and characterization of HCV using blood serum of the infected individual (11). For the confirmation of viremia, initial serological screening is followed by quantitative HCV RNA-based polymerase chain reaction analysis (6). The prime objective of present review was to analyze current epidemiology of HCV infecton in Pakistan through comprehensive literature evaluation of the last decade.

Epidemiology

The WHO estimates that more than 3% of the world population (WHO 2014) is living with hepatitis C. Globally, hepatitis C disease burden has a variable geographical distribution whereas, high prevalence is observed in East and Central Asia followed by North Africa and Middle Eastern counties. As larger population resides in the Asian and African regions, the highest prevalence is observed there as compare to rest of the world (11). Almost 3.7% of Eastern Asia, 3.6% of Middle East and 3.4% of South Asia populations are struggling with hepatitis C (10). The prevalence of HCV infection is estimated at 6% in Pakistan (12), 1.5% in India (13), and 2.2% China (14). However, in underdeveloped countries of Asia, there is lack of authentic data about the disease burden. On the contrary, the developed nations of North America, Western Europe and Australia have low HCV seroprevalence rate i.e. 0.63% in Germany (15), 1% in Canada and 1.1 % in Australia (12). Slightly higher seroprevalence has been reported in some other developed countries like USA (1.7%) (16).

Identification of HCV genotype is critically important for the duration of treatment (17). HCV genotyping is utilized for the production of genotype-specific HCV antibodies. Geographical distribution of genotypes has variable frequency. In West African countries, genetically most diverse genotypes 1 and 2 are more frequent (18). In China and Japan, most HCV infections are due to genotype 1b, whereas genotype 4 is frequently found in Middle East (18) and genotype 5a in South Africa (19). On the other hand, genotype 3a and 1b are more prevalent in Iran, Pakistan and India (20). Patients with HCV genotype 3a are found to be at an accelerated risk of steatosis and hepatic fibrosis whereas, severe liver disease has been reported in patients with chronic HCV genotype 1b (21).

Hepatitis C Prevalence in Pakistan

HCV infection is endemic in Pakistan (22,23,24,25); according to a recent report, the incidence of the disease has increased from 4.7% (41) to 6.8% within a couple of years (28). Unfortunately, low literacy level, inadequate public health facilities and lack of awareness are main reasons behind insufficiency of significant data about the high incidence of the disease in Pakistan. Several studies have reported various HCV infection rates in different geographical areas and ethnic groups in Pakistan (25,26,36,64). In this review, we investigated more than twenty articles on the prevalence of HCV in the country published recently in national and international journals (Table 1).

Majority of the population has never been screened for hepatitis and many individuals have been diagnosed but remained untreated throughout their life. There is no mechanism for the screening of HCV in remote areas and villages of the country where more than 60% of the population is living (68) and ten million people (28,29,30) are reported to have HCV infection. An increase in disease burden is observed in the provinces of Pakistan (31). Data from selected districts of the most populous province Punjab

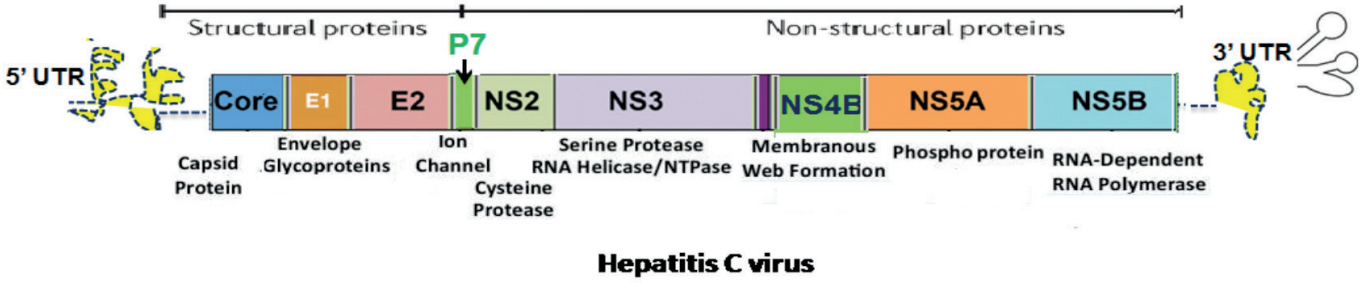

Figure 1. Hepatitis C virus genome

discloses heterogenous results of HCV seroprevalence in recent years. The districts of Punjab include Lahore, Multan, Faisalabad, Gujranwala and Rahimyar Khan (Table 1).

In federal capital Islamabad, the prevalence reported in recent years is 33% in selected group of population. Whereas, studies conducted in different districts of the Khyber Pakhtun Khwa (KPK) province revealed anti-HCV seroprevalence in district Mardan and Mansehra, from Azad Kashmir districts Kotli and Mirpur. Studies of HCV prevalence over the last five years in Sindh Province included the districts of Karachi and Hyderabad. A resent research conducted on HCV frequency in the district of Baluchistan included the provinces of Quetta and Sibi. This review reveals that there are only limited number of studies available in some high prevalence districts, such as Sibi, Mirpur and Kotli. Unfortunately, lack of reports from tribal areas, Gilgit Baltistan, indicates the need for comprehensive studies in these areas (69).

Table 1. Districts wise distribution of anti-hepatitis C virus seroprevalence in Pakistan

Districts	Anti-HCV seroprevalence	Reference
Islamabad	33%	(32)
Faisalabad	22.68%	(33)
Karachi	6.8%	(34)
Karachi	7.6%	(50)
Multan	3.44%	(35)
Multan	6.68%	(45)
Mardan	64.2%	(37)
Gujranwala	5.16%	(30)
Gujranwala	3%	(35)
Lahore	7.3%	(38)
Faisalabad	21.9%	(29)
Islamabad	23.5%	(40)
Karachi	2.61%	(42)
Lahore	4.9%	(41)
Lahore	15.1%	(73)
Mardan	6.46%	(22)
Quetta	20.8%	(43)
Quetta	8.9%	(45)
Sibi	9.3%	(74)
Mirpur	2.5%	(47)
Kotli	6.38%	(70)
Hyderabad	8%	(33)
Mansehra	7%	(50)
Karachi	25.1%	(51)

Anti-HCV: Anti-hepatitis C virus

Hepatitis C Virus Genotype Distribution Pattern in Pakistan

A comprehensive knowledge of HCV genotypes with reference to the geographical location is often significant for determination of severity and treatment regimen. There are various studies that have established a relationship between genotype and their distribution pattern for better understanding of epidemiological complications (40,41,42,43,44,45). However, genotype determination is an expensive procedure which is carried out by sequencing HCV genome at 5'untranslated region or NS5b region (49). Several research groups have shown that HCV infection genotype 3a is most prevalent in Pakistan, followed by untypeable and 1a genotypes (35,50,52). Whereas untypeable infection remains the second most frequent genotype in population studied (46). Similarly, in this review, all reports have stated the same prevalence order of HCV genotypes in Pakistan (Figure 2). Punjab, the largest province of the country, has been reported to have the highest prevalence of HCV infection (55,56). Similarly, different reports based on data from Sindh and KPK province revealed that genotype 3a and 1a were the most prevalent genotypes, respectively. Pakistan shares its long eastern boarders with India where 3a genotype is the most frequent.

However, the data from Baluchistan, geographically the largest province in Pakistan, has shown some variation. According to some studies, genotype 1a, followed by 3a, is predominant in Baluchistan. It is considered that this variation is due to the fact that Iran lies at the western borders of the country. In Iran, 1a is the most frequent genotype (57). Possible shift observed in genotype pattern in KPK is may be due to high mutation or recombination rate in HCV sequence, lack of sensitivity of the present genotyping methods and the migrants from Afghanistan border to tribal regions, and internally displaced people due to war on terror (58). The geographical distribution pattern of HCV genotype is diverse from the rest of the world on the basis of clinical diagnostic methods (59). As untypable, unknown genotypes remain to be second predominant genotype existing in the country. This fact is supported by various studies conducted in Pakistan in the recent years (60,61,62). Therefore, it is a significant challenge for researchers to optimize protocols to sequence the genome of the untypeable genotypes.

Hepatitis C Virus Co-Infections

According to the WHO recommendations, every blood donor should be screened out for at least five transfusion-transmitted

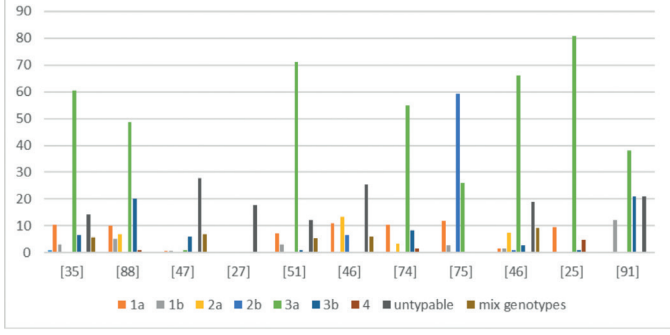

Figure 2. Distribution of hepatitis C virus genotype pattern

infections, such as hepatitis C, hepatitis B, HIV malarial parasite, and syphilis (10). Among donor population, these infectious diseases are the major health concern in developing countries including Pakistan. In case of co-management sound clinical consideration is required to avoid the risks. Limited studies have been conducted in Pakistan which demonstrated an increased rate of co-infection with hepatitis C and hepatitis B virus (HBV), thalassemia and tuberculosis. HBV and HCV co-infection mostly occur in HBV endemic areas, thus, dual infection in Pakistan has been studied by different research groups (64,86,93). Injection drug users (IDU's) have highest rate of HCV and HBV co-infection (69). According to reports from different parts of the country, the prevalence of this co-infection is alarming [0.7% (65) and 3.9% (66)].

Almost 10% of the population of Pakistan is suffering from diabetes mellitus (10). Coexistence of diabetes and HCV infection caused by blood transfusion, surgery, and unsafe insulin syringes is a major health issue. Two studies including data of registered diabetic patients for the screening anti-HCV antibodies has shown the prevalence of 9.3% (68) and 33% (77). Thalassemia is an inherited haemoglobinopathy and the recommended treatment is blood transfusion (70). These transfusions increase the risk of hepatitis C and other transfusion-transmitted diseases if transfusion is made from unscreened donors. According to a study (71), 68.2% of thalassemia patients were screened out for hepatitis C co-infection. During blood transfusion, each time a new, separate syringe can prevent the transmission of HCV in thalassemia patients (72). The frequency of HCV co-infection is illustrated in Figure 3.

Tuberculosis is endemic in regions where blood products are not screened before practice (40). The prevalence of HCV among tuberculosis patients has not been extensively studied in Pakistan (23). HCV and tuberculosis co-infection which increase the risk of liver failure, is 17.02% (67). HIV infection is a serious

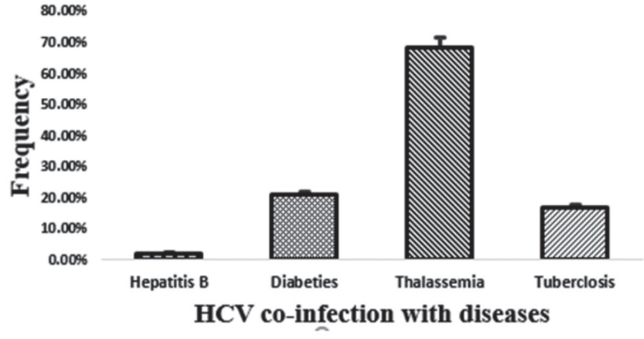

Figure 3. Frequency of hepatitic C infection with hepatitis B, diabeties, thalassemia and tuberclosis
HCV: Hepatitis C virus

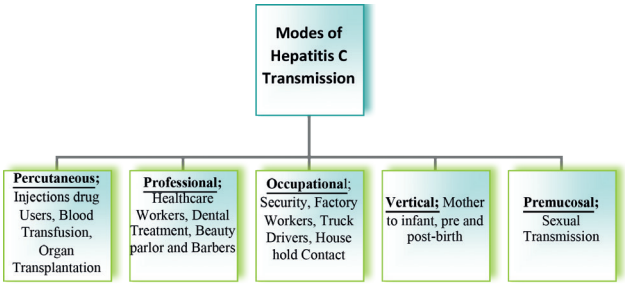

Figure 4. Modes of hepatitis C transmission

life-threatening opportunistic condition. Various reports showed that the incidence of the disease in Pakistan varied between 0.1% (53) and 0.017% (75). Whereas, no subjects were found with HCV and HIV co-infection in other studies (93). The frequency of HCV infection in post-surgery patients in Pakistan is alarming (33). Therefore, pre- and post-surgery serological screening should be performed. Furthermore, the major cause of HCV infection and its co-infections in Pakistan are unsafe blood transfusions (40). In 2003, a comprehensive national blood screening policy was lauched (81) as per recommended protocol of WHO. However, in underdeveloped and remote areas of the country, blood screening policy still needs to be implemented for controlling the frequency of transfusion-transmitted diseases.

Modes of Hepatitis C Virus Transmission

HCV infection is intensely associated with imbalance healthcare facilities in different regions of Pakistan. Major causes of transmission are given in Figure 4. There are 12 billion injections administered throughout the world per annum (80), with more than 45% considered unsafe are practiced in African and Asian countries (81,82). Whereas, Pakistan has the highest frequency of therapeutic intramuscular injections per person annually (83). An elevation in HCV infection rates is observed due to IDU's sharing syringes. Drug equipment and unscreened or inadequately screened blood transfusions (85,86). Nevertheless, hepatitis C is proficiently transmitted through transplantation of infected organs and hemodialysis units (87). Various reports on blood donors have shown reduction in hepatitis C frequency through blood transfusions with different prevalence rates such as, 8.34% (20) 7.1% (89) 20.8% (90), which was 68.2% in another study (Figure 4) (71).

A recent research in Pakistan demonstrated that second major risk factor responsible for blood-borne transmission of hepatitis C was occupation. There have been studies showing the prevalence of HCV infection to be varying between 4.13% and 10% among the healthcare workers (90), 38% (91) and 28.10% (92) among barbers, while dental procedures were found to account for 24.54% (94) and 14.2% (99) (Figure 5). In Pakistan, tattooing is least affecting risk factor on contrary to that in the rest of the world. Some of the hepatitis C infection studies conducted on the prevalence among people belonging to different professions showed 3.13% among Punjab Rangers (94), 14.7% in truck

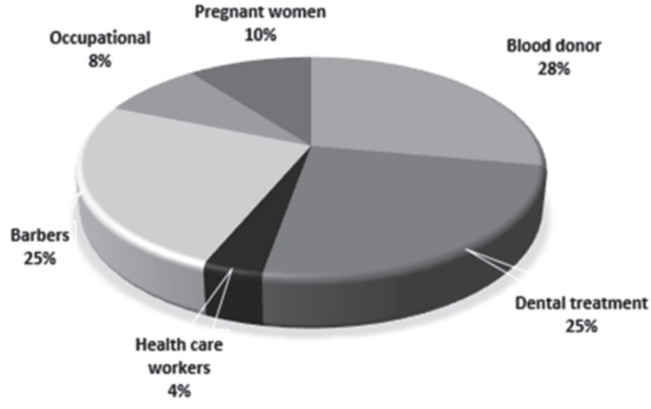

Figure 5. Major risk factors of hepatitis in Pakistan

drivers (95) and 11.38% among factory workers (30). However, it was observed that 36.2% of persons with HCV antibodies were sharing tooth brush, razors, nail cutters and house hold personal belongings (97). Vertical transmission of hepatitis C refers to viral transmission from the mother to the infant. Recently, in a couple of studies, the prevalence of HCV among pregnant women was found to be between 13.3% (98) and 3.45% (99). Viral RNA has been identified in breast milk and colostrum; breast feeding is not a significant risk for mother to infant transmission unless the nipples are intact (100). Even though, HCV RNA has been identified in semen, vaginal fluid, and cervical smears yet, sexual transmission is infrequent (101).

HCV is more frequent among men who have sex with men, heterosexual partners and sex workers (102). Other factors associated with high hepatitis C prevalence comprises of illiteracy, untrained health professionals, and compromised economic background Ford et al. (102). Hence, various awareness programs, basic education and healthcare facilities for all can reduce the prevalence of hepatitis C (104).

Disease Management

With the advances in therapeutic interventions, hepatitis C is curable now and a recovered individual cannot transmit the infection. Previously, the only available therapy was interferon for 6 to 24 weeks (3 million international units) or the same intravenous dose of interferon for 4 to 7 weeks (105). Since 2001, pegylated interferon α with ribavirin has shown favorable results against genotype 2 and 3 (106). Nevertheless, this combination therapy has limited efficacy as well as some adverse effects and low response rate against genotype 1 (107). Currently, for disease management, four new drugs including the protease inhibitors boceprevir, simeprevir, telaprevir are being licensed for HCV genotype 1. A new polymerase inhibitor, sofosbuvir, effective against HCV genotypes 2 and 3 (10), is available on reduced price in Pakistan. These drugs have reduced the risk of hepatocellular carcinoma (107,108). However, telaprevir has been shown to be associated with side effects, such as anemia, rash and pruritus (103,110). This fact emphasizes the inevitability of cost effective, efficient and least toxic hepatitis C therapeutic drugs. Recently, few more HCV inhibitors are at different stages of clinical trials (111).

Conclusion

This systematic review is a pooled analysis to estimate the disease burden attributable to HCV infection. More than 10 million people in Pakistan are suffering from HCV infection, the facts are even worse because there is no data available from remote areas of the country. HCV genotype 3a is the most prevalent genotype in Pakistan. Prediction of genotype assists in selection and time duration of antiviral therapy. Limited studies conducted on HCV co-infections in Pakistan yet their facts and figures are distressing. Moreover, inadequate healthcare services, illiteracy, high cost prognosis and treatment options are barriers in the way to overcome the high prevalence of HCV infection in Pakistan. Furthermore, comprehensive educational awareness seminars concerning preventive measures and risk factors must be arranged on regular basis for the general population. There is an intense need for the establishment of hepatitis clinical research network and administration of database for HCV screening nationwide.

Ethics

Peer-review: Externally and Internally peer-reviewed.

Authorship Contributions

Concept: Sana Riaz, Atia Iqbal, Design: Sana Riaz, Atia Iqbal, Data Collection or Processing: Sana Riaz, Atia Iqbal, Analysis or Interpretation: Sana Riaz, Atia Iqbal, Literature Search: Sana Riaz, Atia Iqbal, Writing: Sana Riaz, Atia Iqbal.

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Research Article

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Hepatitis B Virus Vaccination Rates among Medical Laboratory Workers: A Multi-centered Assessment

Tıbbi Laboratuvar Çalışanlarında Hepatit B Bağışıklama Oranları: Çok Merkezli Bir Değerlendirme

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ABSTRACT

Objective: In this multicenter study, we aimed to determine the rates of hepatitis B virus (HBV) in medical laboratory workers in Turkey and to discuss the current status.
Materials and Methods: We designed this study as prospective, descriptive, epidemiologic research to determine the rates of hepatitis B vaccination in medical laboratory workers. A total of 1359 medical laboratory workers from 26 medical centers, representative of different regions of Turkey, were included in this study. A questionnaire was designed to gather the data on subject planned to apply all the medical laboratory workers. The questionnaire had seven questions in total investigating demographical properties and professional experience of the participants.
Results: We determined that HBV vaccine was administered to the 1118 laboratory workers (82.3%). When anti-HBs titer levels of the vaccinated participants were investigated, 741 (54,4%) of the vaccinated participants stated that they had anti-HBs levels above 10 IU/mL. The results of statistical analysis revealed that vaccination rates and occupation groups were correlated among the laboratory staff ($p<0.05$). However, there was no significant difference between age groups and the duration in work and the vaccination rate ($p>0.05$). Anti-HBs positivity was not correlated with any of the groups ($p>0.05$).
Conclusion: Present study is the first multicenter study to reflect the HBV vaccination rates among laboratory workers across the entire country. Medical laboratory personnel possess the risk of acquiring hepatitis B infection, so that formation of awareness is necessary by education. Anti-HB positivity screened, seronegative all personnel should be vaccinated against hepatitis B and after vaccination anti-HBs should be monitored periodically.
Keywords: Hepatitis B virus, surveys, questionnaire, laboratory personnel, vaccination

ÖZ

Amaç: Bu çok merkezli çalışmada Türkiye genelindeki tıbbi laboratuvar çalışanlarının hepatit B virüsü (HBV) enfeksiyonuna karşı aşılanma durumunun saptanması ve belirlenecek durumun tartışılmasını amaçladık.
Gereç ve Yöntemler: Laboratuvar çalışanlarında hepatit B aşılama oranlarının belirlenmesi için prospektif, tanımlayıcı ve epidemiyolojik bir araştırma çalışması planladık. Bu çalışmaya ülkemizdeki farklı bölgelerdeki 26 sağlık kurumundan 1359 laboratuvar çalışanı katıldı. Konuyla ilgili veri toplamak için demografik bilgiler, mesleki tecrübe ve aşılarla ilgili genel bilgileri içeren toplam 7 sorudan oluşan anket formu kullanıldı.
Bulgular: Çalışmaya katılanların 1118'inde (%82,3) HBV aşısı uygulandığı saptandı. Katılımcılara anti-HBs titreleri sorulduğunda; 741 kişi (%54,5) antikor titresinin 10 IU/mL'nin üzerinde olduğunu belirtti. Aşılama oranları ile tıbbi laboratuvarda çalışan meslek grupları arasında anlamlı ilişki bulunurken ($p<0,05$), yaş grupları, cinsiyet ve çalışma yılları ile aşılama oranları arasındaki fark istatistiksel olarak anlamlı değildi ($p>0,05$). Anti-HBs pozitifliği açısından hiçbir grupta istatistiksel olarak anlamlı bir ilişki bulunamadı ($p>0,05$).
Sonuç: Bu çalışma, HBV aşılanma oranları ile ilgili olarak ülkemiz genelini yansıtabilecek düzeyde ilk çok merkezli çalışmadır. Tıbbi laboratuvar personeli hepatit B enfeksiyonu karşısında yüksek risk altında olduğundan, gerekli farkındalığın oluşması için hizmet içi eğitimler verilmeli, anti-HBs pozitifliği yönünden taranmalı, seronegatif bütün personel hepatit B yönünden aşılanmalı ve aşıdan sonra da periyodik olarak anti-HBs düzeyi yönünden takip edilmesi gereklidir.
Anahtar Kelimeler: Hepatit B virüsü, anket, sorgu formları, laboratuvar personeli, aşılama

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Introduction

Hepatitis B virus (HBV) infection is a major health problem in Turkey as in the whole world. According to 2014 data from the World Health Organization (WHO), approximately 240 million people are chronically infected with HBV worldwide and more than 780.000 people lose their lives due to the complications related with the chronic hepatitis B such as cirrhosis and liver cancer (1). Health care workers are particularly under high risk of blood-transmitted diseases because of occupational exposure. The frequency of HBV infection among health care workers is reported to be 3-8 times more than the normal population, particularly among emergency department, operating room, intensive care unit, and laboratory staff, who are frequently exposed to contaminated patient materials such as blood and other body fluids (2). WHO has reported that approximately 3 million (2 million HBV, 0.9 million HCV and 170.000 HIV) of the 35 million health workers worldwide are exposed to the viruses transmitted via injuries from contaminated medical instruments or direct contact with contaminated blood (1).

Turkey is located in the region with moderate endemicity in terms of HBV carriage. HBV carriage rate is between 2% and -10% in Turkey. It has been reported that this rate is 1.5-2 times more among health care workers (3,4). Hepatitis B is a preventable disease and all health care professionals should be involved in a vaccination program against hepatitis B. Antibody titers of 95-99% can be achieved after 3 dose vaccination in infants, children and adults. This vaccination also contributes to protection of relatives of vaccinated individuals as well. All health care workers should also be vaccinated (2,5). In 2010 the Immunization Advisory Board of the Ministry of Health of Turkey recommended the implementation of vaccines against adult type diphtheria-tetanus, measles-mumps rubella, hepatitis A, hepatitis B, varicella zoster, and seasonal influenza in health care workers (6).

Hepatitis B vaccination rates among health care workers from Turkey, generally evaluate single center data and are not inclusive of all health care workers (7,8). There are few papers from Turkey evaluating hepatitis B vaccination in medical laboratory workers (9). In this multicenter study, we aimed to determine the rates of hepatitis B vaccination in medical laboratory workers in Turkey and to evaluate the precautions to be taken on this special subject.

Materials and Methods

We designed this study as prospective, descriptive, epidemiologic research to determine the rates of hepatitis B vaccination in medical laboratory workers. A total of 1359 medical laboratory workers from 26 medical centers, representative of different regions of Turkey, were included in this study. The 26 medical centers consisted of 17 university hospitals, 8 research and training hospitals, and 1 state hospital (Table 1). A questionnaire was developed to gather data on the subject planned to apply all the medical laboratory workers. The questionnaire had a total of seven questions investigating demographical properties and professional experience of the participants. Vaccination status and the antibody titers related to HBV were also investigated. All the questionnaires were filled upon face-to-face interviews held in the

Table 1. List of medical centres and number of participants

No	Medical institution	City	Participants
1	Ondokuz Mayıs University Training and Research Hospital	Samsun	151
2	Ministry of Health Haydarpaşa Training and Research Hospital	İstanbul	86
3	Adana Numune Hospital	Adana	64
4	Ahi Evran University Faculty of Medicine Hospital	Kırşehir	56
5	Sakarya University Training and Research Hospital	Sakarya	76
6	Haydarpaşa Sultan Abdülhamid Numune Training and Research Hospital	İstanbul	54
7	İnönü University Training and Research Hospital	Malatya	78
8	Atatürk University Training and Research Hospital	Erzurum	74
9	Adnan Menderes University Training and Research Hospital	Aydın	50
10	Bülent Ecevit University Training and Research Hospital	Zonguldak	50
11	Giresun University Training and Research Hospital	Giresun	50
12	Necmettin Erbakan University Training and Research Hospital	Konya	60
13	Erciyes University Training and Research Hospital	Kayseri	48
14	Dicle University Training and Research Hospital	Diyarbakır	48
15	Van Training and Research Hospital	Van	46
16	Kocaeli University Training and Research Hospital	Kocaeli	45
17	Yüzüncü Yıl University Training and Research Hospital	Van	45
18	Osman Gazi University Training and Research Hospital	Eskişehir	44
19	Ankara Training and Research Hospital	Ankara	43
20	Siirt State Hospital	Siirt	36
21	Ordu University Training and Research Hospital	Ordu	32
22	Meram Training and Research Hospital	Konya	53
23	Düzce University Training and Research Hospital	Düzce	25
24	Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital	Ankara	21
25	İzmir University Training and Research Hospital	İzmir	13
26	Siyami Ersek Training and Research Hospital	İstanbul	11
Total			**1359**

working places of the participants. Participants were included in the study after verbal approval; workers not willing to participate were excluded.

Statistical Analysis

Statistical evaluations were performed by commercial statistical software SPSS version 21.0 (SPSS Inc., Chicago, IL, USA). Comparisons between profession groups were analyzed with chi-square test. Comparisons between categorical variables, ages and working years were examined with correlation tests. A p value of less than 0.05 was considered statistically significant.

Results

A total of 1359 laboratory workers (578 male, 781 female) were included in this study. The study population comprised doctors (n=133), research assistants (n=78), laboratory technicians (n=196), biologists (n=750), students (n=24) and cleaning staff (n=161). Seventeen of the participants did not answer the question about the profession. The distribution of occupation of the laboratory personnel, vaccination and anti-HBs positivity rates are listed in Table 2. One thousand one hundred-eighteen of the 1359 laboratory workers replied the question whether they had hepatitis B vaccination as "I completed my vaccines". When anti-HBs titer levels of the vaccinated participants were investigated, 741 (54.5%) of the reported having anti-HBs levels above 10 IU/mL, 116 (8.5%) of the subjects reported below 10 IU/mL and 502 (36.9%) of them stated that they did not know their anti-HBs levels. Forty-eight of the workers did not reply this question (Table 2). The age distribution of the personnel demonstrated that most of the participants were aged 30-40 years (n=443, 32.5%). Occupational groups, age groups and vaccination status according to the duration of employment are summarized in Table 3. Vaccination rate among

Table 2. Vaccination rates of the medical laboratory workers according to professions, age groups and duration at work

	n (%)	Vaccinated n (%)	p value	Anti-HBs positivity n (%)	p value
Occupation groups					
Doctor (Specialist)	133 (9.7)	123 (92.4)	<0.0001	80 (60.1)	0.486
Research assistant	78 (5.7)	71 (91.0)		46 (58.9)	
Student	24 (1.7)	17 (70.8)		12 (50)	
Technician/ Biologist	946 (69.6)	789 (83.4)		517 (54.6)	
Cleaning staff+Other	161 (11.8)	106 (65.8)		78 (48.4)	
Unanswered	17 (0.07)	12 (70.5)		8 (47)	
Age groups					
20-30 ages	252	202 (80.1)	0.898	151 (59.9)	0.859
30-40 ages	443	374 (84.4)		211 (47.4)	
40-50 ages	300	251 (83.6)		183 (61)	
≥50 ages	87	67 (77)		41 (47.1)	
Not answered	277	224 (80.6)		155 (55.9)	
Total years at work					
0-10 years	647	525 (77.8)	0.296	329 (48.8)	0.710
10-20 years	362	319 (88.1)		194 (53.5)	
20-30 years	238	194 (81.5)		147 (61.7)	
>30 years	25	17 (68)		16 (64)	
Not answered	87	63 (72.4)		55 (63.2)	

Table 3. Hepatitis B vaccination rates and anti-HBs titre levels of the medical laboratory workers

Hepatitis B vaccination status	n	%
Vaccinated	1118	82.4
Unvaccinated	241	17.7
Anti-HBs titre level		
<10 IU/mL	116	8.5
>10 IU/mL	741	54.5
Unknown titre	502	36.9

Table 4. Hepatitis B vaccination status and antibody levels of the health care workers in our country reported in the last decade

Article year	Study period	Study group (n)	Vaccination status % (occupation)	Anti-HBs positivity (%)
Öncül et al. (13)	2009	503	83.5 (NU)/78.8 (TEC)/64.2 (HCW)	62.2
Koruk et al. (20)	2013	327	63.8	-
Cılız et al. (11)	2011-2012	309	83.9 (DR)/75.8 (NU)/45.6 (HCW)	84.1
Boşnak et al. (2)	-	199	82.8 (NU)/69.3 (HCW)	81.4
Altun et al. (16)	2010-2011	705	86.9	88.3
Koçak et al. (17)	2012	276	81.2 (DR)/48.2 (HCW)	61.2
İnci et al. (3)	2009	292	80 (DR)/54.2 (NU)/29 (HCW)	62.7
Karacaer et al. (14)	2014	219	90 (DR)/89 (NU)/50 (HCW)	-
Akçalı et al. (8)	2009-2010	256	64	73.4
Baysal and Kaya (18)	2010-2012	823	67.9	81.8
Demir et al. (4)	-	402	55.8 (DR)/74.2 (TEC)/57.5 (NU)	58.3
Karaosmanoğlu et al. (10)	2010	150	89	83
Tekin and Deveci (19)	2008-2009	180	73 (DR)/78.5 (NU)/65.4 (TEC)	68.3
Koruk et al. (15)	2008	303	80.5 (DR)/58.7 (HCW)	63
Omaç et al. (28)	2010	860	70.5	60.6
Aşkar (21)	2005	648	59.6	73

DR: Doctor, NU: Nurse, TEC: Technician, HCW: Health-care worker

female workers was 83.4% whereas the rate was 80.7% among male participants (p>0.005).

The results of the statistical analysis revealed that there was a positive correlation between vaccination rates and occupation groups (p<0.05). However, there was no significant difference between age groups and the duration of employment in respect to vaccination rates (p>0.05).

Discussion

Health care professionals should develop a habit of getting vaccinated in addition to implementation of standard infection control procedures to protect themselves from hepatitis infections (7). Injuries with contaminated needles and other percutaneous injuries appear to be a major problem among health care workers in Turkey. Injury rates specified in various studies range from 46% to 57% (9,10,11,12). Vaccination, use of protective equipment, taking the standard precautions to reduce the risk of exposure, such as hand washing, can prevent the spread of the infectious agents (13,14). The WHO accepted hepatitis B infection as an occupational disease in 1996 for health care workers and the Ministry of Health of Turkey implemented hepatitis B vaccination program determination of the vulnerable workers and vaccination of appropriate population for health care workers (2). HBV seroprevalence and vaccination rates among health care professionals covering the last decade in Turkey are listed in Table 4. Hepatitis B vaccination rate ranges between 29% and 90% (3,14,15,16,17,18,19,20,21). It is observed that vaccination rates increased in recent years. Studies that have investigated vaccination rate among health care professionals in general and were single center studies. There has been only one study including medical laboratory workers in the literature in the last decade (9). This makes it difficult for us to set a clear comment on medical laboratory workers.

The vaccination rate of 82.4% that was found to be quite high in our study can be assumed to represent the profile of the whole country. However, approximately 20% of staff was not protected by vaccination and this vulnerable population needs immediate intervention. Vaccination rates among health care workers across the world differ by country according to the socioeconomic status and range from 11% to 89.8% (22,23,24,25,26). The major challenging problem indicated in these studies is the implementation of the three dose of the vaccine (24,25,27). After implementation of the hepatitis B vaccine schedule for health workers, anti-HBs titers should be checked at the appropriate time. Anti-HBs positivity rate was found to range from 41.2% to 88.3% among health care workers in various studies conducted in our country. Higher anti-HBs levels are remarkable findings of recent studies (Table 4). When different studies across the world were examined, anti-HBs positivity among medical laboratory workers was determined to be 66.7% in Saudi Arabia, which was significantly higher than in other health care workers (21). Anti-HBs seropositivity rate was determined as 75.4% among 474 dentists in Brazil (25). In our study, anti-HBs positivity rate was 54.5% among the participants and 36.9% of the participants were not tested for anti-HBs levels. Despite high rates of HBV vaccination, anti-HBs positivity was relatively low and this fact was the result of participants' unawareness of their antibody titers. It is a remarkable finding that approximately one-third of the staff was not tested for anti-HBs or did not know their titers. Among these staff, there might be ones with antibody titers below 10 IU/mL and surely, they were vulnerable to HBV infection. This indicative situation is due to lack of education and awareness together with personal neglect among our study group. Vaccination rates remained higher among doctors, the research assistants, technicians and biologists compared to that in cleaning staff and other personnel (p<0.05). Except for one study, which was conducted ten years ago, the vaccination rates among doctors working in our country were determined as highest compared to other workers (Table 4). This situation indicates that personnel who are not educated for basic health care, namely the cleaning staff, medical secretaries, technical staff, and security guards should be trained for infection control measures especially for blood-borne diseases. They should be tested before recruitment and in certain intervals thereafter. It will be also logical to include these staff in vaccination programs. In this study, HBV vaccination rates were not correlated with the age and duration of employment (p>0.05).

This study is the first multicenter study to reflect the HBV vaccination rates among laboratory workers across the entire country. According to the findings obtained from this study, it was understood that approximately 20% of the laboratory staff is not vaccinated, and 8% of the staff, although vaccinated, do not have protective levels of antibody titers. By this way, it is clear that approximately 30% of the laboratory workers included in this study were unprotected and were vulnerable to HBV infections.

As a result, medical laboratory personnel possess the risk for acquiring hepatitis B infection, so that awareness raising is necessary by way of education. All laboratory staff should be screened for hepatitis B virus and all seronegative staff should be vaccinated. Periodic monitoring of anti-HBs levels is also essential. Within the scope of workers' health and safety and infection control measures, this assessment is a necessity.

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Ethics

Ethics Committee Approval: The study was approved by the Sakarya University of Local Ethics Committee, Informed Consent: Consent form was filled out by all participants.

Peer-review: Externally and Internally peer-reviewed.

Authorship Contributions

Concept: Özlem Aydemir, Design: Mehmet Köroğlu, Data Collection or Processing: Özlem Aydemir, Lab BioSafety TR working group, Büşra Yüksel, Analysis or Interpretation: Özlem Aydemir, Mehmet Köroğlu, Büşra Yüksel, Tayfur Demiray, Ahmet Özbek, Selma Altındiş, Ferhat Gürkan Aslan, Mustafa Altındiş, Literature Search: Özlem Aydemir, Writing: Özlem Aydemir, Mehmet Köroğlu.

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Research Article

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Seroepidemiology of Hepatitis B Virus Infection in İstanbul: A 20-year Survey

İstanbul'da Hepatit B Virüsünün Seroepidemiyolojisi: 20 Yıllık Bir Çalışma

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ABSTRACT

Objective: Turkey is an intermediate endemic country for hepatitis B virus (HBV) infection, with the prevalence rates showing regional differences. The aim of this study was to determine the recent prevalence of HBV infection among adults in İstanbul and to evaluate the impact of national vaccination program that started in 1998.

Materials and Methods: Data of three healthcare centers from 3 different districts in İstanbul between 1995 and 2015 were obtained. HBV status of the participants was evaluated according to their hepatitis B surface antigen (HBsAg) and anti-HBs results. The change in HBV prevalence was evaluated by dividing the time included in the study into three periods: 1995-2002, 2003-2009 and 2010-2015. Statistical comparison between groups studied in each period was performed. To evaluate the impact of vaccination, the patients of 15-17 years of age were compared with the rest of the last study group.

Results: Totally, 26001 adult patients were included in the study. HBsAg and anti-HBs tested positive in 4.24% and 16.81% of patients, respectively. When the 20 years included in the study were divided into three periods and each studied separately, the positive results for HBsAg decreased from 5.34% to 4.8% and 3.08%, and positive anti-HBs results increased from 14.24% to 15.45% and 19.57%, the difference between the groups being statistically significant (p<0.001). In vaccinated group (n=287), no one tested positive for HBsAg and 30.3% of patients tested positive for anti-HBs, being statistically different from the rest of the group (p<0.001).

Conclusion: Despite the positive effects of vaccination, chronic HB remains a serious health problem.

Keywords: Hepatitis B virus, seroprevalence, vaccination, İstanbul

ÖZ

Amaç: Hepatit B virüsü (HBV) enfeksiyonu Türkiye'de orta düzeyde endemik bir enfeksiyondur ve prevalansı bölgesel olarak farklılıklar gösterir. Bu çalışmanın amacı İstanbul'daki yetişkinlerde HBV enfeksiyonunun son prevalansını belirlemek ve 1998'de başlatılan ulusal aşılama programının etkisini değerlendirmektir.

Gereç ve Yöntemler: İstanbul'da üç farklı ilçenin aile sağlık merkezlerinden elde edilen veriler 1995'ten 2015'e kadar retrospektif olarak tarandı. Katılımcıların HBV açısından durumu hepatit B yüzey antijeni (HBsAg) ve anti-HBs sonuçlarına bakılarak değerlendirildi. HBV prevalansındaki değişim ise çalışmaya dahil edilen süre üç döneme, 1995-2002, 2003-2009 ve 2010-2015 yılları kapsayan dönemler olarak, bölünerek değerlendirildi. Her bir dönemde incelenen gruplar arasındaki farklar istatistiksel olarak karşılaştırıldı. Aşılamanın etkisini değerlendirmek için, 2010-2015 zaman aralığındaki grupta 15-17 yaşındaki hastalar, grubun geri kalanıyla karşılaştırıldı.

Bulgular: Toplamda 26001 yetişkin hasta çalışmaya dahil edildi. HBsAg ve anti-HBs, sırasıyla hastaların %4,24 ve %16,81'inde pozitif tespit edildi. Çalışmaya alınan 20 yıllık süre, üç ayrı döneme ayrılıp her bir dönem ayrı ayrı incelendiğinde, HBsAg'de pozitiflik oranının %5,34'ten %4,8 ve %3,08'e düştüğü; anti-HBs'de pozitiflik oranının %14,24'ten %15,45'e ve %19,57'ye yükseldiği gözlemlendi. Gruplar arasındaki farklar istatistiksel olarak anlamlı bulundu (p<0,001). Aşılanmış grupta (n=287), HBsAg pozitif hasta yoktu, anti-HBs pozitiflik oranı %30,3 ve istatiksel olarak grubun geri kalanından farklı bulundu (p<0,001).

Sonuç: Her ne kadar aşılamanın olumlu bir etkisi olduğu tespit edilmişse de, kronik HBV enfeksiyonu önemli bir sağlık sorunu olmaya devam etmektedir.

Anahtar Kelimeler: Hepatit B virüsü, seroprevalans, aşılama, İstanbul

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Introduction

Hepatitis B (HB) is one of the most common infectious diseases in the world with approximately 2 billion infected people (1,2). HB virus (HBV) is a major pathogen in the etiology of chronic hepatitis and approximately 40% of all chronic HB cases develop cirrhosis, liver failure, or liver cancer (3,4). HB prevalence is highest in sub-Saharan Africa and East Asia; high rates are also found in the Amazon and southern parts of eastern and central Europe. Western Europe and North America have lower prevalence of HBV infection (1). Turkey is one of the countries with intermediate (28%) endemicity for HB. It is determined that the overall rate of HB surface antigen (HBsAg)-positive population in Turkey is 4.6%. Wide range of seropositivity, from 3% to 12%, has been reported in different studies. These studies were performed in specific populations and in certain cities, and HBV prevalence changes according to the region of the country and the occupation of the population studied (2,5,6,7,8,9,10). An effective and safe vaccine against HBV has been available since 1982. In Turkey, a national vaccination program for HBV was launched in 1998. All infants are vaccinated at birth, at 1 month and at 6 months of age; adults are vaccinated on request.

The aim of our study was to determine the HBV prevalence in İstanbul by studying a large population that do not belong to specific occupational group such that can better represent the general population; also, we aimed to evaluate the periodical change in seroprevalence of HBV markers over 20 years, and to determine the effect of HBV vaccination.

Materials and Methods

After obtaining the local Ethics Committee Approval, HBsAg and anti-HBs results of patients having applied to the healthcare centers in the Fatih, Üsküdar and Kadıköy districts of İstanbul were obtained from the patients' file records. Although being called differently throughout the time period of the study, "family health centers" in 2015, are the centers where primary care health service is given. Because of the characteristics of the patient population (regular check-up, chronic disease follow-up), the parameters, such as anti-HBc, HB envelope antigen (HBeAg) and anti-HBe, were not evaluated. The patient's serum samples were studied using an automatic analyzer (Abbott C8000).

Three periods, namely 1995-2002, 2003-2009 and 2010-2015, were determined and studied separately. Adult patients between the ages of 15 and 90 years were included and children, accepted as individuals younger than 15 years of age, were excluded from the study. All patients whose HBV records were available were included in the study.

Statistical Analysis

The sex ratios and mean age of each group were determined. HBsAg and anti-HBs prevalence was determined for each group and compared statistically calculating chi-square value on Microsoft Excel (2011). After forming the contingency tables by determining expected frequencies, the deviances of each cell of the table were calculated and the general sum representing the chi-square value was converted to p value using quick p value calculator on web (11). The comparison was done for female patients, male patients and total numbers separately for both HBsAg and anti-HBs prevalence. Patients being 15-17 years old in the last group represented the patients having been vaccinated as a part of national vaccination program for HBV that started in 1998. This group of patients was also evaluated separately. Those patients were compared with the rest of the group by one-sample t-test. A p value of less than 0.05 was considered statistically significant.

Results

Totally, 26001 patient's HBsAg and anti-HBs results were evaluated (8791 females and 17210 males). The prevalence of HBsAg and anti-HBs in all of the patients included and according to the sex is presented in Table 1 and Table 2.

The first time interval was the 8-year-period from 1995 to 2002. The average age of patients included was 27±5.2 years. Totally, 5098 patients were evaluated (2951 females and 2147 males), 27 (0.91%) females and 245 (11.41%) males, totally 272 (5.34%) patients tested positive for HBsAg; 343 (11.62%) females and 383 (17.84%) males, totally 726 (14.24%) patients tested positive for anti-HBs.

The second time interval was the 7-year-period beginning from 2003 and ending at 2009. The average age of the patients was 29±6.3 years. Totally, 10843 patients were included (1198 females and 9645 males); HBsAg and anti-HBs tested positive in 4.8% and 15.45%, respectively. Of the females, 46 (3.84%) and 699 (58.35%) tested positive for HBsAg and anti-HBs, respectively. In male patients, those rates were found to be 4.92% and 10.12%, respectively.

The last time interval studied was the 5-year period covering the years from 2010 to 2015. The average age of the patients in this group was 30±5.7 years. Sixty-eight (1.46%) female patients and 242 (4.47%) male patients, totally 310 (3.08%) patients tested positive for HBsAg and 957 (20.62%) female, 1012 (18.68%) male, totally 1969 (19.57%) patients were found to be positive for anti-HBs. In this group, the patients between 15 and 17 years of age were evaluated separately as vaccinated group and in this group, there were 115 females and 172 males, totally 287 patients. Among those patients, there was no HBsAg-positive patient and anti-HBs positivity rate was determined as 30.3%. Vaccinated group showed a statistically significant difference with the rest of the group for HBsAg and anti-HBs prevalence ($p<0.001$).

For the total of 20-year period, 1103 out of 26001 patients (4.24%) were HBsAg-positive and 4370 (16.81%) were anti-HBs-positive. The positive results for HBsAg decreased from 5.34% to 4.8% and 3.08%, and positive anti-HBs results increased from 14.24% to 15.45% and 19.57% when the whole 20 years included were divided into three different periods, namely 1995-2002, 2003-2009 and 2010-2015. There was a statistically significant difference in the prevalence rates between the periods ($p<0.001$).

Discussion

HBV is still a risk for developing countries and the mode of transmission shows differences in these countries when compared with the developed ones. In developing countries, horizontal and vertical transmissions, in developed countries, parenteral drug use, sexual transmission, hemodialysis and surgical interventions

are responsible for HBV transmission (12,13). HBV prevalence in Europe and USA was reported to be about 0.1-0.5%, and in Far East, China and tropical countries the HBV prevalence increases to 5-20% (14,15,16). As HBV prevalence shows differences in different geographic areas and countries, it can also be reported different in various areas inside a country. This is the case for Turkey. The large regional differences in HBV prevalence in Turkey are mainly due to differences in socioeconomic status, lifestyles, infrastructure, and access to health services. In a meta-analysis of 399 studies, the estimated overall population prevalence in Turkey was calculated as 4.57%, and the outcomes of the age-specific groups varied from 2.84%, for the 0-14-year olds to 6.36% in the 25-34-year-old group (5).

With the aim to determine the HBV prevalence in İstanbul, the city of Turkey with the highest socioeconomic status, we conducted this study. Also, we wanted to find out the changes in of HBV prevalence in the last 20 years and the effects of national HBV vaccination program (17).

Several studies aiming to determine HB seroprevalence in İstanbul have been conducted. Koçak et al. (18) analyzed HBV seropositivity in blood donation centers in İstanbul from 1987 to 2003 and showed a decrease from 5.98% in 1987 to 2.07% in 2003. Özsoy et al. (19) determined HBV seroprevalence in healthcare workers in a training hospital in İstanbul from 1998 to 2000 as 3%. That rate was 2.1% in blood donors in that hospital for the same period of time. Erden et al. (20) studied HBsAg and anti-HBs frequencies in randomly selected patients attending a university hospital in İstanbul. The prevalence for HBsAg was determined as 6.6% and for anti-HBs as 28.1%. These data belong to the period of the years 1998 to 2001. In a survey from a hospital in İstanbul, the seroprevalence of HBV positivity was determined as 2.39% in blood donors from 1998 to 2004 (21). Another survey performed in İstanbul found HBV seroprevalence as 5% in emergency patients (22). Tigen et al. (23) investigated the HBsAg positivity rate in blood donors in a university hospital in İstanbul from 2004 to 2011. HBsAg positivity rate was found to be 1.54%, decreasing each year. Doğan et al. (24) retrospectively screened the seropositivity rate of HB in pregnant women in İstanbul from 2008 to 2013. The seropositivity was found to be 1.2% for HBsAg and 26.3% for anti-HBs. From the same cohort of that study, we analyzed pregnant women in a separate study and found it to be 1.5% for HBsAg and 11.5% for anti-HBs. Totally, 7605 pregnant women were evaluated for the period of 20 years, from 1995 to 2015 (25). It can be seen that the HBV prevalence is lower in blood donors, probably because those who are aware of their HBV status do not apply for blood donation. The rates are higher in patients and healthcare workers as expected. It also can be seen that the HBV prevalence decreases by time.

The results of our study are important in aspect that they show the change in HBV prevalence in the last 20 years in İstanbul. In the first period studied, 1995-2002, HBsAg was found positive in 5.3% and anti-HBs in 14.2% of patients. Routine newborn HBV vaccination started at that period, in 1998. Since our study group included adults only, the effect of vaccination was not seen and anti-HBs levels were found to be quite low. In the second period, 2003-2009, the effect of the routine vaccination could not be seen, because the study group began with 15-year-old patients. However, the fall in HBsAg prevalence to 4.8% and the increase of anti-HBs prevalence to 15.4% can be explained by the increased awareness of the illness and adult vaccination. In the third period, between 2010 and 2015, the effect of vaccination can be partly observed. In this period, the decrease in the rate of HBsAg-positive patients to 3.08% and the increase in anti-HBs positives to 19.5% were found to be statistically significant ($p<0.001$).

After vaccination started, good results have been reported from Turkey and all over the world. Since the implementation of national vaccination program for HBV for all children and risk groups, a decline in the prevalence of HBV has been observed (26). A study from the United States shows patterns in the success of vaccination in healthcare workers (27). In our study, we evaluated the vaccinated population in our third group. The patients of 15-17

Table 1. The seroprevalence of hepatitis B surface antigen in three different periods

Time period	Female			Male			Total		
	Positive	Total	%	Positive	Total	%	Positive	Total	%
1995-2002	27	2951	0.91	245	2147	11.41	272	5098	5.34
2003-2009	46	1198	3.84	475	9645	4.92	521	10843	4.80
2010-2015	68	4642	1.46	242	5418	4.47	310	10060	3.08
1995-2015	141	8791	1.60	962	17210	5.59	1103	26001	4.24

Table 2. The seroprevalence of anti-hepatitis B in three different periods

Time period	Female			Male			Total		
	Positive	Total	%	Positive	Total	%	Positive	Total	%
1995-2002	343	2951	11.62	383	2147	17.84	726	5098	14.24
2003-2009	699	1198	58.35	976	9645	10.12	1675	10843	15.45
2010-2015	957	4642	20.62	1012	5418	18.68	1969	10060	19.57
1995-2015	1999	8791	22.74	2371	17210	13.78	4370	26001	16.81

years of age were totally 287 patients with no HBsAg positivity and 30.3% anti-HBs positivity that are significantly higher than the rest of the group that is 19.2% ($p<0.001$). The study sample was quite small, when the number of vaccinated patients reaches that of non-vaccinated ones, more clear results can be obtained. Among the people born before the vaccination started, the prevalence of HBV is still relatively high. Although the awareness against the disease has increased, the routes of transmission of the virus and the need for vaccination are not known enough, thus, the prevalence rates of HBV in Turkey are much higher than those in the developed countries.

Despite the availability of safe and effective vaccine for more than 17 years, because of its asymptomatic nature, chronic HB remains a serious health problem.

Ethics

Ethics Committee Approval: Medical Park Local Ethics Committee, Informed Consent: None (a retrospective study).

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: Yavuz Furuncuoğlu, Recep Öztürk, Design: Yavuz Furuncuoğlu, Füsun Bölükbaş, Data Collection or Processing: Yavuz Furuncuoğlu, Filiz Sağlam, Cengiz Bölükbaş, Analysis or Interpretation: Yavuz Furuncuoğlu, Filiz Sağlam, Literature Search: Yavuz Furuncuoğlu, Filiz Sağlam, Writing: Yavuz Furuncuoğlu, Filiz Sağlam.

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Research Article

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Real-life Outcomes of Tenofovir Disoproxil Fumarate Monotherapy in Nucleos(t)ide Analogue-naive and Nucleos(t)ide Analogue-experienced Chronic Hepatitis B Patients: A Single-center Experience

Nükleozit-naive ve Nükleozit Deneyimli Kronik Hepatit B'li Hastalarda Tenofovir Disoproksil Fumarate Monoterapisi Gerçek-yaşam Sonuçları: Tek Merkez Deneyimi

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ABSTRACT

Objective: The aim of the study was to evaluate the long-term results of treatment efficacy and safety in nucleos(t)ide analogue-naive (NA-naive) and NA-experienced chronic hepatitis B (CHB) patients receiving tenofovir disoproxil fumarate (TDF) therapy.
Materials and Methods: Data of 99 patients treated with the diagnosis of CHB (hepatitis B surface antigen-positive for more than 6 months) with TDF monotherapy between February 2008 and May 2014 were evaluated retrospectively.
Results: In total, 99 patients (median age: 50 years, 68.7% male, 21.2% hepatitis B e-antigen-positive) were included in the study. Thirty patients were NA-naive and 69 patients were NA-experienced. No significant difference was determined between NA-naive and NA-experienced patients regarding the rate of achieving complete virological response at the 1st, 2nd, 3rd, 4th, and 5th years of the treatment (p>0.05). Additionally, no significant difference was determined between NA-naive and NA-experienced patients regarding achieving a biochemical response rate at the 1st, 2nd, 3rd, 4th, and 5th years of the treatment (p>0.05). In our study, resistance at the end of the 5th year was not found. No patient required discontinuation of the treatment due to adverse effects during treatment.
Conclusion: Data analysis indicates that TDF monotherapy provides an efficient viral suppression in NA-naive and NA-experienced patients at the end of the 5th year of the treatment.
Keywords: Tenofovir, nucleos(t)ide analogue-naive, nucleos(t)ide analogue-experienced, complete virological response

ÖZ

Amaç: Bu çalışmada tenofovir disoproksil fumarat (TDF) tedavisi alan nükleos(t)ide analoğu (NA)-naive ve NA-deneyimli kronik hepatit B (KHB) hastalarında tedavi etkinliği ve güvenilirliğine ait uzun dönem sonuçlarının değerlendirilmesi amaçlanmıştır.
Gereç ve Yöntemler: Şubat 2008-Mayıs 2014 tarihleri arasında KHB (6 aydan uzun süredir hepatit B yüzey antijeni-pozitif) tanısıyla TDF tedavisi alan 99 hastaya ait kayıtlar retrospektif olarak değerlendirildi.
Bulgular: Toplamda 99 hasta (medyan yaş 50, %68,7 erkek, %21,2 hepatit B e-antijeni-pozitif) çalışmaya dahil edildi. Otuz hasta NA-naive, 69 hasta NA-deneyimli idi. NA-naive ve NA-deneyimli hastalarda 1., 2., 3., 4., 5. yıllarda komplet virolojik yanıt elde etme oranı açısından anlamlı bir fark tespit edilmedi (p>0,05). NA-naive ve NA-deneyimli hastalarda 1., 2., 3., 4., 5. yıllarda biyokimyasal yanıt elde etme oranı açısından anlamlı bir fark tespit edilmedi (p>0,05). Çalışmamızda TDF tedavisi alırken 5. yıl sonu direnç tespit edilmemiştir. Tedavi süresi boyunca hiçbir hastada yan etki dolayısıyla tedavi sonlandırılmak zorunda kalınmamıştır.
Sonuç: TDF monoterapi tedavisi NA-naive ve NA-deneyimli hastalarda tedavinin 5. yılı sonunda etkin bir viral süpresyon sağlamaktadır.
Anahtar Kelimeler: Tenofovir, nükleos(t)ide analoğu-naive, nükleos(t)ide analoğu-deneyimli, komplet virolojik yanıt

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Introduction

Tenofovir disoproxil fumarate (TDF) is a prodrug of nucleotide analogue tenofovir, a potent and selective inhibitor of hepatitis B virus (HBV) DNA polymerase-reverse transcriptase (1). Initially, TDF was used for the treatment of HIV infection and was then approved for the treatment of chronic hepatitis B (CHB) in 2008 (2). TDF is recommended as a first-line treatment in the guidelines (3,4). Long-term TDF therapy was found to be associated with regression in fibrosis and cirrhosis in CHB patients at the 5th year of treatment (5). With TDF therapy, an efficient viral suppression without developing resistance was achieved at the 7th year of treatment in clinical trials (6).

TDF has been shown to have a good efficacy in nucleos(t)ide analogue-experienced (NA-experienced) patients (7,8). However, there are a limited number of studies about the efficacy of TDF therapy in NA-experienced patients in our country (9,10,11). Since TDF therapy is a frequently preferred treatment for CHB in Turkey, long-term data-based outcomes are necessary to determine its efficacy in both NA-naive and NA-experienced patients.

This study aimed to evaluate the long-term, data-based outcomes of treatment efficacy and safety in NA-naive and NA-experienced CHB patients receiving TDF therapy between February 2008 and May 2014.

Materials and Methods

Patients

Data of 99 NA-naive or NA-experienced patients, who received TDF therapy with the diagnosis of CHB [hepatitis B surface antigen (HBsAg)-positive for more than 6 months] between February 2008 and May 2014, were retrospectively evaluated. Data were obtained from the outpatient clinic files of the patients. The study was approved by the ethics committee of Eskişehir Yunus Emre State Hospital. The treatment was initiated in hepatitis B envelope antigen (HBeAg)-positive patients with alanine aminotransferase (ALT) ≥2 upper limit of normal (ULN), HBV DNA ≥2 upper IU/mL and in HBeAg-negative patients with ALT ≥2 ULN, HBV DNA ≥2 UL IU/mL, and/or concomitant moderate-severe histological injury at liver biopsy (3). TDF 300 mg/day oral therapy was given as monotherapy/combined therapy [lamivudine (LAM) in 9 patients whose therapy had been switched from LAM to TDF during the first 6 months]. The patients with a history of alcohol consumption, hepatitis C virus, hepatitis D virus, HIV coinfection, hepatocellular carcinoma, decompensated cirrhosis, or autoimmune disease were excluded from the study.

Study Design

Patient records were accessed for physical examination, complete blood count test and biochemical tests (ALT, aspartate aminotransferase, alkaline phosphatase, gama glutamil transferase, albumin, bilirubin and creatinine) that were performed in all of the patients at the beginning of the TDF treatment. The patients were followed up every 3 months. Complete blood count test, biochemical tests, and HBV DNA measurement were performed every 3-6 months. Viral markers (HBsAg, anti-HBs, HBeAg, and anti-HBe) were monitored at the beginning and every 6-12 months thereafter. The patients were followed up for hepatocellular carcinoma screenings with abdominal ultrasonography and serum alpha-fetoprotein level. Biopsy was performed in patients without contraindication for liver biopsy. Fibrosis and histology activity indexes were scored according to the Ishak and Knodell scoring systems. HBsAg, anti-HBs, HBeAg, anti-HBe, hepatitis B core antibody, antibody to hepatitis D, antibody to hepatitis C, and antibody to HIV were detected using ELISA assay (DiaSorin, Saluggia, Italy). HBV DNA was detected using polymerase chain reaction (PCR) (2007-2009 COBAS TAGMAN RT-PCR with lower detection limit of 6 IU/mL, 2010-2011 Rotorgene Q RT-PCR with lower detection limit of 20 IU/mL, 2012-2013 Qiagen Artus with lower detection limit of 11 IU/mL, 2014-Rotorgene 6000 RT-PCR with lower detection limit of 3.8 IU/mL).

Treatments and Endpoints

The primary endpoint was defined as an HBV DNA level undetectable by PCR during TDF treatment. Secondary endpoints were ALT normalization, HBeAg seroconversion, safety, and tolerability. Virologic breakthrough was defined as an increase in serum HBV DNA level of >1 log10 copies/mL above the treatment nadir during treatment. HBeAg seroconversion was defined as loss of HBeAg and appearance of anti-HBe antibody in HBeAg-positive patients (3,4).

Statistical Analysis

Data analysis was performed with the SPSS 20.0 program and relevant graphs were created using MS Excel. During analyses, classified knowledge was interpreted using frequency and percentage and continuous data was interpreted using mean, median, and deviations. Statistical significance was investigated with independent and dependent t-tests in parametric conditions. When parametric hypothesis was not provided, statistical significance was investigated with Wilcoxon and Mann-Whitney U tests and interpreted. A p value of less than 0.05 was considered statistically significant.

Results

Ninety-nine patients were included in the study. Sixty eight (68.7%) patients were male and 32 (32.3%) were female. The

Table 1. Baseline characteristics of the patients

Baseline demographics	Total (n=99)	HBeAg negative (n=78)	HBeAg positive (n=21)	p value
Male (%)	68 (68.7%)	54(69.2%)	14 (66.7%)	>0.05
Age (years, mean)	49.77±12.56	52±11.6	41.43±12.73	≤0.05
Follow-up, months (median)	37	38	29	>0.05
Prior ADV treatment (%)	41 (41.4%)	32 (41%)	9 (42.9%)	>0.05
NA-naive patients (%)	30 (30.3%)	22 (28.2%)	8 (38.1%)	>0.05
Mean ALT (U/L)	70.59±103	73.17±112	61±58.9	>0.05
Mean HBV DNA (log10 IU/mL)	4±2.55	3.5±2.3	5.96±2.4	<0.05
Mean Knodell	9.11±3.12	9.32±3	8.47±3.44	>0.05
Mean fibrosis	2.56±1.28	2±1.34	2.33±1	>0.05

HBeAg: Hepatitis B envelope antigen, ADV: Adefovir, NA-naive: Nucleos(t)ide analogue-naive, ALT: Alanine aminotransferase, HBV: Hepatitis B virus

mean age of the patients was 50±13 years. Baseline characteristics of the patients' status as HBeAg-positive and -negative group and totals are given in Table 1. Patients' previous treatments were investigated. They included the use of more than one drug [adefovir (ADV), LAM, entecavir, interferon, pegylated interferon] in 41 patients (56.2%), use of LAM in 16 patients (21.9%), use of ADV in 8 patients (10.9%), use of interferon in 4 patients (5.5%) and use of entecavir in 4 patients (5.5%).

The rate of complete virologic response (CVR) was determined to be 78.7%, 89%, 97.9%, 95%, 96.5%, 97.1%, and 92.8% at the 3rd month, 6th month, and 1st, 2nd, 3rd, 4th, 5th and 6th years of the treatment, respectively. When HBeAg-positive and negative patients were evaluated, CVR at the end of 3rd month and 6th month was statistically significantly higher in HBeAg-negative patients, but no significant difference could be determined between the two groups in all other periods. CVR rates achieved in HBeAg-positive and negative patients by years are given in Figure 1.

When CVR in NA-naive and NA-experienced patients was evaluated by month/year, CVR at the end of the 3rd month was found to be significantly higher in the NA-experienced group compared to the NA-naive group ($p<0.05$). No significant difference was determined between the two groups regarding CVR in all of the other periods ($p>0.05$) (Figure 2). ALT normalization rate was determined to be 86.8%, 92.5%, 94.9%, 96.3%, 94.7%, 97.1%, and 100% at the 3rd month, 6th month, 1st, 2nd, 3rd, 4th, 5th and 6th years of the treatment, respectively. A total of 2 patients have completed 6 years of the treatment and virologic and biochemical responses have been maintained so far. ALT normalization rates in HBeAg-positive and -negative patients by month/year are given in Figure 3. ALT normalization rates in NA-naive and NA-experienced patients by month/year are given in Figure 4. When the patients with and without virologic response at 3rd month and 6th month were compared, it was observed that statistically significantly higher virologic response rates were achieved in HBeAg-negative patients, in patients of higher ages, and in patients with lower mean baseline ALT values and lower log HBV DNA values (p.1% with).

The HBeAg seroconversion rate was 9.5%. None of the patients developed loss of HBsAg. Virologic breakthrough developed in a total of 7 patients during treatment. We could not evaluate TDF resistance. Also, we could not evaluate drug resistance in any of our NA-experienced patients. Poor drug compliance and discontinuation of the treatment at their own request was determined in these 7 patients. TDF therapy was maintained and CVR was achieved. None of the patients in this study had to discontinue treatment due to TDF-associated adverse effects, and there was no significant elevation in serum creatinine levels during the treatment period. It was determined that two patients developed hepatocellular carcinoma during TDF treatment. Both patients were male, of advanced age, and treatment-experienced. Also, both had long-term CHB history and had developed HCC in the presence of cirrhosis.

Discussion

TDF monotherapy is efficient and safe in the long-term suppression of HBV (5,6,12). In a study performed by Marcellin et al. (5) the CVR rate at the end of the 5th year of treatment in HBeAg-positive and negative patients was found to be 97% and 99%, respectively. In a study by Buti et al. (6) the CVR rate at the end of the 7th year of treatment in HBeAg-positive and -negative patients was found to be 99.4% and 99.3%, respectively. However, both of the studies reporting long-term results of TDF treatment were multi-center studies. Also, in these cases, emtricitabine could be added to TDF treatment for confirmed viremia at week 72. In daily practice, we cannot add emtricitabine to TDF because of financial barriers. Therefore, our study is important to demonstrate real-life data for clinicians because of the high CVR rates achieved in both NA-naive and NA-experienced patients with TDF monotherapy.

Advantages of oral NA treatment compared to interferons are the more potent antiviral effect, good tolerance, lower side-effect profile, and good compliance (3). However, antiviral resistance

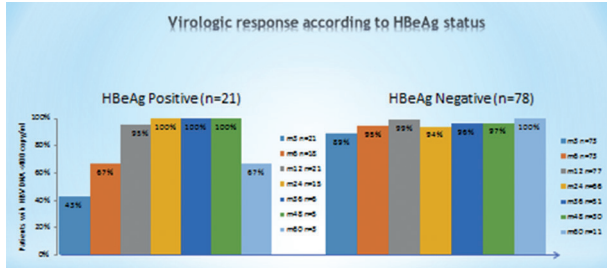

Figure 1. Virological response according to the hepatitis B envelope antigen status
HBeAg: Hepatitis B envelope antigen, HBV: Hepatitis B virus

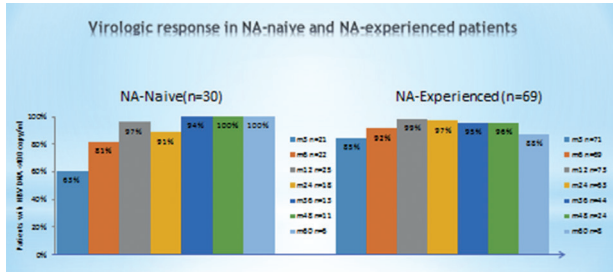

Figure 2. Virologic responses in nucleos(t)ide analogue-naive and nucleos(t)ide analogue-experienced patients
NA: Nucleos(t)ide analogue, HBV: Hepatitis B virus

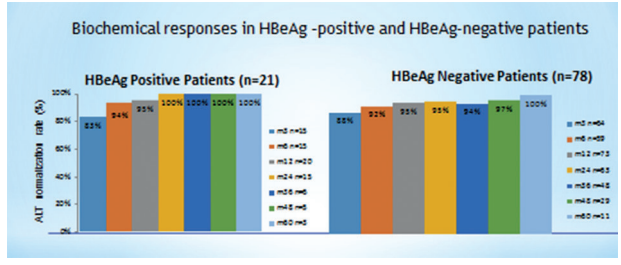

Figure 3. Biochemical responses in hepatitis B envelope antigen-positive and hepatitis B envelope antigen-negative patients
HBeAg: Hepatitis B envelope antigen, ALT: Alanine aminotransferase

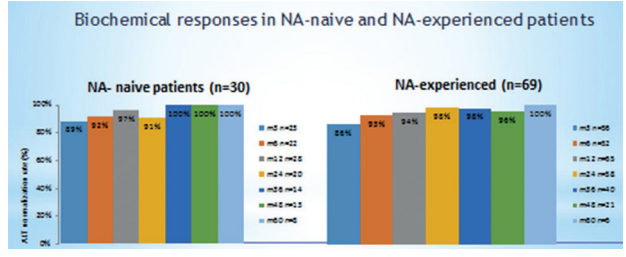

Figure 4. Biochemical responses in nucleos(t)ide analogue-naive and nucleos(t)ide analogue-experienced patients
NA: Nucleos(t)ide analogue, ALT: Alanine aminotransferase

that can be seen during NA treatment causes treatment failure. Currently, there are an increasing number of patients who used multiple NA treatments and experienced treatment failures (13). In our study, no statistically significant difference was found between CVR rates in NA-naive and NA-experienced patients at the 1st, 2nd, 3rd, 4th, and 5th years of the treatment. TDF monotherapy was found to be efficient in NA-experienced patients as it was in NA-naive patients (8,9,10,11,14). However, data on long-term efficacy and safety in daily practice are still limited, especially in NA-experienced patients. When we evaluated the current studies of TDF treatment in NA-experienced patients, we observed that CVR rates over 4 years of therapy were given in only one study. Most of the other studies reported only short-term results. For this reason, we thought that our data reporting results over 5 years of TDF treatment in NA-experienced patients is important because it reports long-term results.

Multiple failures of NA therapies are a growing global problem. LAM is commonly used, especially in some parts of Asia, due to the high prevalence of CHB and its availability at a low cost (15). However, in the era when drugs with a high genetic barrier were not available, ADV and/or entecavir therapy was begun to manage LAM resistance (13). It has been determined that treatment response to TDF therapy was not affected in patients who were determined as having LAM resistance (8,10,14,16,17,18,19). So far, TDF resistance has not been described in NA-experienced patients (20). In our study, drug resistance related to NA analogues previously used was not studied, but it was determined that being NA-experienced did not affect CVR rates ($p \leq 0.05$).

Virologic breakthrough developed in total of 7 patients during treatment. This resulted from poor drug compliance and the discontinuation of treatment on their own request in these patients. The drug had not been switched, TDF therapy was maintained regularly, and CVR was achieved. These results show us that poor drug compliance is an important factor in the development of virologic breakthrough. In accordance with our study, Jung et al. (14) reported that virologic breakthrough developed in a total of 5 patients due to poor medication compliance. The drug had not been switched, therapy was maintained regularly, and CVR was achieved in all of these patients.

In our study, virologic response rates achieved at 3rd month and 6th month were statistically significantly higher in the patients with advanced age, HBeAg-negative, lower baseline ALT values, and lower log HBV DNA values. In a study performed by Bakhshizadeh et al. (21) age, HBeAg positivity, higher baseline ALT values, and HBV DNA values were determined to be factors affecting CVR in univariate analyses, but HBeAg positivity and higher baseline HBV DNA levels were found to be independently associated with CVR in multivariate analyses.

Study Limitations

Our study design is retrospective and we could not evaluate drug resistance in our NA-experienced patients. Also, the number of patients who reached five years of treatment is quite low.

Conclusion

The long-term results of TDF monotherapy in both NA-naive and NA-experienced patients of our study comprising real-life data showed that TDF monotherapy was efficient and safe. Our long-term results with TDF therapy, especially in CHB patients who have developed multiple treatment failures to NA treatments that have caused problems in clinical practice, indicate that TDF therapy is a highly efficient and safe treatment option in this patient group.

Ethics

Ethics Committee Approval: The study were approved by Eskişehir Yunus Emre State Hospital of Local Ethics Committee, Informed Consent: Consent form was filled out by all participants.

Peer-review: Externally and Internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Pınar Korkmaz, Figen Çevik Çağlan, Nevil Aykın, Hasan Naz, Concept: Pınar Korkmaz, Figen Çevik Çağlan, Design: Pınar Korkmaz, Figen Çevik Çağlan, Data Collection or Processing: Pınar Korkmaz, Figen Çevik Çağlan, Nevil Aykın, Hasan Naz, Analysis or Interpretation: Pınar Korkmaz, Onur Toka, Literature Search: Pınar Korkmaz, Figen Çevik Çağlan, Writing: Pınar Korkmaz, Figen Çevik Çağlan.

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

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Research Article

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Interleukin 28B rs12979860 CT, rs12980275 GA, rs8099917 GT and TT genotypes are the Predictors of Rapid Viral Response in Hepatitis C Virus-Infected Patients

Hepatit C Virüs Enfeksiyonu Olan Hastalarda İnterlökin 28B *rs12979860* CT, *rs12980275* GA, *rs8099917* GT ve TT Genotipleri Hızlı Viral Yanıtın Göstergesidir

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ABSTRACT

Objective: In this study, the effects of genotypic differences on the clinical course of the disease, response to treatment and fibrosis were investigated in patients with hepatitis C virus (HCV) infection.
Materials and Methods: Ninety-nine chronic HCV-infected patients and 95 controls were enrolled. The patients received pegylated interferon (PegIFN) + ribavirin (RBV) for 48 weeks and followed up for the next 48 weeks. Aspartate aminotransferase/platelet ratio index was used to determine the stage of liver fibrosis. DNA specimens were extracted from peripheral blood mononuclear cells and the interleukin (IL) 28B gene *rs12979860*, *rs12980275*, and *rs8099917* were genotyped by the immune polymerase chain reaction-restriction fragment length polymorphism method. Results were analysed using the SPSS 16.0 and OpenEpi 2.2 softwares.
Results: All patients had HCV genotype 1. Among the 99 HCV+ patients, in 26.3% spontaneous viral clearance, in 42.8% rapid viral response, 92% early viral response and in 72.6% sustained viral response was observed. The allele frequencies of IL28B single nucleotide polymorphisms (SNP), *rs12979860*, *rs12980275*, and *rs8099917* were not identical in all samples (p<0.005). SNP *rs12979860* CT genotype (p=0.010); *rs12980275* GA genotype (p=0.010); and *rs8099917* GT and TT genotypes (p=0.019 and 0.020, respectively) were strongly associated with rapid viral response in the overall sample.
Conclusion: The determination of IL28B polymorphisms may be useful to individualize treatment options when using PEG/RBV-based therapies for chronic HCV infection but genetic characteristics of populations of the countries must be known.
Keywords: Interleukin 28B, hepatitis C virus, single nucleotide polymorphisms, polymorphism, genotype

ÖZ

Amaç: Bu çalışmamızda hepatit C virüs (HCV) enfeksiyonu olan hastalarda genotipik farklılıkların hastalığın klinik gidişi, tedavi yanıtları ve fibrosis üzerine etkileri araştırılmıştır.
Gereç ve Yöntemler: Çalışmaya 99 kronik aktif HCV enfeksiyonu olan hasta ve 95 sağlıklı kontrol dahil edildi. Hastaların tümü 48 hafta boyunca pegile interferon (PegIFN) + ribavirin (RBV) tedavisi aldı ve tedavi sonu 48 hafta takip edildi. Karaciğer fibrosis evresi için aspartat aminotransferaz/platelet skoru kullanıldı. DNA örnekleri deneklerin peripheral kan mononükleer hücrelerinden elde edildi ve immün polimeraz zincir reaksiyonu-restriksiyon parça uzunluk polimorfizmi yöntemiyle interlökin (IL) 28B *rs12979860*, *rs12980275* ve *rs8099917* genotiplendirmeleri yapıldı. Sonuçlar SPSS 16,0 ve OpenEpi 2,2 yazılımı ile analiz edildi.
Bulgular: Tüm hastalar HCV genotip 1 hastası idi. Çalışmaya alınan deneklerde IL28B tek nükleotid polimorfizmi (TNP) (*rs12979860*, *rs12980275* ve *rs8099917*) dağılımı farklılık gösteriyordu (p<0,005). TNP *rs12979860* CT genotipi (p=0,010); *rs12980275* GA genotipi (p=0,010); ve *rs8099917* GT ve TT genotipleri (p=0,019 ve 0,020 sırasıyla) ve hızlı viral yanıt arasında kuvvetli ilişkili bulundu.
Sonuç: Kronik HCV enfeksiyonu olan hastaların PegIFN+RBV ile tedavilerinin bireysel olarak belirlenmesinde IL28B polimorfizmlerinin bilinmesi faydalı olabilir. Fakat her ülkenin kendi genotipik karakteristiklerini bilmesi gereklidir.
Anahtar Kelimeler: İnterlökin 28B, hepatit C virüs, tek nükleotid polimorfizmi, polimorfizm, genotip

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Introduction

Hepatitis C virus (HCV) is the etiological factor for hepatitis C, which is one of the most important pathogenic factors of chronic liver diseases, cirrhosis and, even hepatocellular carcinoma. When infected with HCV, only a small proportion of patients clear the virus spontaneously and the majority develops chronic hepatitis C (CHC) (1). There are viral and host factors that are important in the development of chronic infection. Baseline viral load, rapid virologic response (RVR) and host characteristics (e.g. alcohol consumption, steatosis, liver fibrosis, metabolic syndrome, ethnicity, and host genetic polymorphisms) are the examples that have impact on chronicity (2).

Hepatocytes are the target cell of the virus. After infection, the innate immune system reacts to the virus and after 4 to 8 weeks, CD8+ T cells recognize viral peptides bound to human leukocyte antigen class 1 molecules on virus-infected hepatocytes. This initiates signaling pathways that lead to the synthesis of interferon (IFN) and a variety of other cytokines. IFN-lambda3 (λ3) belongs to the type 3 IFN family (IFN-λ). IFN-λ is rapidly induced during HCV infection and has antiviral activity against HCV (3,4). The virus is eliminated during the acute phase of the infection by T cell-mediated antiviral mechanisms. The rate of spontaneous viral clearance in acute HCV infection is approximately 26% (range: 15%-40%) (5,6,7). In the remaining patients who do not defeat the virus at first glance, HCV persists for decades unless treated. Until recently, the effective treatment of chronic HCV infection includes pegylated interferon (PegIFN) and ribavirin (RBV) regimen (1). IFN, especially IFN-λ3 interacts with its acceptor, a heterodimer [IFN-λR1 x interleukin (IL)-10R2]. Even the most perfect therapeutic molecules (PegIFN+RBV) do not guarantee 100% efficacy and sustained virologic response (SVR) remains 40% (2,8).

There are variations that contribute to therapeutic success of HCV infection. Genotype of HCV is the most important parameter that has impact on treatment response. Genotype 1 is regarded as "difficult-to-treat" (2). According the HCV genotypes involved, SVR rates of genotypes 2, 3, 5 and 6 is 70%-90%, but it is less than 50% for genotypes 1 and 4 (1,9,10). Two postdoctoral thesis including 500 and 115 patients (11,12) conducted in Turkey revealed that HCV genotype 1b was the most common (81.7-90%), followed by genotype 1a (5.2-7.2%).

Besides HCV genotype, host genetic background could impact HCV infection, viral clearance, and treatment. Although studies demonstrated associations between cytokine gene polymorphisms and outcome of HCV infection, no general consensus has been reached, possibly due to differences between ethnic groups. Four recent studies (13,14,15,16) demonstrated that predictive role of single nucleotide polymorphisms (SNPs) of the IL28B locus was more likely to be associated with spontaneous viral clearance and treatment effectiveness of HCV in genotype 1 patients who were cured by PegIFN combined with RBV: IL28B rs12979860 C (good-response allele) versus T (poor-response allele) and rs809917 T (good-response allele) versus G (poor-response allele) showed the strongest association with SVR.

SNPs of the IL28B gene has been extensively described in the literature but allele frequencies, in particular rs809917, differs somewhat between world-wide populations (17,18,19,20). Therefore, the predictive power of SNPs may vary between different cohorts. For example rs809917 was only a weak predictor of SVR in African-American patients (13). The aim of this study was to examine the prevalence and clinical significance of the outlined SNPs in a population from Turkey, a region with a high prevalence of HCV infection and a high prevalence of genotype 1b.

Materials and Methods

A total of 99 HCV-infected patients (26 spontaneous clearance and 73 chronic HCV genotype 1b patients) and 95 healthy control subjects were included in the study by Gaziosmanpaşa University Faculty of Medicine, Department of Infectious Diseases and Clinical Microbiology. CHC patients, who had received weekly injections of PegIFNepartment of Infectious Diseases and Clinical Microbiology. CHC patients, who had received weegIFNepartment of Infectious Diseases and Clinical Microbiology. CHC paegIFNepartment of Infectious Diseases and Clinical Microbio.5 µg/kg body weight. Ribavirin was orally administered daily in two divided doses (1.000 mg for ≤75 kg, 1.200 mg for >75 kg) (21).

Genomic DNA was extracted from blood samples using an Invitrogen Genomic DNA Isolation Mini Kit K1820-02 (Invitrogen Life Technologies, Carlsbad, CA, USA). Polymerase chain reaction (PCR) of *rs12979860, rs12980275,* and *rs8099917* polymorphisms of IL28B gene were performed in a total volume of 25 µL, using 100 ng of genomic DNA with 20 pmol primers each (for *rs12979860* F:5′-AGG GCC CCT AAC CTC TGC ACA GTC T-3′, R: 5′- GCT GAG GGA CCG CTA CGT AAG TCA CC-3′; for rs12980275 F:5′- GAG AGC AAG AGG AGG GAA GGA A-3′, R: 5′-GTG TGC CAT TAG CCA GTC AGA T-3′; and for rs8099917 F:5′-TTC ACC ATC CTC CTC TCA TCC CTC AT -3′, R: 5′- TCC TAA ATT GAC GGG CCA TCT GTT TC-3′), 0.2 mM each dNTP, 1X buffer, 2 mM MgCl2 and 1 U Taq DNA polymerase (Invitrogen Life Technologies, Carlsbad, CA, USA). Cycling was performed in a Techne TC-4000 Thermal Cycler (Bibby Scientific Limited, Staffordshire, UK) as follows: amplification consisted of a 2-minute denaturation step at 94 °C; 40 cycles for 60 seconds at 94 °C, 40 seconds at 58 °C, 60 seconds at 72 °C and final extension of 7 minutes at 72 °C followed by cooling to 4 °C.

Genotype analysis of three IL28B gene loci (*rs12979860*, *rs12980275* and *rs8099917*) was conducted using restriction fragment length polymorphism for all three polymorphic loci. PCR products were digested with specific restriction enzymes: BstU I for *rs12979860*, Bsl I for *rs12980275*, and *Mae III* for *rs8099917*. The digested PCR products were resolved by electrophoresis on 2.5% agarose gels containing 0.5 µg/mL ethidium bromide. Restriction fragments were visualized with the use of a Vilber-Lourmat Gel Quantification and Documentation System QUANTUM-ST4 (Vilber Lourmat BP 66 Torcy, France).

Statistical Analysis

Statistical analysis was performed by SPSS 16.0 Software (SPSS Inc., Chicago, IL, USA). The distribution of IL28B gene polymorphisms between HCV patients and healthy controls and their deviations from Hardy-Weinberg equilibrium were compared by using the Fisher's exact chi-square test. A p value of less than 0.05 was considered statistically significant.

Odds ratios (ORs) and 95% confidence intervals (CIs) were used to determine the association of IL28B allelic and genotypic variants, compound genotypes and haplotypes with the occurrence of HCV disease were also calculated by Win PEPI version 11.39 software.

Results

Seventy-four patients completed treatment with PegIFN-a plus RBV (two patients could not receive the treatment because of the side effects). There was no significant difference between the patient and the control group in terms of age, gender, and viral genotype (p>0.05). Genotype and allele frequencies are given in Table 1. SNP *rs12979860* C allele (OR, 0.56; 95% CI, 0.37-0.83; p<0.005) and CC genotype (OR, 0.42; 95% CI, 0.23-0.77; p<0.006); *rs12980275* A allele (OR, 0.57; 95% CI, 0.38-0.87; p<0.009) and GG genotype (OR, 3.96; 95% CI, 1.41-11.12; p<0.007); *rs8099917* T allele (OR, 0.56; 95% CI, 0.36-0.88; p<0.014) and TT genotype (OR, 0.50; 95% CI, 0.28-0.87; p<0.022) were strongly associated with the disease development compare to controls.

To evaluate the clinical applicability of individual SNPs, we calculated the predictive ORs for each SNP between rapid RVR, early virologic response, and SVR (Table 2, 3, 4). There were 25 patients who had spontaneous viral clearance. Rapid viral response was seen in 27 patients who had SNP *rs12979860* CT genotype (p=0.010), *rs12980275* GA genotype (p=0.010), and both *rs8099917* GT and TT genotypes (p=0.019, p=0.020, respectively)

Table 1. Genotype and allele frequencies of interleukin 28B gene loci among patients and control

Loci	Genotypes	Patients N (F)	Controls N (F)	p (OR, 95% CI)	Alleles	Patients N (F)	Controls N (F)	p (OR, 95% CI)
rs12979860	CC	24 (0.2424)	42 (0.4330)	0.006(0.42, 0.23-0.77)	C	105 (0.5303)	130 (0.6701)	0.005 (0.56, 0.37-0.83)
	CT	57 (0.5758)	46 (0.4742)	0.198(1.50, 0.86-2.64)				
					T	105 (0.5303)	130(0.6701)	
	TT	18 (0.1818)	9 (0.0928)	0.097(2.17, 0.93-5.09)				
p for HWE		0.1212	0.4746					
rs12980275	AA	26 (0.2680)	37 (0.4022)	0.064 (0.54, 0.30-1.00)	A	105 (0.5412)	124 (0.6739)	0.009 (0.57, 0.38-0.87)
	GA	53 (0.5464)	50 (0.5435)	1.000 (1.01, 0.57-1.79)	G	89 (0.4588)	60 (0.3261)	
	GG	18 (0.1856)	5 (0.0543)	0.007 (3.96, 1.41-11.12)				
p for HWE		0.3234	0.0233					
rs8099917	TT	40 (0.4040)	56 (0.5773)	0.022 (0.50, 0.28-0.87)	T	129 (0.6515)	149 (0.7680)	0.014 (0.56, 0.36-0.88)
	GT	49 (0.4949)	37 (0.3814)	0.116 (1.59, 0.90-2.80)				
					G	129 (0.6515)	149 (0.7680)	
	GG	10 (0.1010)	4 (0.0412)	0.164 (2.61, 0.80-8.58)				
p for HWE		0.3706	0.4872					

N: Number, F: Frequency, OR: Odds ratio, CI: Confidence interval, HWE: Hardy Weinberg equilibrium

Table 2. Genotype and allele frequencies of interleukin 28B- *rs12979860* for disease symptoms

CC		Genotypes		Alleles		
		CT	TT	C	T	
Patients	CHC (n=73)	14 (0.1918)	45 (0.6164)	14 (0.1918)	73 (0.5000)	73 (0.5000)
	Carriers (n=25)	10 (0.4000)	11 (0.4400)	4 (0.1600)	31 (0.6200)	19 (0.3800)
p		0.057	0.161	1.000	0.189	
OR, 95% CI		0.36, 0.13-0.94	2.05, 0.83-5.05	1.25, 0.38-4.12	0.61, 0.32-1018	
RVR	Yes (27)	9 (0.3333)	10 (0.3704)	8 (0.2963)	28 (0.5185)	26 (0.4815)
	No (36)	4 (0.1111)	26 (0.7222)	6 (0.1667)	34 (0.4722)	38 (0.5278)
p		0.060	0.010	0.240	0.719	
OR, 95% CI		3.75, 1.03-13.67	0.23, 0.08-0.65	2.11, 0.64-6.89	1.20, 0.60-2.43	
EVR	Yes (58)	13 (0.2241)	32 (0.5517)	13 (0.2241)	58 (0.5000)	58 (0.5000)
	No (5)	0	4 (0.8000)	1 (0.2000)	4 (0.4000)	6 (0.6000)
p		0.574	0.381	1.000	0.744	
OR, 95% CI		3.26, 0.22-49.08	0.31, 0.04-2.33	1.16, 0.15-9.01	1.50, 0.43-5.26	
SVR	Yes (45)	9 (0.2000)	26 (0.5778)	10 (0.2222)	44 (0.4889)	46 (0.5111)
	No (17)	4 (0.2353)	9 (0.5294)	4 (0.2353)	17 (0.5000)	17 (0.5000)
p		0.739	0.780	1.000	1.000	
OR, 95% CI		0.81, 0.22-3.00	1.22, 0.41-3.63	0.93, 0.26-3.37	0.96, 0.44-2.09	

OR: Odds ratio, CI: Confidence interval, CHC: Chronic hepatitis C, RVR: Rapid virological response, EVR: Early virological response, SVR: Sustained virological response

predicted the most positive response to treatment outcome in the overall study population.

We did not find any difference between aspartate aminotransferase/platelet ratio index (APRI) and genotype frequencies.

Discussion

HCV infection is a major health problem worldwide. The virus is the main cause of chronic hepatitis and liver cirrhosis. Studies on entire viral genomes split HCV into seven major genotypes (22).

Table 3. Genotype and allele frequencies of interleukin 28B- *rs12980275* for disease symptoms

GG		Genotypes			Alleles	
		GA	AA	G	A	
Patients	CHC (n=72)	13 (0.1806)	42 (0.5833)	17 (0.2361)	68 (0.4722)	76 (0.5278)
	Carriers (n=24)	5 (0.2083)	10 (0.4167)	9 (0.3750)	20 (0.4167)	28 (0.5833)
p		0.768	0.166	0.196	0.616	
OR, 95% CI		0.84, 0.27-2.60	1.96, 0.78-4.92	0.52, 0.19-1.36	1.25, 0.65-2.41	
RVR	Yes (27)	8 (0.2963)	9 (0.3333)	10 (0.3704)	26 (0.4727)	29 (0.5273)
	No (35)	5 (0.1429)	24 (0.6857)	6 (0.1714)	34 (0.4857)	36 (0.5143)
p		0.209	0.010	0.089	1.000	
OR, 95% CI		2.53, 0.73-8.70	0.23, 0.08-0.66	2.84, 0.89-9.04	0.95, 0.47-1.91	
EVR	Yes (57)	12 (0.2105)	29 (0.5088)	16 (0.2807)	53 (0.4649)	61 (0.5351)
	No (5)	1 (0.2000)	4 (0.8000)	0	6 (0.6000)	4 (0.4000)
p		1.000	0.360	0.315	0.516	
OR, 95% CI		1.07, 0.4-8.36	0.26, 0.03-1.96	4.37, 0.29-65.25	0.58, 0.17-2.03	
SVR	Yes (44)	9 (0.2045)	24 (0.5455)	11 (0.2500)	42 (0.5385)	36 (0.4615)
	No (17)	4 (0.2353)	8 (0.4706)	5 (0.2941)	16 (0.4706)	18 (0.5294)
p		1.000	0.776	0.752	0.543	
OR, 95% CI		0.84, 0.23-3.09	1.35, 0.45-4.03	0.80, 0.24-2.70	1.31, 0.59-2.91	

OR: Odds ratio, CI: Confidence interval, CHC: Chronic hepatitis C, RVR: Rapid virological response, EVR: Early virological response, SVR: Sustained virological response

Table 4. Genotype and allele frequencies of interleukin 28B- *rs8099917* for disease symptoms

GG		Genotypes			Alleles	
		GT	TT	G	T	
Patients	CHC (n=73)	7 (0.0959)	42 (0.5753)	24 (0.3288)	56 (0.3836)	90 (0.6164)
	Carriers (n=25)	3 (0.1200)	7 (0.2800)	15 (0.6000)	13 (0.2600)	37 (0.7400)
p		0.712	0.019	0.020	0.126	
OR, 95% CI		0.78, 0.19-3.19	3.48, 1.32-9.21	0.33, 0.13-0.82	1.77, 0.87-3.60	
RVR	Yes (27)	3 (0.1111)	11 (0.4400)	13 (0.4815)	17 (0.3148)	37 (0.6852)
	No (36)	4 (0.1111)	23 (0.6389)	9 (0.2500)	31 (0.4306)	41 (0.5694)
p		1.000	0.190	0.067	0.200	
OR, 95% CI		1.00, 0.21-4.76	0.44, 0.16-1.24	2.79, 0.98-7.96	0.61, 0.29-1.27	
EVR	Yes (58)	7 (0.1207)	29 (0.5000)	22 (0.3793)	43 (0.3707)	73 (0.6293)
	No (5)	0	5 (1.0000)	0	5 (0.5000)	5 (0.5000)
p		1.000	0.056	0.153	0.503	
OR, 95% CI		1.60, 0.10-25.05	0.09, 0.01-1.34	6.78, 0.46-100.26	0.59, 0.17-2.02	
SVR	Yes (45)	5 (0.1111)	24 (0.5333)	16 (0.3556)	34 (0.3778)	56 (0.6222)
	No (17)	2 (0.1176)	10 (0.5882)	5 (0.2941)	14 (0.4118)	20 (0.5882)
p		1.000	0.780	0.768	0.837	
OR, 95% CI		0.94, 0.17-5.14	0.80, 0.27-2.41	1.32, 0.41-4.30	0.87, 0.39-1.92	

OR: Odds ratio, CI: Confidence interval, CHC: Chronic hepatitis C, RVR: Rapid virological response, EVR: Early virological response, SVR: Sustained virological response

The HCV genotype 1 is the most prevalent genotype worldwide (46% of all HCV cases), followed by genotype 3 (30%) but the distribution of these genotypes are different between countries (23).

Human hepatocytes are the primary target cell for HCV infection. The first line of immune defense comprises activation of innate immunity following HCV recognition. Local production of IFNs disrupts HCV genome replication and spreading in the liver parenchyma (24). The rate of the treatment of chronic HCV infection (SVR) varies under the influence of ethnicity. For example, it was found that patients of European ancestry were cured more successfully than patients of African ancestry (25).

Besides ethnicity, genetic polymorphism of certain genes influences treatment response. A cohort study with 1000 patients infected with HCV genotype 1 revealed that carrying the IL28B *rs12979860* CC genotype was associated with two-fold chance of SVR compared to TT genotype (13). Its effect has been shown in HCV+HIV co-infected patients as well (25,26). This CC genotype was also reported to be associated with a higher rate of spontaneous clearance in European and Asian populations (20,27). On the other hand, these significant SNPs observed in Europe and Asia were not strongly associated with Japanese population (17). Moreover, it was found that genomic ancestry did not interfere with therapy response among HCV genotype 1 patients with C/C genotype in a Brazilian study (28).

The frequency of homozygote genotype (*rs12979860* CC) is different among countries (29). It is found in 24.2% of the patient group and 43.3% of the control group in our study. In a German study, the *rs12979860* CC was 33.9% in genotype 1 and 49% in the control group, which is pretty much, same as in our study (30). When we compared the genotype frequencies between the each group, *rs12979860* CC, *rs12980275* GG, and *rs8099917* GT genotypes and *rs12979860* C, *rs12980275* A, and *rs8099917* T alleles were found to be higher in the patients but *rs12979860* CT (57.6%), *rs12980275* GA genotype (54.6%); and *rs8099917* GT genotypes were the most common genotypes and all were associated with RVR and the RVR was found to be the best indicator for treatment outcome (31).

The main focus of the present study was the importance of the SNP of the IL28B gene. However, not only the rs12979860 CC variability may influence the treatment response but also the *rs12980275* and *rs8099917*. We wanted to bring out the importance of differences between variabilities among countries so that, focusing only the *rs12979860* CC genotype should not accurately identify those patients who would respond to the therapy and who would not need longer treatment period. Although our study revealed results consistent with that in many studies, the frequency of the *rs12979860* CC genotype has been found lower in HCV genotype 1 vs. genotype 2/3 patients in a German study (30). It was the same in a Spanish cohort: the CC genotype was overrepresented among patients infected with viral genotypes non-1 (66.7% versus 39.1% in patients) (32). In Taiwan, not the *rs12979860* but the *rs8099917* TT genotype had benefit from a shorter duration of combination therapy in HCV-1 patients (33). In Uzbekistan, SNP *rs8099917* was found the most predictive of outcome for Central Asians (18) and in chile, all the three genotypes (the IL28B *rs12979860* CC, *rs12980275* AA and *rs8099917* TT) have been found frequent in patients with SVR compared to null responders (38%, 44% and 50% vs. 2%, 8.2% and 8.2%, respectively) (34). Two recent studies have failed to show such an association: status of IL-28B polymorphism neither affected nor had an impact on virologic response in France and Japan (35,36).

In this study, we also evaluated fibrosis. Although we did not search HAI and Ishak fibrosis scoring, based on APRI, there was no association observed in terms of fibrosis and IL28B polymorphism in this study. A study observed an association between IL28B and fibrosis progression in CHC patients with IL28B CC genotype had significantly higher portal inflammation (2.4 versus 2.2) and ALT levels (37).

Study Limitations

The study was conducted before the start of the use of new treatments.

Conclusion

The determination of IL28B polymorphisms may be useful to individualize treatment options when using Peg/RBV-based therapies for CHC but countries must know their population's genetic characteristics.

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Ethics

Ethics Committee Approval: The study were approved by the Gaziosmanpaşa University of Local Ethics Committee, Informed Consent: Consent form was filled out by all participants.

Peer-review: Externally and Internally peer-reviewed.

Authorship Contributions

Medical Practices: Aydın Rüstemoğlu, Özgür Günal, Concept: Aydın Rüstemoğlu, Özgür Günal, Didem Yalçın, Design: Özgür Günal, Didem Yalçın, Betül Çelik, Data Collection or Processing: Şener Barut, Ömer Ateş, Analysis or Interpretation: Didem Yalçın, Betül Çelik, Literature Search: Aydın Rüstemoğlu, Şener Barut, Ömer Ateş, Writing: Özgür Günal, Didem Yalçın, Betül Çelik.

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Hepatitis B Virus Carrying Drug-resistance Compensatory Mutations in Chronically Infected Treatment-naive Patients

Tedavi Almamış Kronik Hepatit B Olgularında İlaç Direnci İlişkili Kompansatuvar Mutasyonlar

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ABSTRACT

Objective: The prevalence of hepatitis B virus (HBV) is highly variable throughout the world. Geographical regions are classified according to the prevalence of hepatitis B surface antigen in the general population as high (>8%), moderate (2-7%), and low endemicity (<2%). Turkey has a moderate endemicity level of HBV infection which is a serious health problem. Currently, there are various nucleos(t)ide analogues with anti-HBV activity and they are mostly used in the treatment of chronic hepatitis B (CHB) and cirrhosis. The risk of drug resistance increases because these drugs are still being used as monotherapy. It has been reported that HBV drug resistance-related mutations can occur also in patients who are classified as treatment-naive and who have not received any oral anti-HBV treatment.

Materials and Methods: This prospective and descriptive epidemiological study aimed to determine the genotype/subgenotypes of HBV and to investigate the drug resistance mutations in treatment-naive CHB patients. The study included 149 CHB patients who had no chronic co-infections, and have not received treatment for CHB infection. In 53 of the samples collected from the patients, the amount of viral DNA was enough for sequence analysis to search for drug resistance. BigDyeTM Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, Calif., USA) was used for sequencing of the serum samples from these patients and drug resistance mutations were determined and genotype/subgenotype detection was performed.

Results: The mean viral load value was calculated as $9.84x10^6$, and there was no primary drug resistance in any of these 53 samples which were sequenced. There were compensatory resistance-related amino acid changes in 19 samples. Genotype D was determined as HBV in all cases.

Conclusion: The early detection of drug resistance-related mutations can be important in determination of treatment protocol, and prevention of unnecessary drug use, complications, and economic loses.

Keywords: Hepatitis B virus, naive patients, drug resistance, compensatory mutation

ÖZ

Amaç: Dünya çapında hepatit B virüs (HBV) prevalansı oldukça değişkendir. Yüksek (>%8); orta (%2-7) ve düşük (<%2) endemik bölgeler içinde sınıflandırılmıştır. Türkiye HBV enfeksiyonu açısından orta endemisiteye sahip olup ülkemiz için ciddi bir halk sağlığı sorunudur. Günümüzde anti-HBV aktivitesi olan çeşitli nükleozit analogları mevcuttur ve kronik hepatit B (KHB) ve siroz tedavisi için çoğunlukla kullanılmaktadır. Bu ilaçlar monoterapi şekilde kullanıldığından ilaç direnci riski artmaktadır. HBV ilaç direnci ile ilişkili mutasyonların, herhangi bir oral anti-HBV tedavisi almamış naif hastalarda da meydana geldiği bildirilmiştir.

Gereç ve Yöntemler: Prospektif, tanımlayıcı temelinde gerçekleştirilen bu epidemiyolojik çalışmada, naif KHB hastalarında ilaç direnç mutasyonlarının araştırılması ve HBV genotip/subgenotiplerinin belirlenmesi amaçlanmıştır. Çalışmaya KHB olduğu bilinen, başka bir kronik koenfeksiyonu bulunmayan, KHB enfeksiyonu için tedavi almamış 149 hasta dahil edilmiştir. Hastalara ait örneklerden 53'ünde viral DNA miktarı ilaç direnci araştırmasında sekans analizi için yeterli olmuştur. Hastalara ait serum örneklerinde BigDyeTM Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, Calif., ABD) kullanılarak yapılan sekanslama yöntemi ile ilaç direnç mutasyonları araştırılmış, genotip/subgenotip tayini yapılmıştır.

Bulgular: Katılımcıların ortalama viral yük değeri $9,84x10^6$ olarak hesaplanmış, sekanslaması yapılan 53 örneğin hiçbirinde primer ilaç direnci saptanmazken, 19 olguda kompansatuvar direnç ile ilişkili aminoasit değişiklikleri tespit edilmiştir. Olguların tamamında genotip D olarak belirlenmiştir.

Sonuç: KHB tedavisindeki amaç hastalığın progresyonunu önlemek ve sağ kalımı sürdürmektir. İlaç direnci ile ilişkili mutasyonların erken dönemde tespiti, tedavi protokolünün belirlenmesinde yol gösterici olması, gereksiz ilaç kullanımı, komplikasyon gelişimi ve ekonomik kayıpların önlenmesi açısından önemli olabilir.

Anahtar Kelimeler: Hepatit B virüs, naif olgular, ilaç direnci, kompansatuvar mutasyon

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Introduction

Hepatitis B virus (HBV) is a viral factor leading to acute and chronic infections by affecting the liver (1). Even though an effective hepatitis vaccine has been commonly used, approximately 2 million individuals (almost one third of the world's population) encounter the hepatitis virus and it is known that HBV is a growing global public health problem (2). Probably 240 million people have chronic HBV infection and more than 686.000 individuals die every year due to hepatitis B infection-related diseases such as cirrhosis and liver cancer (1). It has been specified in studies conducted in our country that the prevalence of hepatitis B surface antigen varies between 0.8% and 14.3% and it has been reported that our country has a moderate endemicity level among other regions of the world (3).

HBV can lead to different clinical outcomes such as asymptomatic infections, fulminant hepatitis, inactive carrier state, and even life-threatening diseases such as liver cirrhosis and liver cancer. Therefore, early detection and treatment of HBV is crucial. Non-specific factors, such as age, and specific genetic factors in the host as well as genetic features of the virus can affect the prognosis of the HBV infections (4). Genetic variance can occur due to the genotypic differences in carriers or mutations in the infected host (5,6). Although it has not yet been clarified, findings support that genotypic differences have effects on the HBV pathogenicity and the clinical course of the infection. Therefore, determination of the genotype in the beginning of the disease can contribute to clinical approaches in a more conscious way (4). It has been determined that there are 10 different HBV genotypes (from A to J) in addition to the recently defined genotypes (5,6). However, there have been a limited number of studies on HBV genotype distribution in Turkey. In the work done in the obtained data, genotype D and subgenotype D1 have been found to be a widely prevalent genotype in Turkey (7,8,9).

Developments in viral techniques used in the diagnosis of chronic HBV infections ensure that individual treatments can be more accurately decided. Recently, hepatitis treatment is better managed by the application of molecular techniques, which can quantitatively detect HBV DNA, genotype detection, and determination of the antiviral resistance. It is very important that the right patient is treated conveniently and the right drug is used for the sake of the future of both the patient and the drug. Therefore, the treatment goals and treatments should be accurately determined (10,11).

Recently, there have been various approved chronic hepatitis B (CHB) treatments which prominently decrease the mortality and morbidity rates. They include two interferons (IFNs) (conventional and pegylated alfa-2a) analogues and five nucleoside/nucleotide analogues (NAs): lamivudine (LAM), telbivudine (TBV), entecavir, adefovir (ADV), and tenofovir. NA inhibits the activity of HBV polymerase and suppresses its replication by binding it competitively. However, the drug resistance is a common problem in long-term treatments (12). Furthermore, recent studies showed that there is an antiviral resistance even in HBV isolated from non-treated patients (13).

In this study, the pol and s gene kinetics İN treatment-naive HBV patients were evaluated by using the sequencing technique and drug resistance-related mutations were examined. The aim of the study was to determine the HBV genotype and subgenotypes and thus contribute to the epidemiological data.

Materials and Methods

In this prospective and descriptive epidemiological study, we included a total of 149 patients who had no any infection except CHB and who were treated for CHB between January 2012 and May 2013. Blood samples obtained from the patients were used for routine laboratory tests and then remaining blood serum samples were stored at -80 ºC till they were used. The amounts of viral DNA in 53 samples were sufficient for drug resistance sequence analysis assay. The demographic and clinical data of participants were obtained from the hospital records. The ethical approval of this study (15/10/2015-12797) was obtained from Sakarya University.

HBV DNA levels were measured by the real-time polymerase chain reaction (RT-PCR) technique with the help of a Cobas TaqMan 48 kit (Roche Diagnostics, USA). The degenerate primers were used to amplify the 16S gene, from HBV samples and from isolates. The PCR products were purified with a QIAquick PCR Purification Kit (Qiagen) and used for direct sequencing using a BigDyeTM Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, Calif., USA), and an ABI-3100 at the Center for Comparative Genomics at the University of Iowa (Iowa City, Iowa, USA). The sequences for phylogenetic analysis were retrieved from DDBJ/EMBL/GeneBank. Alignments were carried using CLUSTALW [http://clustalw.ddbj.nig.ac.jp/top-e.html] and neighbor-joining tree was formed. Statistical differences were analyzed by DNA polymorphism levels within locations and mutations were summarized in haplotype diversity (h) and nucleotide diversity (π) with standard deviation. Population expansions were assessed by neutrality tests implemented in DnaSP v.5.10.01, for the Tajima's D and the Fu and Li's F.

Results

Gene sequences of PCR products in 53 patients (41 male and 12 female) were obtained by using PCR and sequencing techniques. The amount of DNA samples in the rest of patients was not sufficient for sequence analysis. The average age of the patients was 49.3 years (range: 24-74). The average viral load was $9.84x10^6$ IU/mL (range: $1.346x10^1$ IU/mL-$4.383x10^8$ IU/mL. Some demographical characteristics and laboratory findings of patients are shown in Table 1. All the 53 patients had genotype D. Subgenotype distribution was as follows: 46 patients had genotype D1, 4 patients had genotype D2, and 3 patients had genotype D3. There was no primary drug resistance in any of the patients (Table 1). There were amino acid alterations in 19 patients and these alterations were associated with compensatory resistance which had roles in viral replication repair and increased viral loads (Table 2). It was detected that there were compensatory changes associated with only TBV, LAM and ADV, and only ADV in 4, 13, and 2 patients, respectively.

Discussion

The final goal of CHB treatment is to prevent the progression of the disease and to maintain survival. It is possible to achieve these goals by suppressing HBV replication and maintaining this

suppression (14). Recently, seven antiviral agents have been approved in order to be used in the CHB infection treatment. Two of them were IFN-α(IFN/pegylated IFN) analogues and five of them were NAs. NAs suppress HBV replication by affecting the reverse transcriptase enzyme. The most important issue during long-term CHB treatment with NAs is the mutagenesis which is responsible for the antiviral agent resistance. Amino acid alterations in the HBV reverse transcriptase cause NA resistance which is an important problem in CHB treatment (6,14). Even though it is specified that there is no need to examine drug resistance in patients who have not been previously treated (10,14,15), there can be spontaneous drug resistance mutations in treatment-naive patients (6). NA resistance mutations can occur in non-treated patients because of the viral and patient factors. The reasons for the variance of resistance in non-treated patients are: 1) these mutations can naturally occur during the replication (10^{-5}-10^{-4} substitution/base/cycle), 2) the patient can be infected by a mutant virus from another patient who was previously treated, and 3) the patient can be directly and unconsciously exposed to equivalent components with anti-HBV activity (12,16). Thus, resistance mutations can be observed in non-treated patients. These mutations can be primary drug resistance mutations (drug unresponsiveness) or they can increase the replication capacities of reduced resistant HBV variances (11).

In recent studies conducted with non-treated patients in different countries, it was shown that the frequencies of HBV strains which had clinically important drug resistance mutation (NA resistance) were between 0.5% and 1% (17,18,19,20,21,22,23,24,25). In our country, Akarsu et al. (26) evaluated 71 inactive HBV carriers who have not received any treatment and they detected YMDD variants (18.3%) in 13 cases. Yıldız et al. (27) detected a primary resistance against LAM in 8 of 202 LAM-naive CHB patients (4%). Sayan et al. (28) evaluated 88 treatment-naive CHB patients and they showed that there were amino acid alterations in HBV polymerase genes in 17 of these patients (19%). Ergünay et al. (29) evaluated 30 CHB patients who were not previously treated and they determined HBV NA resistance-related mutations in 3 of them (10%).

Table 1. Demographical characteristics and laboratory information of 53 patients whose DNA was sequenced

Clinical factors	Value
Male/Female	41/12
Average age (year)	49.3 years
HBeAg positive (n)	26
Average ALT (U/mL)	16.7
Average AST (U/mL)	39.8
Average HBV DNA (IU/mL)	$9.84x10^6$
HBV genotype (subgenotype)	46 (D1) 4 (D2) 3 (D3)

HBeAg: Hepatitis B envelope antigen, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, HBV: Hepatitis B virus

Table 2. Number of compensatory mutation cases in treatment-naive hepatitis B virus patients

Reverse transcriptase mutations (compensatory)	Antiviral agent	Case number
Q149K	LAM and ADV	4
Q215S	LAM and ADV	4
Q215H	LAM and ADV	4
L91I	TBV	4
Q249K	LAM and ADV	1
N238D	ADV	1
V214A	LAM and ADV	1

LAM: Lamivudine, ADV: Adefovir, TBV: Telbivudine

In our study, there was no primary drug resistance in any of the 53 patients. In order to determine the nucleotide alterations in targeted region of the viral genome, DNA sequence analysis method was applied by using PCR products. This technique has an advantage of showing all mutations in the amplified region and it is accepted as the gold standard for the detection of nucleotide alterations. However, mutant genomes should constitute at least 20% of the whole viral population in order to detect all of the mutations in the genome directly by using sequence analysis. In our study, it is possible that we did not detect primary drug resistance amino acid alterations because of their low levels. Recent and new generation sequencing systems can be used to analyze the whole viral genomes and thus all viral genome pool of infected people can be examined. However, these approaches cannot be easily used in all institutions because they require equipments and experienced staff (29,30). Furthermore, emergence of resistance variants against approved NAs is the main reason for treatment failure in CHB infections. Therefore, the treatment can be regulated timely with the help of the early detection of these mutations and thus aggravation of the disease can be prevented. Meanwhile, early detection of these mutations can also contribute to detection of compensatory mutations which can repair the viral replication and increase the resistance risk against other drugs (16).

Population sequencing method can be used to detect the known NA resistance mutations as well as compensatory mutations. It has been reported that rtQ215H/Q/P/S compensatory mutations are frequently observed in CHB patients who receive both NA-naive and LAM and/or ADV treatments (11,12). In our study, there were compensatory mutations in 19 patients and 4 of them had rtQ149K compensatory mutation which was associated with LAM and ADV treatment. Other compensatory mutations were Q215S mutation associated with LAM and ADV (4 patients), Q215H mutation associated with LAM and ADV (4 patients), rtL91I mutation associated only with TBV (4 patients), Q249K mutation associated with LAM and ADV (1 patients), N238D mutation associated with only ADV (1 patients), and V214A mutation which was associated with LAM and ADV (1 patient) (Table 2).

Compensatory mutations which can repair the viral replication and increase the viral load and particularly the mutations related to drug resistance in NA target region of the HBV polymerase gene can be associated with treatment failure. Compensatory mutations can lead to unnecessary or incorrect medication changes in CHB patients. Mutation detection in HBV patients can have important effects on the development of different treatment strategies (31).

It has been specified that there are at least 10 HBV genotypes (A-J) in case of differences more than 8% in the HBV genome. Some genotypes are divided into subgenotypes in case there is

a 4-8% difference between nucleotide sequences. Recent data has shown that genotypes are determining factors affecting the severity of the liver disease and the response against the antiviral drug (7). Furthermore, HBV genotype D is the most commonly determined genotype in our country and the most commonly observed subgenotype is the genotype D1. Even though Kaklikkaya et al. (7) have reported that the most commonly observed genotype was genotype D2, other studies claimed that the genotype D1 was the most frequently detected one in our country. In our study, all patients were classified as HBV genotype D and the most frequently detected genotype was genotype D1. Genotype D2 and genotype D3 were the other genotypes that can be observed in Turkey.

NA combinations are important in treatment-resistant patients and initial treatment selection and subsequent treatment decisions should depend on the resistance rates (6). Consequently, early detection of antiviral drug resistance-related mutations can be important in determination of the most convenient treatment protocol. Thus, toxicity and economic losses due to unnecessary drug use can be prevented and severe complications can be reduced (32,33). Although we did not detect such mutations in our study, there are other studies in which drug resistance mutations are detected in treatment-naive CHB patients. Furthermore, it will be clinically useful to determine the resistance profile since treatment-naive CHB patients develop primary drug resistance against LAM and ADV (29). Further studies investigating mutations in the viral polymerase region and determining the clinical importance of these mutations in treatment-naive patients are warranted.

Ethics

Ethics Committee Approval: The ethical approval of this study (15/10/2015-12797) was obtained from Sakarya University.

Peer-review: External and Internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Mustafa Altındiş, Concept: Mustafa Altındiş, Design: Mustafa Altındiş, Ferhat Gürkan Aslan, Mehmet Köroğlu, Data Collection or Processing: Mustafa Altındiş, Ferhat Gürkan Aslan, Mehmet Köroğlu, Leyla Demir, Mustafa İhsan Uslan, Savaş Aslan, Mehmet Özdemir, Analysis or Interpretation: Mustafa Altındiş, Ferhat Gürkan Aslan, Mehmet Köroğlu, Ayla Eren, Mahmut Baykan, Literature Search: Mustafa Altındiş, Ferhat Gürkan Aslan, Ayla Eren, Writing: Mustafa Altındiş, Ferhat Gürkan Aslan, Ayla Eren.

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Case Report

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Acute Viral Hepatitis B with a Severe Clinical Course in Pregnancy: A Case Report

Gebelikte Ciddi Seyirli Akut Viral Hepatit B: Olgu Sunumu

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ABSTRACT

The most common cause of non-pregnancy-specific acute hepatitis during pregnancy is hepatitis B. The clinical course of viral hepatitis B in pregnancy is similar to that in non-pregnant women, and about 1% of patients may develop acute liver failure. In this paper, we present a patient at 16 weeks of pregnancy who was administered tenofovirdisoproxil fumarate due to acute hepatitis B.
Keywords: Pregnancy, acute hepatitis B, tenofovir

ÖZ

Gebelik esnasında ortaya çıkan ve gebeliğe özgü olmayan en sık akut hepatit nedeni viral hepatit B'dir. Gebelikte viral hepatit B hastalarının klinik seyri gebe olmayanlarla benzer olmasına karşın hastaların yaklaşık %1'i akut karaciğer yetmezliğine ilerleyebilir. Bu yazımızda 16 haftalık gebeliği olan akut hepatit B hastasının takiplerinde ciddi seyirli olması üzerine tenofovir disoproksil fumarat başlanan bir hasta sunulmuştur.
Anahtar Kelimeler: Gebelik, akut hepatit B, tenofovir

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Introduction

Hepatitis B virus (HBV) is a DNA virus that belongs to the Hepadnaviridae family. HBV infection is an important health issue leading to acute and chronic hepatitis, cirrhosis, and hepatocellular carcinoma in clinical course (1).

Şanlıurfa is in a HBV endemic region where birth rates increase each year (2). Pregnancy-specific liver diseases are responsible for approximately 60% of the liver diseases in pregnancy, whereas 40% of these diseases are caused by acute viral hepatitis (3,4). The most common cause of jaundice is acute hepatitis B during pregnancy, and the treatment of acute infection is essentially as supportive (5). Antiviral therapy should be considered in patients with acute liver failure or severe chronic hepatitis (6). In this paper, we present a pregnant woman who was using tenofovir disoproxil fumarate due to acute hepatitis B.

Case

An 18-year-old pregnant woman was admitted to our gastroenterology department with the complaints of fatigue, yellowing of the skin, and itching. In history, she was at 16 weeks of pregnancy and itching and yellowing on her body was increased gradually in the past 2-3 days. She had no known any previous disease. Other systems were normal on physical examination. The laboratory findings were as follows: WBC: 12.300/mcL, hemoglobin: 14.4 g/dL, platelet: 258.000/mcL, urea: 8 mg/dL, creatinine: 0.5 mg/dL, aspartate aminotransferase: 2038 U/L, alanine aminotransferase: 2223 U/L, alkaline phosphatase: 183 U/L, gamma-glutamyltransferase: 45 U/L, total bilirubin: 15.4 mg/dL, direct bilirubin: 10.4 mg/dL, albumin: 3.4 g/dL, prothrombin time: 18.9 seconds, international normalized ratio (INR): 1.45, activated partial thromboplastin time: 32.8 seconds, sodium: 134 mEq/L, K: 3.3 mEq/L, P: 2.6 mg/dL, and C-reactive protein: 0.87 mg/dL. Trace amounts of urobilinogen, bilirubin in urine tests: was (++). Patient's viral serology were: hepatitis B surface antigen (HBsAg) (+), anti-Hbs (-), anti-hepatitis C virus (HCV) (-), anti-HBc, immunoglobulin (Ig) G (+), anti-HBc IgM (+), anti-hepatitis A virus (HAV) IgG (+), anti-HAV IgM (-), anti-HDV (-), HBV-DNA:1,27x106 IU. Ultrasonographic examination showed that the fetus was

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alive at the 16th week of gestation. The patient was hospitalized with the diagnosis of acute hepatitis B. She was hydrated and her liver function tests were monitored daily. During monitoring, there was an increase in INR and total bilirubin (2.01, 19.4 mg/dL, respectively). Hence, severe acute hepatitis B was considered and tenofovir 245 mg 1x1 was started. Because the liver function tests were decreased after treatment, the patient discharged with follow-up recommendation.

Discussion

Although pregnancy-specific liver diseases are more common in pregnant women who present with acute hepatitis, acute viral hepatitis should be considered in the differential diagnosis particularly in endemic regions (7,8). The differential diagnosis of liver diseases in pregnancy and starting treatment without delay is very important in terms of morbidity and mortality in both mother and baby. In our case, the patient had no characteristic history. The complaints of jaundice and pruritus indicated a pregnancy-specific liver disease at first. The patient's physical examination and abdominal ultrasonography were normal except for jaundice and 1 cm hepatomegaly. Transaminases were increased more than 10-fold, and the patient was diagnosed with acute hepatitis B upon detection of HBsAg (+), anti-HBsAg (-), anti-HCV (-), anti-HBc-IgM (+), and anti-HBc-IgG (+) in the viral serology.

The treatment of acute hepatitis B is supportive (9). Acute HBV infection becomes chronic in 5-10% of adults, and approximately 1% of patients may develop acute liver failure (10). In a study, 22 pregnant women were compared with 87 non-pregnant women with acute HBV infection and no difference was found between the two groups in terms of risk of fulminant hepatic failure (11). In another study investigating the etiologic causes of fulminant hepatic failure in 52 pregnant patients, the most common cause was found to be acute hepatitis B following hepatitis E (10). Antiviral therapy should be considered in patients with acute liver failure and severe chronic hepatitis (9). Our patient was considered as having acute hepatitis B in the second trimester, and was hospitalized and followed up. In follow-up, bilirubin and prothrombin levels were increased progressively, thus, severe hepatitis B was considered and an antiviral treatment was started.

The perinatal transition rate has been reported to be 10% (12) in early acute hepatitis B, whereas this rate was reported as 60% (5) in the time of delivery. If the mother has high serum HBV DNA levels before the delivery, antiviral treatment should be considered to reduce viral load in the mother (13,14). Tenofovir disoproxil fumarate (245 mg/day) or lamivudine (100 mg/day) are recommended for antiviral treatment and both drugs can be used safely during pregnancy (9,15). Since the risk of resistance is low, tenofovir disoproxil fumarate should be preferred (16). In our series of 296 HBsAg-positive patients whom we followed between Dec 2011 and Apr 2012 in the Şanlıurfa region, there were 7 pregnant patients (approximately 2%), and tenofovir was started in 3 of these patients in the last trimester because of the higher viral load (2). In our patient, severe hepatitis B was considered and tenofovir disoproxil fumarate therapy was started. Since the liver function test values were decreased after treatment, the patient discharged on follow-up recommendations.

Conclusion

Acute hepatitis B might be seen during pregnancy. These patients should be strictly followed for acute liver failure and antiviral treatment should be administered if necessary.

Ethics

Informed Consent: It was taken.

Peer-review: Externally and Internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Ahmet Uyanıkoğlu, Concept: Ahmet Uyanıkoğlu, Design: Ahmet Uyanıkoğlu, Data Collection or Processing: Ferzan Aydın, Ahmet Uyanıkoğlu, Analysis or Interpretation: Ahmet Uyanıkoğlu, Literature Search: Hacer Uyanıkoğlu, Ahmet Uyanıkoğlu, Necati Yenice, Writing: Ahmet Uyanıkoğlu, Hacer Uyanıkoğlu.

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An Important Financial Burden: Unnecessary Test Requests for Viral Hepatitis

Önemli Bir Ekonomik Yük: Viral Hepatitlerin Gereksiz Test İstekleri

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Keywords: Hepatitis, unnecessary test, cost
Anahtar Kelimeler: Hepatit, gereksiz test, maliyet

Karacaer Z. An Important Financial Burden: Unnecessary Test Requests for Viral Hepatitis. Viral Hepat J. 2016;22:111-112.

Dear Editor;

Cost-effective approaches for using limited financial resources correctly are essential in the healthcare field. Life expectancy has been increasing in developed and developing countries, and nowadays, acute and chronic diseases are being treated with processes that are becoming more expensive. Consequently, increasing pressure on the health budgets of countries around the world is now a reality. The health expenditure percentage of the gross domestic product is expected to climb in future years (1).

To decrease this health expenditure, initially, misapplications in health care should be detected. An article by Demiray et al. (2) in the recent issue of the Viral Hepatitis Journal emphasized the costs of unnecessary tests, and provided beneficial information on this subject for clinicians.

Unnecessary test requests for viral hepatitis, in particular, are a significant problem in various centers in Turkey. Alpay Ozbek et al. (3) found that 14% of anti-hepatitis A virus total tests and 18% of (anti-HBc) tests were unnecessarily repeated over a three-year period at a university hospital. The rate of inappropriate tests used for the diagnosis of hepatitis A infection was 52.2% in a two-year study at a state hospital. At this same hospital, it was detected that 12.9% of anti-HBs, 12.9% of anti-HBc total, 74.8% of anti-HBc immunoglobulin M (IgM), 83.9% of hepatitis B envelope antigen (HBeAg), and 75.2% of anti-HBe tests were unnecessary, at a total cost of 56.153 Turkish liras (TL) (4). Genc and Aksu (5) found that the percentages of unnecessary test requests were 2.23% in anti-HBs, 0.7% in anti-HBc total, 37.41% in anti-HBc IgM, 44.86% in HBeAg, and 37.75% in anti-HBe at a tertiary care hospital, and those tests led to an average annual financial burden of 14.000 TL.

The causes of unnecessary test requests were as follows: i) failure of healthcare workers to check previous tests results due to lack of time; ii) difficulty for healthcare workers to retrieve previous results; iii) distrust of previous test results; iv) no familiarity of diagnostic algorithms; v) requests for tests made by non-physician healthcare providers; vi) failure to determine if the patient had already received the hepatitis B vaccine; vii) requests for screening tests for preventive medicine (5). These reasons can all be corrected. Consequently, unnecessary tests might decrease.

The total economic burden of chronic hepatitis infection in terms of treatment, monitoring, and complications is high (6), all costs which might be inevitable. The results of health policies that will be developed to prevent diseases may require a long time. In addition, we cannot afford prevention campaigns. However, it is possible to lessen this financial burden with some preventative measures.

A test may be studied in accordance with another test, as appropriate diagnostic algorithms. This practice is called reflex tests. Therefore, if the first requested test is not appropriate, the second and subsequent tests may be blocked (7). Determining test interval is important. Warning signals that are situated within hospital network systems may report to the physician previous test results. Thus, the physician may not request the tests again (5). Another technique for preventing duplicate tests is organizing education about diagnostic algorithms for health professionals (4). Also, if the patient is infected with hepatitis B or C virus, this information can be saved on hospital network systems. History of

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hepatitis B or A vaccine, the presence of hepatitis B and C infected person in the family, and previous hepatitis test results performed in another hospital can be questioned.

Ethics

Peer-review: Externally and Internally peer-reviewed.

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Human Pegivirus (GB Virus Type C) and Its Relationship with HIV

İnsan Pegivirus (GB Virüs Tip C) ve HIV ile Olan İlişkisi

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Keywords: Human pegivirus, GB virus C, Hepatitis G virus, HIV
Anahtar Kelimeler: İnsan pegivirus, GB virus C, Hepatit G virus, HIV

Aslan FG, Altındiş M. Human Pegivirus (GB Virus Type C) and Its Relationship with HIV. Viral Hepat J. 2016;22:113-114.

Dear Editor;

Human pegivirus (HPgV) is a RNA virus which is classified in *Flaviviridae* family. The virus is named as hepatitis G virus (HGV) or GB virus type C (GBV-C) (1).

HPgV was initially considered as hepatotropic depending on its definition in humans with non-A, non-B, and non-C hepatitis. Then, no association was found between acute or chronic hepatitis and HPgV. Therefore, virus (HGV or GBV-D) together with primate and bat viruses (GBV-A, GBV-Cczp, GBV-D) were included in a new genus (Pegivirus). It was again named as HPgV. HPgV infection can occur via exposure to infected blood, sexual contact, and mother-to-child transmission (1).

HPgV can cause infections either alone or in combination with other factors such as hepatitis C virus (HCV) and HIV. The effects of this virus on chronic infections with hepatitis B virus or HCV have not yet been clarified (2). Furthermore, it was shown that the prevalence of HPgV increased in individuals infected with HCV and the HCV RNA levels were permanently high in livers of individuals infected by the combination of HCV/HPgV (1). It was specified that HPgV infection can have effects on chronic infection development or drug resistance (2). Viral hepatitis is commonly observed in chronic dialysis patients. The risk of HPgV infection increases in hemodialysis patients. However, this risk is lower in continuous ambulatory peritoneal dialysis patients (3).

Besides HPgV is associated with beneficial effects in HIV infection. Various studies and a meta-analysis found that survival in HIV-infected individuals was longer in patients with HPgV viremia compared to those without viremia (4). It was concluded that GBV-C viremia was associated with lower mortality in HIV-infected patients. As a result of T cell activation which happens due to HIV infection, immune functions deteriorate and AIDS progresses after the loss of CD4 (+) T cells. In contrast, compared to chronic HIV-infected patients without HPgV infection, HPgV infection in patients with acute HIV infection is associated with significant decrease in the expression of T cell activation markers. This is independent from treatment (1). In comparison to inactive cells, HIV replication decreases in active peripheral blood mononuclear cells and this finding supports the relationship between HPgV and T cells proliferation (5).

Consequently, when its clinical outcomes and co-infections are considered, even though HPgV is a non-cytopathic factor, there should be further studies investigating the relationship between HIV-1 subtypes and HPgV infection as well as the possible molecular and cellular mechanisms behinds its effects.

Ethics

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Authorship Contributions

Concept: Mustafa Altındiş, Ferhat Gürkan Aslan, Design: Mustafa Altındiş, Ferhat Gürkan Aslan, Data Collection or Processing: Ferhat Gürkan Aslan, Mustafa Altındiş, Analysis or Interpretation: Mustafa Altındiş, Ferhat Gürkan Aslan, Literature Search: Mustafa Altındiş, Ferhat Gürkan Aslan, Writing: Ferhat Gürkan Aslan, Mustafa Altındiş.

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VİRAL HEPATİT DERGİSİ

Yazarlık, Yayın Hakkı Devri, Maddi Yardım ve Teşekkür-Kabul İzni

TEŞEKKÜR VE KABUL BEYANI BÖLÜMÜ, SORUMLU YAZAR TARAFINDAN İMZALANMALI. SON BÖLÜM İSE MAKALEDE İSMİ GEÇEN BÜTÜN YAZARLAR TARAFINDAN İMZALANMALIDIR.

MAKALE BAŞVURUSUNDA FORM DOLDURULARAK ONLİNE SİSTEME YÜKLENMELİDİR.

BU FORM GEREKİRSE, İMZA İÇİN HER BİR YAZAR TARAFINDAN DOLDURULMAK ÜZERE FOTOKOPİ İLE ÇOĞALTILABİLİR.

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Telif HAKKI

Telif hakkı oluşturulmuş olup toplum tarafından kullanıma açıktır. Orijinal olduğunu, daha önce yayınlanmadığını ve yayınlanmak üzere değerlendirme aşamasında olmadığını beyan ederim.

YAYIN HAKKI ŞARTNAMESİ

Bu başvuru ile makalemizin değerlendirme ve düzeltilmesinin, *Viral Hepatit Dergisi* tarafından yapılma haklarını; imza yetkisi, kopyalama ve başka şekillerde çoğaltılmasını da içeren yayın haklarını ve basım haklarını Galenos Yayınevi'ne veriyorum.

Bu çalışmanın daha önce yayınlanmadığını, yayınlanması için değerlendirilmek üzere gönderilmediğini ve değerlendirme aşamasında olmadığını, belirtilen sunum(lar) dışında başka bir yerde yayınlanmadığını onaylıyorum.

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DOĞRUDAN DESTEK KAYNAKLARI

- ❍ Bu çalışmaya destek veren kaynak(lar) yoktur.
- ❍ Bu çalışma için maddi ve materyal destek kaynakları makalede tanımlanmış ve aşağıda sıralanmıştır.

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- ❍ Ben ve arkadaş(lar)ımın birbiriyle çelişen maddi veya kişisel ilişkimiz olmamıştır.
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